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Ministry of Health
& Family Welfare
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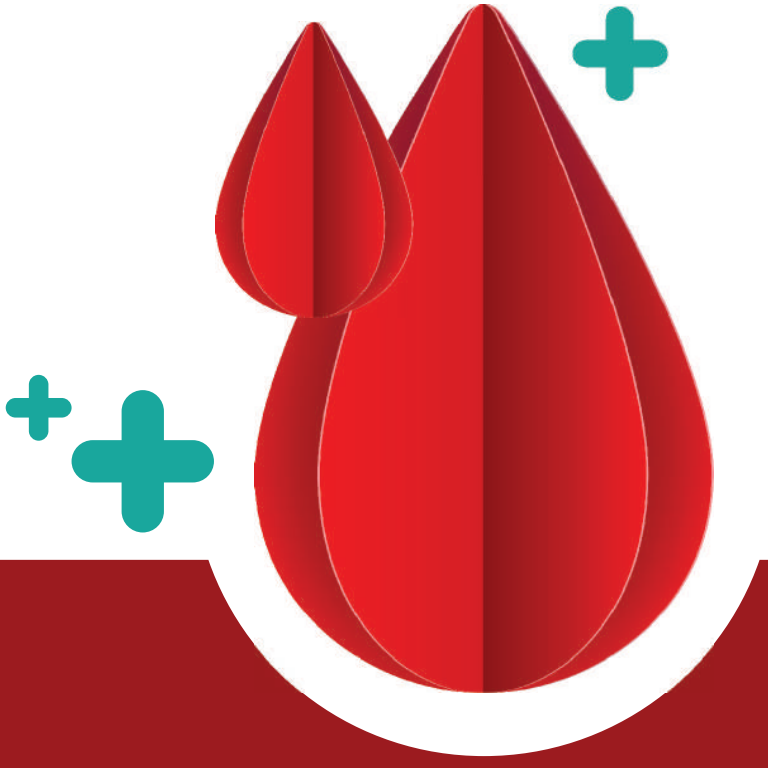

TRANSFUSION MEDICINE TECHNICAL MANUAL

Third Edition 2023

NBTC and Blood Transfusion Services Division
Directorate General of Health Services
Ministry of Health & Family Welfare, Government of India

Reviewed and Published - 2023

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NBTC and Blood Transfusion Services Division
Directorate General of Health Services
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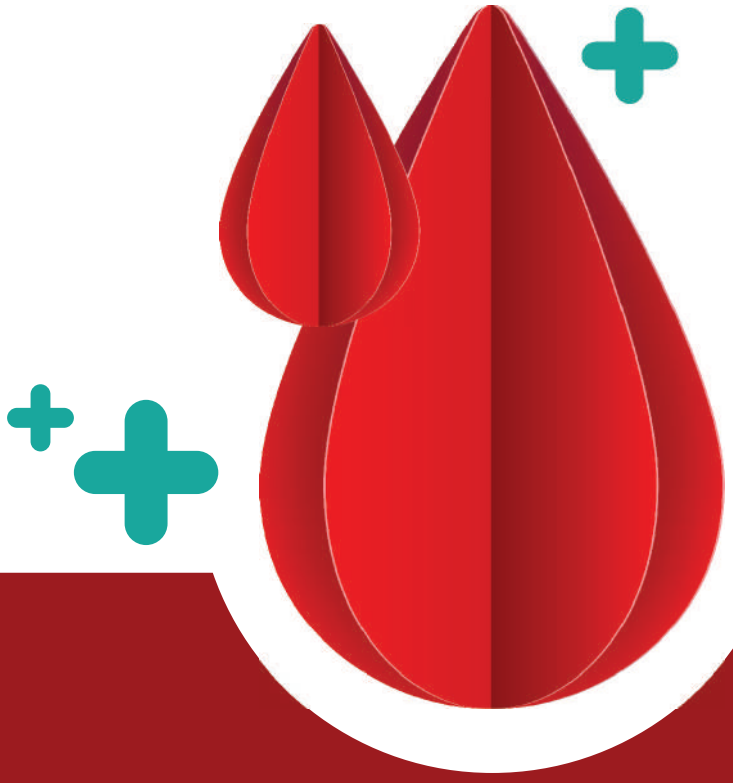

TRANSFUSION MEDICINE TECHNICAL MANUAL

Third Edition 2023

NBTC and Blood Transfusion Services Division

Directorate General of Health Services

Ministry of Health & Family Welfare, Government of India

डॉ. मनसुख मांडविया
DR. MANSUKH MANDAVIYA

मंत्री
स्वास्थ्य एवं परिवार कल्याण
व रसायन एवं उर्वरक
भारत सरकार
Minister
Health & Family Welfare
and Chemicals & Fertilizers
Government of India

MESSAGE

Blood Transfusion Services are an essential part of any national healthcare delivery system and timely transfusion of safe blood can save lakhs of lives every year. It is necessary to keep pace with the rapid scientific and technological advancements occurring in the field to ensure blood safety. Trained manpower is a prerequisite for the safety and efficacy of blood and blood components, thus emphasizing the need of a Technical Manual with recent updates.

The last edition of Transfusion Medicine Technical Manual was published in 2003 and has been now thoroughly updated in line with international practices in the field. It is noteworthy that this revised technical manual has come through the coordinated and dedicated inputs and efforts of the experts in this field from all over the country.

I would like to commend the efforts of National Aids Control Organisation for initiating the process of reviewing of the technical manual. I also appreciate the efforts of Directorate General Health Services, Government of India and National Blood Transfusion Council for taking this initiative forward and in finalizing the manual

I hope that this third edition of Transfusion Medicine Technical Manual would be of immense help in improving and standardizing the Blood Transfusion Services in the country and keep the personnel in this field abreast of the advances in the field of transfusion medicine.

(Dr. Mansukh Mandaviya)

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डॉ. भारती प्रविण पवार
Dr. Bharati Pravin Pawar

सत्यमेव जयते

स्वास्थ्य एवं परिवार कल्याण राज्य मंत्री
भारत सरकार

MINISTER OF STATE FOR
HEALTH & FAMILY WELFARE
GOVERNMENT OF INDIA

MESSAGE

Blood transfusion is one of the oldest and is a widely used modern medical therapy. Transfusion of blood and blood components is an essential segment of health care system. It is a lifesaving measure for patients during emergency situations e.g. road side accidents and transfusion dependent patients suffering from diseases like Thalassemia, Sickle cell anemia, Hemophilia and other conditions.

Rapid advancements have been witnessed over the years in the field of transfusion medicine. Introduction of latest technologies and scientific transfusion practices is necessary for making blood transfusion processes safe, thus stressing the importance of a Technical Manual with recent updates.

The 3rd edition of Transfusion Medicine Technical Manual has now been published incorporating most up-to-date information on all the recent developments including regulatory changes in the field of transfusion medicine.

It is pertinent to emphasize that the Government of India, under the visionary guidance of Hon'ble Prime Minister Shri Narendra Modi, is taking new initiatives to meet all the health needs of the people of India. I would like to appreciate the efforts of National Aids Control Organisation, Directorate General Health Services, Government of India, National Blood Transfusion Council and the technical experts who have contributed immensely in bringing out this edition.

I am sure that this revised manual will serve as a repository of knowledge and skills in the practice of transfusion medicine and will be gainfully utilized by the personnel working in the field.

BP

(Dr. Bharati Pravin Pawar)

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आज़ादी का अमृत महोत्सव

भारत सरकार
स्वास्थ्य एवं परिवार कल्याण विभाग
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
Government of India
Department of Health and Family Welfare
Ministry of Health and Family Welfare

MESSAGE

An efficient Blood Transfusion Service is an indispensable component of healthcare delivery system in the country to achieve universal access to quality blood and blood components. Blood Transfusion Services must work on the principle of 5 A's viz. accessibility, availability, affordability, assured quality & safety and academic excellence.

To ensure quality, safety and efficacy of blood and blood components, all the personnel involved should be well versed with the latest techniques and developments in the field of transfusion medicine. The practice of transfusion medicine has evolved significantly in the last two decades. In view of above, an updated technical manual is thereby essential.

Considering the need to revise the 2nd Edition of Transfusion Medicine Technical Manual, the Technical Resource Group on Blood Safety of National AIDS Control Organization and National Blood Transfusion Council initiated the process of revision and updation. It is heartening to know that the experts from all over the country have given their valuable inputs for revising this technical manual.

I am certain that this technical manual will serve as a reference guide for transfusion medicine practitioners, post graduate students, medical officers and technical staff working in this field. This will go a long way in further improving the safety and efficacy of Blood Transfusion Services in our country.

(Rajesh Bhushan)

Place: New Delhi
Date : 22.03.2023

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स्वास्थ्य एवं परिवार कल्याण मंत्रालय
स्वास्थ्य सेवा महानिदेशालय
Government of India
Ministry of Health & Family Welfare
Directorate General of Health Services

प्रो.(डॉ.) अतुल गोयल
Prof. (Dr.) ATUL GOEL
MD (Med.)
स्वास्थ्य सेवा महानिदेशक
DIRECTOR GENERAL OF HEALTH SERVICES

MESSAGE

Blood Transfusion Services are integral to any health care delivery system in India being no exception. The Transfusion Medicine Technical Manual was first published in the year 1991 and subsequently updated in 2003. It is one of the most referred documents by transfusion medicine fraternity. I am glad that this 3rd edition of technical manual has been brought by the BTS Division covering latest scientific and technological aspects in the field.

Many new sections viz. Quality Management System; Biosafety; Patient Blood Management; and Documentation, etc. have been incorporated. Disaster Management and use of convalescent Plasma for treatment of patients (as was done during the recent Covid-19 pandemic) have also been incorporated in this edition.

I thank the Blood Transfusion Services, NBTC Division and National AIDS control Organization (NACO) for successful completion of this task of updation this special technical manual. A special word of appreciation for experts from all over the country for sparing valuable time and making considerable efforts in creating this very important manual.

I am sure that various technical skills included in this revised manual will benefit practitioners, postgraduate students as well as the technical staff in their day-do-day work in blood centers and their related laboratory work. It will also help in standardizing blood transfusion practices and improving blood safety in the country.

(Atul Goel)

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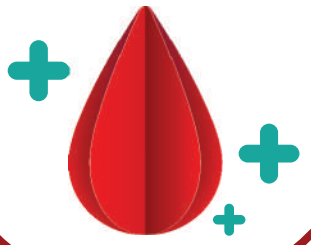
Acknowledgement



Transfusion Medicine today is a vast and expanding field. Significant developments have taken place in the scientific and technological aspects of transfusion medicine, related practices, regulatory and quality management aspects since the second edition of the Transfusion Medicine Technical Manual was published in 2003.

We are thankful to the Technical Resource Group on Blood Safety of National AIDS Control Organization and National Blood Transfusion Council for recognizing the need for revision and updation of 2nd Edition of Transfusion Medicine Technical Manual. We thankfully acknowledge the contribution of President and members of NBTC for forming a Technical Committee and continued guidance for successful revision of the manual and its finalization. We would like to express our sincere gratitude to the authors and members of the Editorial Board for sparing their valuable time and enriching the manual with insightful chapters.

We are grateful to Prof. (Dr) Atul Goel, Director General of Health Services for his unflinching support and leadership in completing the project. The contribution of the technical officers of Blood Transfusion Services division, Directorate General of Health Services including Dr Megha Pravin Khobragade, ADG is most gratefully acknowledged. We are thankful for the valuable assistance of the WHO India team including Dr Hilde De Graeve, Dr Madhur Gupta, Dr Vimlesh Purohit, and Dr Smriti Chawla.

It is envisaged that this third edition of the technical manual will provide the transfusion medicine practitioners, medical technologists, various categories of blood centre staff, residents and postgraduate students, a thorough and concise guidance for improving and standardizing the blood transfusion services.

Dr Anil Kumar
Director NBTC and Additional DDG
Directorate General of Health Services
Ministry of Health & Family Welfare
Government of India

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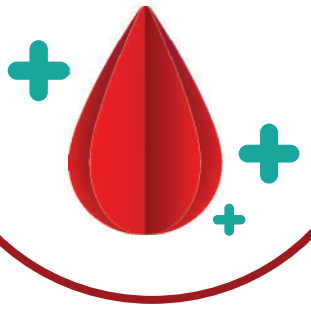

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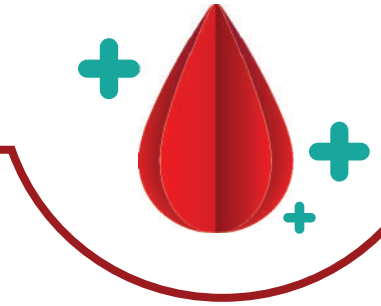

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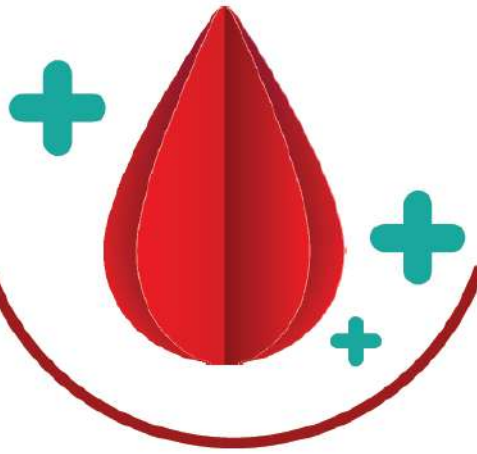
IM11012/06/2022-NBTC/BTS
3260197/2023/NBTC

Contents

Section 1



Blood Safety and Blood Transfusion Services in Ministry of Health and Family Welfare, Government of India

1. Introduction:

Blood is an intrinsic requirement for health care and blood transfusion services (BTS) are an integral part of healthcare systems throughout the world. BTS in most countries is centralized and managed by Government and/ or non- governmental organizations (NGOs).

WHO recommends that "National blood system should be governed by national blood policy and legislative framework to promote uniform implementation of standards and consistency in the quality and safety of blood and blood products". It also reiterates that countries must have "well-organized and coordinated BTS". Coordination may happen at national or sub-national levels.

BTS have to ensure that blood/ components (Whole Blood/ Packed Red Cells/ Plasma/ Platelets) are:

- Available (Adequate blood collection to fulfil need)
- Accessible (Enough reach where it is needed)
- Affordable (At reasonable costs)
- Safe (Not cause harm, especially transfusion transmitted infections [TTI])
- Standard quality (Provide clinical gain)

The following basic conditions are required for ensuring the safety of blood used for transfusion:

a) **Safe donor:**
   - o Blood collection from regular, repeat, non-remunerated voluntary blood donors.
   - o Pre-donation counselling & deferral of people with "risk behaviour."

b) **Safe blood/ components:**
   - o Quality in testing & processing- Good Laboratory Practice (GLP) & Good Manufacturing Practice (GMP)
   - o Sensitive screening for TTIs
   - o Blood component separation
   - o Proper storage

c) **Safe transfusion:**
   - o Optimum and appropriate transfusion practice using components
   - o Proper bedside practices

d) **Haemovigilance -** A look-back system to track donor and transfusion related adverse reactions.

2. Historical perspective:

- Blood centres were under the mandate of Directorate General of Health services (DGHS) Emergency Medical Relief (EMR) division up to 1992. The standards of the blood banks were variable, and the blood centres were managed by different organizations. Thus, with fragmented management, blood centres operated without a common standard. Besides licensing was not mandatory for government and municipal blood centres and all blood centres were not licensed. Paid donors were the main source of blood collection and the rest was from the relatives and the friends of the patients who were coerced to donate blood.
- In January 1992, a public interest litigation (PIL) was filed by Common Cause, a voluntary association in the Honorable Supreme Court of India seeking the court's directive for Government of India (GOI) for achieving adequate, safe and efficient blood supply for the whole country.
- The landmark Supreme Court (SC) judgment in January 1996, directed the GOI to set the house in order by complying with the mandates as follows:
  a) Establish National Blood Transfusion Council (NBTC) and State Blood Transfusion Councils (SBTC)
  b) Eliminate professional donors
  c) Enact legislation for controlling blood centre operations
  d) Introduce mandatory licensing for all blood centres
  e) Strengthen enforcement machinery
  f) Develop trained manpower
  g) Encourage research in the subject
- In 1992 the entire BTS was entrusted to National AIDS Control organization (NACO) as a blood safety programme. Easy availability of funds was the justification for this action. NBTC along with SBTC was set up in 1996 for monitoring the activity of blood centres in India.
- Licensing of blood centres in the country was made mandatory in 1997 with blood and its components and products being considered to be drugs according to Indian Pharmacopeia. Blood centres require a manufacturing license under the Drugs and Cosmetic Act (D and C Act), 1940 and Drugs and Cosmetics Rules, 1945 and as amended from time to time. The Drug Controller General (India) is the license approving authority, but the regulatory control remains under the dual authority of state and centre.
- Paid blood donors were banned from January 1998.
- The National Blood Policy (NBP) was published by the GOI in 2002. The NBP reiterates government commitment to safe blood and blood components and has well documented strategies, for making available adequate resources, technology, and training for improving transfusion services. It also outlines methods for donor motivation and appropriate clinical use of blood by clinicians. It has also indicated steps for research and development in transfusion medicine.
- In the interest of maintaining and monitoring quality as well as to take advantage of economy of scale, the NBP of India and Action Plan for Blood Safety, 2007, proposed centralization of the BTS. With collection, testing and component production at a few large Regional Blood Transfusion Centers (RBTCs), the components could be issued to peripheral hospitals and storage centres thus ensuring uniform and uninterrupted access to blood.
- For nearly two decades, i.e. four phases of the NACO Programme, blood centres of India in the government and charitable sectors continue to be supported for a one time grant for set up and supplementary grant for maintenance. Blood centres however were not governed by NACO or NBTC and were only recipients of government support through the AIDS control programme. National Health Mission (NHM) has also been supporting the strengthening of health care delivery system including blood centres/ blood storage centres in the district hospitals and below.
- Regulatory control of the State Food and Drug Administration (FDA) and Central Drugs Standard Control Organization (CDSCO) and licensing of blood centres is based on the compliance to basic minimum

requirements/ standards, but licensing is only the first step towards quality.

- NACO played a pivotal role in improving blood safety by infrastructure development, setting up component separation units, promoting voluntary blood donation, controlling the infection rates, training of blood centre staff and also laid down standards for blood centres in India. BTS have been transitioned out from NACO and are now governed from within the office of Director General of Health Services, Ministry of Health and Family Welfare (MoHFW).

3. Structure of the blood transfusion services in India

The blood transfusion services in India are directly under the purview of Directorate General of Health Services (DteGHS). Drug controller general of India (DCGI) and the blood cell, National Health Mission (NHM) along with DteGHS together coordinate the various activities of the blood transfusion services. NBTC is the policy making body working under the DteGHS within Ministry of Health and Family Welfare (MoHFW) for all matters pertaining to the organization, operation and standards for a sustainable and safe BTS for the country. At the state level, the activities related to blood transfusion services and blood safety are coordinated by the SBTC and blood safety division of state AIDS control society (SACS). Blood centres and blood storage centres which are regulated by DCGI, and state Drug Controller (DC, State) are ultimately responsible for the operations of blood bank right from recruiting the blood donors, collecting blood, processing into components, their testing, storage and distribution (Figure 1)

Current status:

Blood transfusion services have grown tremendously since the landmark Supreme Court (SC) judgment in January 1996. BTS in India are managed by both Government and/ or non- governmental organizations (NGOs). Majority of blood centres are hospital based. Blood centres can transfer blood and blood components to other centres with facilities to store and distribute. The BTS is committed to open a blood centre or blood storage centre at each district level to fulfil the requirement in peripheral areas. The aim is to have a blood supply which is collected from voluntary non-remunerated blood donors and the services are managed by a well-coordinated and networked blood transfusion service. Transfusion Medicine has also evolved in the last two decades into a medical specialty and there are more than 50 medical institutions offering post graduate courses (MD, DNB and Diploma) in Transfusion Medicine.

Blood transfusion services have also started to play an important role in managing the donors who are

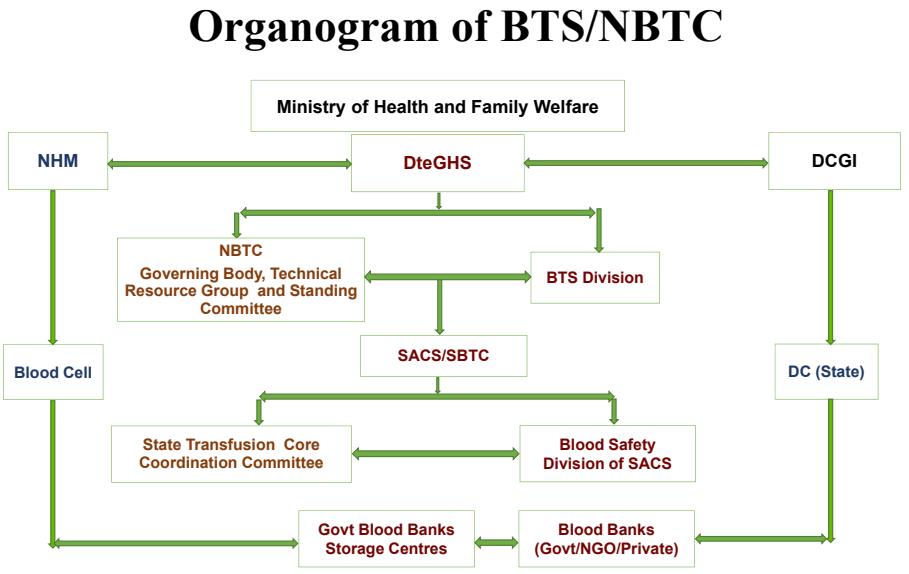


Figure 1: Organogram of blood transfusion services in India.

identified to be sero-reactive thus contributing to limit these infections across the nation. BTS has been collaborating with the ICTCs and with National Viral Hepatitis Control Programme for early identification and appropriate management of TTIs. The Hemovigilance Programme of India has also grown tremendously and has been monitoring both recipient and donor adverse reactions, thus increasing both patient and donor safety.

The mandate of NBTC includes:

3.1. Policy formulation:

- National Blood Policy in 2002
- National Plasma Policy in 2014
- Processing Charges for blood and blood components
- Standards for blood centres and transfusion services
- Guidelines for voluntary blood donation (VBD)
- Norms for issuance of No Objection certificate (NOC), RBTC status, VBD camp permission etc.

3.2. Capacity Building:

- Coordination with 26 Regional Training Institutes across medical colleges/ large NGO blood centres
- Technical manual of transfusion medicine
- Development and roll-out of standardized modular trainings on blood centre procedures, strengthening quality management systems and counselling of blood donors

3.3. Research:

- Baseline assessment of all licensed blood centres across the country
- Estimation of blood requirement in India
- Discordance in HIV testing at Integrated Counselling and Testing Centre (ICTC) and HIV screening at blood centres

3.4. Quality Strengthening

- Identification of proficiency testing providers and establishment of external quality assurance (EQAS) Programme for Blood centres
- Quality management system (QMS) training for staff

3.5. Information Technology (IT) and Information, Education, and Communication (IEC):

- Development and maintenance of NBTC Website
- Communication Strategy
- Development and dissemination of standardized messages using multimedia campaigns

3.6. Voluntary Blood Donation promotion:

- Observance of National Voluntary Blood Donation Day and World Blood Donor Day (1st October and 14th June)
- Coordination for the organization of nationwide blood drives
- Engagement with professional associations
- Engagement with NGOs
- Engagement with different ministries and stakeholders in public and private sectors

3.7. Review and Monitoring:

- Collation of monthly reports from all licensed blood centres
- Data analysis and preparation of status notes and reports

- Periodic review meetings of SBTC
- Supervisory visits to states and blood centres

4. State Blood Transfusion Councils (SBTC):

There are 35 SBTCs, each headed by the Principal Secretary of health, to implement policy guidelines of NBTC, but are otherwise independent societies in themselves.

NBTC receives grant in aid from GOI through MoHFW/ NACO and works as per the action plan approved by the governing body. All administrative matters, finance, procurement, audit, human resources (HR) of NBTC etc. are handled by staff of the BTS division. SBTCs receive a grant in aid through funds made available in the SACS BTS budget. Some SBTCs also receive matching grants from respective state governments and donor partners.

5. Key challenges

The highly fragmented BTS with over 3108 blood centres poses unique challenges.

5.1. Multiplicity of control:

While the Drugs Controller is the regulatory authority, NBTC is the main technical body to frame policy and guidelines for the practice of transfusion medicine. Besides NBTC/SBTC and DCGI/State FDA, which are the main bodies, there are various health programmes that are supporting the blood centres, National Institute of Biologicals (NIB), Noida (Uttar Pradesh) is running the Haemovigilance Programme, Directorate General of Medical Services (DGMS) deals with the medical education part for transfusion medicine, but there is no single vertical of the ministry responsible for this task in totality.

NBTC is presently functioning as a subset of NACO and is unable to fulfil its role as envisaged. The medical and quality aspects of transfusion medicine are neglected. Involving carefully chosen experts in transfusion medicine under the umbrella of NBTC will strengthen blood transfusion services. It will also enable co-opting public relation persons for multi-sectoral engagement and promotion of VBD, communication, information technology, monitoring and evaluation and quality experts to evolve transfusion services holistically and not as a mere medical entity.

5.2. Decentralized blood transfusion services:

There are too many blood centres, and a majority of them too small to adequately focus on quality strengthening, have blood component facilities or be economically viable. The baseline assessment of blood centres of India, 2016 revealed a vast variation in quality across blood centres in different states, sectors, based on the annual collection, based on whether they prepare components and other parameters. Most of the cities have numerous blood centres, many of which are opportunistic “for-profit” blood centres, which do not follow the NBP with respect to the principle of non-profit. Too many small blood centres also create an artificial scarcity of skilled manpower and fail to achieve economies of scale, consuming excessive capital disproportionate to the quantity of blood collected and supplied.

5.3. Delay in the adoption of newer technologies:

In the light of having so many blood centres, standards are often prepared to keep the compliance by the weakest and smallest of them in mind. Hence, adopting additional layers of safety like nucleic acid amplification testing (NAAT) of all units, universal leukoreduction, pathogen reduction techniques etc., are only employed by the larger blood centres.

5.4. Cord blood, Stem Cell and Bone Marrow transplantation unaddressed:

Although cord blood, stem cells and bone marrow transplants come within the purview of transfusion medicine, only cord blood is considered a drug and is regulated under the Drugs and Cosmetics Act and Rules 1945 thereof. There is presently no vertical either in the DGHS or MoHFW, which deals with all these activities.

5.5. Poor access to blood and components in the rural hinterland:

Access to safe blood continues to be limited, especially in rural areas of states like Uttar Pradesh (UP), Uttarakhand, Jharkhand, Bihar, Chhattisgarh, and Northeast. This issue is pertinent and requires resolution through policy reforms, networking, and regional coordination of BTS and strengthening linkages with NHM.

There are 76 districts where there is no blood centre. States set up blood storage centres in these districts or provide linkage to the nearest blood centre in a neighbouring district to cater to blood needs with the support from NHM.

5.6. Continued reliance on replacement blood donation:

Blood centres usually insist on replacement blood donation so as to issue blood or components against it, even though the NBP reiterates that blood should be collected from voluntary non remunerated blood donor and replacement donation should be phased out.

6. Way forward:

The key strategies should be targeted towards enhancing availability, accessibility, quality, safety and affordability of blood and blood components for end-users. The main areas include

1. Developing a nationally coordinated blood transfusion system**:** This strategy aims to achieve a national blood transfusion system with a single decision/policy-making body for BTS at the national and state level, i.e., NBTC –the apex central body and SBTC at the state level. The national BTS will be working as per the guidelines and mandate of NBTC. Strengthening the functioning of NBTC and SBTC in all states through the provision of adequate resources will be emphasized.
2. Taking steps towards review and legislation of the existing NBP and NBTC guidelines to give it the necessary authority to streamline and improve the functioning of blood centres
3. Developing common national standards for BTS and blood centres enforceable by law.

Though there exists a coordination mechanism among the different stakeholders responsible for blood centres and BTS, there is considerable verticality in all agencies. Similarly, there are multiple monitoring systems/ e-initiatives.

The way forward should include:

6.1 Creation of a separate vertical in the MoHFW for blood transfusion services

- There should be a single decision/policy making body in the country related to all matters of BTS at the national and state level.
- It should be mandatory for all states to establish SBTC as separate entities and not a subset of an existing health programme.
- The NBTC and SBTC should be directly under the umbrella of MoHFW both at the centre and state levels.
- The BTS of India should be working as per the guidelines and mandate of NBTC implemented through SBTC.

6.2 Strengthening and recognition of NBTC/ SBTC-

- As an autonomous body with authority, responsibility, and accountability.
- Provide financial and budgetary allocation and full-fledged managerial team.
- Identify needs for infrastructural and management reforms.
- Appoint workforce for implementation at central and state level providing them infrastructural facilities.
- Review and formulate working groups to assign the roles and responsibilities of various functions, including VBD, technical matters and standardization, quality management and accreditation, training, regulation, accessibility and adequacy of safe blood supply, logistics, and procurement, finance, public relations etc.

6.3 Review and revision of national blood policy:

- NBP 2002 should be reviewed and revised to the present context and a strategic framework with resources and action plan developed in conjunction with the respective state governments.
- The processing charges decided by NBTC should be complied with by all licensed blood centres, and non-compliance should be dealt with by cancellation or revoking the licensure.

As suggested in NBP, the blood centres in hospitals attached to medical colleges should be encouraged to set up the Department of Transfusion Medicine, acting as nodal training and teaching centres.

6.4 Networking and coordination between blood centres:

- All licensed blood centres should be registered with SBTC and follow the policy norms of the NBTC as adopted or adapted by SBTC.
- SBTC representative should be a part of the inspection team for license issuance and renewal.
- SBTC should network the blood centres of the state to allow for fair sharing of blood and blood components.

6.5 Regulatory coordination:

- Periodic amendments in the Drugs and Cosmetics Rules need to be done as per the recommendations of NBTC.
- Fresh licenses should only be issued to blood centres with blood component separation except in the government sector catering to underserved areas. Renewal of whole blood licenses should not be done after 5 years.
- Inter blood centre transfer of blood units should be permitted. The creation of a blood grid with territorial limitation should be made official under appropriate rules and regulations.
- Permission should be granted for a centralized testing centre to utilize resources efficiently and enhance blood testing quality.

6.6 Legislation of National Blood Policy:

- Advocacy and guidance for the development of a legislative framework of National Blood Policy should be provided to identify NBTC as authority and CDSCO to assist as a regulatory arm to license blood centres and monitor GMP and GLP.
- Legislation should suggest changes in the regulatory framework as blood centres have evolved into transfusion medicine with advanced technologies including stem cells, cord blood centres, regenerative cell therapy, gene therapy, organ donation and transplant etc.
- There should be an independent body of experts from transfusion medicine for grant of license and regulation of the new technologies such as apheresis, cord blood centres, stem cell registries, bone marrow transplant, regenerative therapies, organ transplants and gene therapy etc.
- There is a need for legal provision for audits by experts regularly, rather than only during licensing.

6.7 Inter-sectoral collaboration for strengthening voluntary blood donation

- Ministries of Government of India/ State Governments like Ministry of Human Resource Development (MHRD) for supporting school programmes and Ministry of Youth and Sports for engagement with young persons, Ministry of Civil Aviation, Ministry of Communication etc.
- Non-government organizations
- Professional associations
- Corporate sectors
- Social organizations
- Institutions and workplaces

6.8 Human Resource Development:

- All teaching hospitals having licensed blood centres collecting more than 10,000 units per annum must establish a Department of Transfusion Medicine and accept the subject as a speciality.

- Undergraduate and postgraduate medical courses curriculum should include transfusion medicine as a subject, especially clinical use of blood and bedside transfusion practices.
- Nursing Council of India should include transfusion medicine as part of the curriculum of BSc or MSc Nursing.
- A separate cadre should be created for the blood centre (Doctors, Laboratory Technicians, Social Workers, Quality Managers).

6.9 Set up of Centres of Excellence:

- Set up of centres of excellence in transfusion medicine, which are state-of-the-art, as envisaged in the metro blood centre project.
- All major blood centres collecting > 20,000 units per annum should be modernized to implement advanced/ automated techniques for testing donated blood in the initial phase. NAAT testing may be introduced in a phased manner, starting with a centralized facility.
- Development of rare donor registries, apheresis registries and bone marrow donor registries.

6.10 Emphasis on Quality Assurance Programmes

- Designated centres that can support solving problems in labs and introduce External Quality Assurance Scheme (EQAS) programmes for TTI and red cell serology.
- Sensitization to quality management systems should be provided to all blood centres.
- Encouraging and supporting accreditation of blood centres by National Accreditation

Board of Hospitals (NABH).

- Introduce automation and adoption of newer technologies in high volume blood centres, e.g., radio-frequency identification (RFID) and other “intelligent” transport and storage systems, addressing issues of bacterial contamination, pathogen reduction technology, molecular testing in immunohaematology, therapeutic apheresis, promotion of cellular therapy, an extension of blood centre expertise to banking and supply of cord blood, stem cells and other tissues of human origin.

6.11 Research and Development

- Research in transfusion medicine and the indigenization of technologies should be encouraged.
- Collaboration to be developed with Indian Council of Medical Research (ICMR) and its allied institutes for undertaking operations in research and explore research funding from multilateral and bilateral agencies through Department of Health and family welfare (DoHFW), Department of Biotechnology (DBT) or Department of Science and Technology (DST).
- More active involvement of the industry in attaining self-sufficiency for equipment, consumables, and products.
- Encourage public-private partnerships for a national plasma fractionation facility.

6.12 Management Information System

- Uniform IT-based electronic blood centre system (E-RaktKosh) to be a single window for all blood centres and BTS concerning reporting.
- Citizen centric management information system (MIS) portals and helplines.
- Use international standards like ISBT 128 system for identification, labelling and information transfer of medical product of human origin.

7. Conclusion:

In the increasingly public and legislative environment at present, GOI needs to respond towards strengthening the BTS quickly and totally, moving towards creating a vertical at the Central and State level to ensure the safety and security of the nation’s blood supply. This will enable locally responsive service delivery by a coordinated

network of blood centres managed at national and state levels based on the approach of non-profitability, total quality management and voluntary, altruistic donation to meet the requirements of blood and its components. Funding support is essential for strengthening blood transfusion services and improving access to an adequate quantity of affordable, safe, and quality blood and blood components. All available resources, i.e., support from the Government of India through its various health programmes and state governments, as well as entities such as the World Health Organization (WHO), and other bilateral partners, must be leveraged and put into a common resource envelope from where prioritized activities may be carried out with coordinated planning at state and national level. The autonomy of NBTC and SBTC to receive donations with a 100% income tax exemption under Section 80 G should also be leveraged to its optimum capacity.

This will ensure clear demarcation and minimum overlap in activities carried out by each agency. Responsibility will lie with NBTC and SBTC for all matters about blood centres and blood transfusion services, as they function as verticals at the Central and State level, respectively. Note: At the time of publication blood transfusion services are under the office of Director General of Health Services (DGHS), Ministry of Health and Family Welfare, Government of India.

Reference:

1. National Blood Transfusion Council; http://nbtc.naco.gov.in/

Section 2

Quality Management Systems in Blood Centres

1. Introduction

The blood centre is envisaged as the key functional entity of the blood transfusion system of the country. In this role, ensuring quality is implicit in ensuring satisfactory delivery of the expected standard of service to patients and clinicians. The World Health Organization (WHO, 1975) has urged its member organizations to put into place legislative and structural measures to ensure safe and quality blood, and products sourced from voluntary blood donors and are made accessible to all patients at the time of need.[1] The National Blood Policy of India (2002) also envisages the implementation of quality systems (Strategy 3) to ensure that stated national goals are met. Fulfilment of regulatory requirements will ensure compliance with standards laid down in the Drugs and Cosmetics Act 1945 and the Rules therein. Going beyond minimum requirements, blood centres realize that donated blood and its components are tested and processed in better quality for administration to patients as a drug. To maintain its intrinsic properties and effect desired pharmacological properties, the entire process must be carefully standardized and adhered to so that we are as compliant as possible with good manufacturing processes. There are different approaches for quality systems implementation, such as WHO (2005) and approach detailed in the Blood Bank QMS Training Manual of NACO (2016). In India, the NABH is the organization that offers accreditation to blood centres, and its requirements are aligned with the regulatory framework. Other options are also available. Blood centre management must select the most appropriate system and fulfil its mandated requirements while ensuring that all regulatory aspects are fulfilled.

- Quality is defined as the degree to which inherent characteristics fulfil requirements (ISO900:2005).
- Quality control (QC) includes evaluating a test or procedure within a process, and it also determines whether it works as expected or not at a particular time. In this organization, performs QC of reagents, blood components, equipment, etc.
- Quality assurance is defined as a system designed to produce a good quality product from input source materials by implementing a consistently performed manufacturing process. Quality assurance aims to implement a safe and effective process that ensures continuous safety and quality of the product. Quality assurance includes detection, investigation, prioritization, and correction of errors with the ultimate aim of error prevention.
- Quality management systems encompass all aspects, ranging from defining the organizational structure, roles and responsibilities of all stakeholders and policies, processes and procedures, and the commitment of resources essential to achieving and sustaining the stated goal at all levels – from top management to the employee.

Quality planning is required to ensure that all issues are identified, prioritized, and addressed systematically, including stating policy, goals, and objectives. The next step involves the activities to be carried out to achieve these goals. This is followed by monitoring the outcomes (data), which allows for control and continuous improvement.

2. Essential elements of quality management system:

2.1. Organization and leadership
2.2. Customer focus
2.3. Human resources
2.4. Equipment management
2.5. Suppliers and materials management
2.6. Process control and management
2.7. Documents and records
2.8. Information management
2.9. Management of non-conforming events
2.10. Monitoring and assessment
2.11. Process improvement
2.12. Facilities, work environment, and safety

The purpose of this section is to provide an outline of standards and principles relating to the preparation, use and quality assurance of blood and its components. It does not extend to the plasma-derived products (PDPs) obtained by fractionation, which will be under the ambit of the Indian Pharmacopeia (IP).

2.1. Organization and management:

A clearly defined organizational structure is an important component of a quality management system. The depiction of organizational structure, also referred to as an organogram, clarifies the organization as to its functional entities and flow of responsibilities and authority. Clarity of the organizational structure is important to ensure that adequate resources are available for executing activities that are essential to achieve the processes within the quality system. There must be no ambiguity within the organization about leadership roles.

The organogram also ensures access to top-level management to transmit important information about the employees complying with the required processes and procedures and hindrances, if any, to achieving the stated objectives. The establishment of the quality management system is evidence that the top management is committed to ensuring quality as envisioned.

The top-level management of QMS has some specific duties such as:

1. Designing the quality goal, quality objectives and quality policies.
2. Providing adequate resources to maintain and bring about improvement of quality in an organization.
3. Implementation or enforcement of quality policies, processes, and procedures.
4. Periodically review the effectiveness of policies.

Top-level management can assign quality managers, who take care of all quality policies in the organization and report them.

2.2. Customer focus:

Towards this, it is important to ascertain the needs of the patients and clinicians who treat them (the customers). It is, therefore, vital to sensitize clinicians about the advances in the domain and evidence that will support the appropriate use of this scarce resource. This knowledge will enable better planning of operations beginning with voluntary donor recruitment, implementation of automation, and provision of appropriate solutions, be they products or services. Customer feedback is also important for continuous improvement, so there should be some mechanism from which the organization can assess customer satisfaction and receive feedback from them.

2.3. Personnel focus or human resource management:

Personnel management is the process of efficiency in staffing, training, recruiting, selecting appropriate human capital to make the best use of the human resources for which it includes the recruitment of the right

kind of people according to jobs, their training and development to do the work, their welfare to maintain their morale and incentives to boost their interest to reach the ultimate objectives of the organization. Well planned and formulated human resource strategy can bring high productivity for the organization.

Personnel management is the responsibility of all those employed as specialists, who manage people and the work of those who are employed. Characteristics of a successful personnel management program are adequate and appropriate training programs for employees, favourable and conducive working conditions, appreciation and recognition at work, competency assessment and fair leadership.

In summary, personnel management involves both management and operative functions: managing functions include planning, organizing, directing and controlling. The operative functions involve selecting, recruiting, and placement the right kind of personnel in the right places to accomplish organizational objectives. After selecting a suitable person, there should be a well-written job description, adequate orientation and training, and competency assessment at regular intervals of new employees.

2.4. Equipment management:

Equipment management is one of the essential elements of a quality management system. Proper management of the equipment in the blood centre is necessary to ensure accurate, reliable, and timely testing. It is also essential to maintain a high level of performance, lengthen the instrument's life, reduce interruption of services due to breakdowns and failures, and improve the technologist's confidence and knowledge.

A blood centre should procure requisite equipment as per their scope of activities and based on their services and workload. While selecting an instrument, attention should be paid to facility requirements, quality control requirements, training requirements, cost and ease of operation, the supply of reagents, technical support and validation, maintenance, and service contracts, mean time between failures.

2.4.1. Equipment specification:

Equipment specification defines what do you expect from the equipment and how the equipment will be installed, operated, maintained, stored and how the equipment should perform during regular use in its typical operating environment. Before installation, equipment should be verified physically and confirm that equipment is inspected as per specifications given in the supply order by the user department. No equipment must be used before proper installation.

2.4.2. Calibration:

Calibration includes the set of operations which establish, under specified condition, the relationship between values indicated by a measuring instrument or system and the corresponding known value of a reference standard. Calibration is done to ensure that equipment always works efficiently and reliably with accuracy. It must be done at regular intervals and must be traceable to national/international standards.

2.4.3. Validation:

Validation is the confirmation and provision of objective evidence that requirements for a specific intended use or application have been fulfilled. Validation of new equipment used in a process should include design qualification, installation qualification, operational qualification, and performance qualification.

1. Design qualifications are the documented verification that the specifications of the equipment and its components are adequate for the requirements stated by the user.
2. Installation qualification demonstrates that the instrument is correctly installed in environmental conditions that meet the manufacturer's specifications.
3. An operational qualification is a validation protocol that provides documented verification that equipment or a system functions according to written and pre-approved specifications. It demonstrates that the installed equipment operates as intended. Equipment should be challenged to ensure that their results meet all defined user requirements under all anticipated conditions of manufacturing.
4. Performance qualification demonstrates that the equipment performs as expected for its intended use in the processes established by the blood centre and that the output meets the centre's specifications.

2.4.4. Records of equipment management:

Records of all the equipment must be maintained, and equipment must have a unique identification number displayed on them. Blood centres should have a standard operating procedure (SOP) for replacement/ repairing of equipment malfunction, failure, or adverse events during working. The defective equipment should be labelled and taken out of service; once repaired, it should be calibrated before putting in use, and the procedure should be specified in an SOP.

2.5. Suppliers and materials management:

Many of the services offered by the blood centre depend on the consumables and reagents that are purchased from different suppliers. Some of these, such as blood bags, blood grouping reagents and infectious disease testing kits, are termed critical when they directly bear the primary purpose of the blood centre, which are related to the collection, testing, and preparation of components. Therefore, it is important in the quality system to address the cycle of procedures that contribute towards the acquisition of these supplies and the interactions with suppliers. The quality oversight also extends to other areas, such as equipment used for the testing, which has been dealt with in an earlier section.

In line with this, there are three important aspects that the quality system should address, the first of which is related to the supplier.

Supplier criteria:

1. All suppliers must be assessed for their ability to supply the material sourced from them reliably.
2. They must have appropriate licensing and registration.
3. Should be able to provide relevant documents when needed.
4. Should be willing to be audited or provide reports of third-party audits of their facilities and processes (such as cold chain).
5. Should be reasonable in the costs charged for services or products.
6. Should have a reliable distribution service and provide good after-sales support.

It is good to have a checklist against which suppliers are assessed, which may occur at least annually at the onset of the procurement relationship. Any incidents related to short supply, damaged supplies, or non-compliant supply must be reviewed and discussed, and the ability of the supplier to put in corrective action must be documented. Critical supplies must be acquired only from proven and reliable suppliers. A list of such suppliers must be maintained, and backup plans for failed supply must be in place to avoid interruption of critical services.

The second point to consider is contracts and agreements. Where required, contracts must be put in place to ensure continuity of services. This may be related to equipment placed at the blood centre for which reagents are to be supplied over a defined period (reagent rental contracts), or provision of consumables are to be provided at a pre-agreed rate for a defined period (rate control contracts). These must conform to the legal requirements of the organizations and relevant regulatory bodies. These contracts help in planning the supply chain and projecting demand for the supplier, therefore making it possible to provide interruption-free services. Agreements for the supply of blood to storage centres and surplus plasma supply to fractionators should also be maintained as applicable. The blood centre must be involved in the framing of these agreements as they will ensure commitments that will mitigate the impact on critical services.

The third issue is related to the review of supplies at the point of receipt to ensure adherence to expected norms of the supply chain compliant with the manufacturers' stated requirements. The consumables that affect the quality of the blood collected and distributed must also be highly controlled to ensure regulatory compliance. There must be protocols for acceptance checking and verification at the receipt. There must be procedures to ensure that reagents transported without a cold chain or goods that are damaged are returned and replaced without affecting the delivery of services. Maintaining records of such events are important to future planning and vendor qualification steps must be ensured.

2.6. Process control and management:

A process is defined as a series of actions or steps taken to reach the desired end. These must be developed, keeping in mind the policies of the organization and the environment in which service delivery is to occur. In the blood centre, it is important that all processes are listed and mapped. Once this is done, a list of procedures (laboratory or system-related) can be formulated to achieve the processes. Any process management must begin with planned process development. This will need inputs from customers (clinicians and donors), management and the staff working in the blood centre, besides review of relevant literature taking into account regulatory framework. This is followed by validation of the process and then implementation of the same. Specific procedural documents must be put into place, and those involved must be trained.

Once these are implemented, there must be methods of ensuring that the policies and processes are adhered to. These may be through training, audits, outputs (reports), and systematic periodic review. Therefore, it is important to institute quality control for the processes – which may be analytic or process-based. The results of the quality control program must be analyzed and reviewed periodically to ensure that the processes and procedures are being followed. In the blood centre, the components that are prepared should be inspected to detect any non-adherence to the stated processes.

Records must be created during the processes and, as far as possible, must be concurrent, i.e., must be strongly associated in a temporal manner with the execution of the process itself rather than *post hoc* recording. This will help in the root cause analysis of non-conformances.

Specific processes may be designated as critical and be reviewed and scrutinized more frequently as decided by the blood centre. It is important to ensure the traceability of the process and records. This will assist in ascribing cause when non-conformances are reported or defects are detected. Computer systems lend themselves to the ease of recording, creating reports and detecting outliers when designed appropriately. Finally, there should be a system for change control implemented such that when the process needs to be altered, the effect of relevant policies and procedures are reviewed to avoid potential conflicts. The change must be made with the knowledge of those involved, and finally, the staff who are responsible for the process are trained with the newly revised process before implementation, and relevant documentation should be maintained.

2.7. Documentation and records:

These are explained in Section 13: Documentation in Transfusion Medicine.

2.8. Information management:

Blood centres have various critical and confidential information about patients and donors. This information should be properly managed and accessed to very limited responsible persons. Unauthorised access to this information should not be allowed. If information is stored electronically, it should be encrypted, and there should be a backup of this information.

2.9. Management of non-conforming events:

Based on the awareness of numerous risks of transfusion treatment and their potentially disastrous consequences, quality has always had a prominent place in transfusion medicine. An important component of ensuring quality is systematic and efficient error management.

A meticulously designed Corrective Action & Preventive Action (CAPA) System provides a process by which we can proactively and retrospectively correct errors and problems identified through the quality system. Therefore, the QMS should contain processes and procedures to detect, document, investigate, correct, and follow up on a quality incident. Such processes and procedures must be in line with regulations and applicable standards.

Definitions:

- Quality incident: An event that can adversely affect the quality of our services or products.
- Corrective action: It eliminates the cause of nonconformities to prevent a recurrence.
- Preventive action: It determines and eliminates the causes of potential nonconformities to prevent occurrence.

2.9.1. Steps for CAPA

1. Identification and documentation of quality incidents: Quality incidents include but are not restricted to non-conformities (NC), deviations from SOPs and laid down processes, errors, near-misses, and adverse reactions. They are detected during routine work, internal and external audits, haemovigilance reports, customer and stakeholder feedback and complaints. Staff should be trained to find and report quality incidents and non-conformances and capture the event's facts in sufficient detail to allow a complete and thorough investigation.
2. Determination of the effect, if any, on the quality of products or services and evaluation of the effect on interrelated activities. A risk-based review should be conducted to determine if the problem needs to be escalated to CAPA. If the issue is minor and can be resolved with some containment, it can be closed at this stage. If the problem has to be solved immediately and sufficiently, immediate corrective action should be taken—for example, adverse events needing product recall.
3. Investigation, root cause analysis and corrective action: If the quality issue/problem is more serious, a CAPA process should be initiated. The first step is a root-cause analysis to determine causes and contributing factors, which is generally done using a fishbone diagram. Once the root cause is determined, the appropriate corrective action should be performed, including notification to hospitals or patients or recall of the blood unit as necessary. These immediate remedial steps constitute a short-term corrective action. In addition, a long-term CA should be instituted to prevent its recurrence. The corrective action has to be verified and validated to ensure that it resolves the problem on a long-term basis.
4. Preventive action: Opportunities for preventive action can be identified by using tools such as a failure mode effects analysis (FMEA)- a step by step approach for identifying all possible failures, deficiencies, and potential risks in design, processes, product or services. Based on the analysis, safeguards and process changes to prevent the occurrence of a non-conformance and priorities for improvement should be instituted. Documentation, trending and tracking to enable monitoring over time should be done for all NCs, even for the NCs not escalated to CAPA. Notification of NCs and CAPA to regulatory bodies or accrediting agencies may be needed as well. Studies have shown that near-miss events are several times more common than events causing harm. Therefore, comprehensive reports and analysis of all events, especially near-miss events, generate more accurate information on process functioning, including shortcomings, and allows for the timely introduction of corrective and preventive actions.

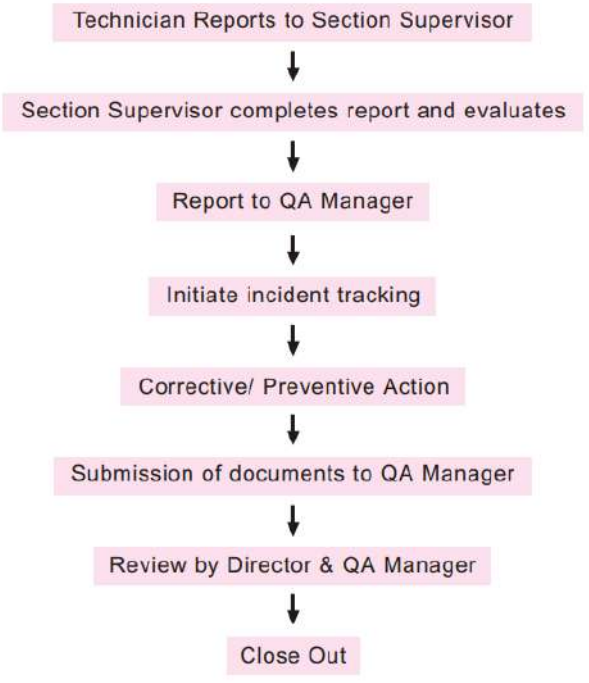


Figure 1: Workflow for initiating CAPA

2.10. Monitoring and evaluation:

Organizations should have a system for continuous monitoring and evaluation to know the effectiveness of the quality management system. It can be done by a periodical audit of the system and management review. Blood centres can conduct audits in various areas like blood donation centre, component laboratory, transfusion-transmitted infection screening testing laboratory, crossmatch testing, etc., details of which are mentioned in section 11.

A management review is a formal, structured meeting that involves top management and takes place at regular intervals throughout the year and must be done once a year after an internal audit. The purpose of a management review meeting is to review and evaluate the effectiveness of the quality management system, helping to determine its continued suitability and adequacy. Management review also ensures that all levels of management are made aware of any changes, updates, revisions, etc., to the day-to-day workings of the management system itself. It is recommended that a meeting should take place at least once a year, but meetings can be held quarterly or even monthly if preferred. All the non-conformities detected during the audit process need to be discussed, and any change in process has to be discussed and approved. Also, other feedback, complaints and external audit findings with their corrective and preventive actions are discussed. The management review meeting is attended by the top management of the organization from all the departments. The agenda should be shared with the members in advance so that members are prepared for the same. The result of the review is incorporated into a plan that includes goals, objectives, and action plans.

2.11. Process improvement:

Managing quality in any organization centres around three fundamental processes: planning, control, and improvement (Juran's Quality Trilogy). The third of this trilogy, process improvement, is to improve the overall effectiveness and the efficiency of the QMS of the blood centre. The strategies should focus on ongoing improvement, measured through quality indicators. Processes can always be made more efficient and effective, even when they are producing conforming products. There are several process improvement methodologies available, of which total quality management, six sigma and lean methodologies are a few.

2.11.1. Total quality management (TQM):

TQM focuses on customer satisfaction as the most important quality indicator. Blood centres implementing TQM should have defined processes, monitor the performance, and continually look for ways to be more effective. The PDCA (Plan, Do, Check, Act) cycle, also called the Deming cycle and control circle, is used for continual process improvement. The cycle lists the proposed plan (including objectives and the steps in the process), the process for testing that plan, and checking that plan's success. If the plan is successful, that process becomes the new standard.

Performance monitoring is an important tool of TQM, which is used for setting priorities for process improvement. It is defined as a method by which procedures, activities, or human resources can be assessed on certain parameters keeping the pre-established criteria and objectives as a benchmark. While record review and analysis are an ongoing form of monitoring, internal and external assessments of the processes are very useful. Assessments may include comparing actual to expected results and can consist of quality assessments, peer reviews, self-assessments, and proficiency testing.

Benchmarking is a structured, continuous, collaborative process in which comparisons of selected indicators are used to identify factors, which when implemented, will improve performance. Performance monitoring is one type of internal audit, which helps us to improve our quality standards in transfusion practices.

Quality indicators are statistical measures used to adjudge and improve quality performance. Depending on what we measure, there can be input, output and outcome quality indicators. Blood centres need to ensure that the quality indicators they institute are appropriately selected and analyzed and acted upon to be effective and efficient monitors of quality. They are useful in the evaluation of customer requirements, personnel, inventory management, process control and so on. Organizations should communicate quality indicator results frequently, so stakeholders are aware of how the organization is performing.

2.11.2. Six Sigma:

Six sigma helps to measure defects or inconsistencies in a process to deliver perfect products and services. It relies on data and statistics to make decisions. Within six sigma, two sub-methodologies are used, DMAIC (Define, Measure, Analyze, Improve and Control) for improving existing processes and DMADV (Define-Measure- Analyze-Design- Verify) for creating new processes.

DMAIC follows these steps:

- Define the opportunity for improvement (project goal).
- Measure the performance of your existing process.
- Analyze the process to find any defects and their root causes using tools such as a fishbone diagram.
- Improve the process by addressing the root causes you found.
- Control the improved process and future process performance to correct any deviations before they result in defects.

DMADV follows similar steps, though users will look at different factors since a process does not exist yet:

- Define the process goal based on overall strategy and customer needs.
- Measure the factors that are critical to quality.
- Analyze various design and development options.
- Design the process.
- Verify that the design meets process goals and customer needs. Pilot the process and, if successful, implement the process.

2.11.3. Lean manufacturing:

While TQM and Six Sigma focus on eliminating defects for quality assurance, 'lean' focuses on eliminating any waste in order to improve efficiency. Any action that does not add value or is not required as part of a policy or regulation is waste. Waste can include unnecessary inventory, overproduction, periods of inactivity, the effort involved in checking for and fixing defects, and underutilizing employees' knowledge and skills.

2.11.4. Risk-based quality planning:

Risk-based quality planning and risk management should be an integral part of strategic decision-making and change management in blood centres and blood transfusion services. There should be a risk management framework with relevant policies, procedures, and assigned roles and responsibilities.

ISO 31000 definitions applicable to this area of quality planning are:

1. Risk: It is the effect of uncertainty on objectives.
2. Risk management: Systematic application of management policies, procedures, and practices to the tasks of analysing, evaluating, controlling, and monitoring risk. Risk management aims to identify potential issues or problems before they occur and have planned activities in place to reduce or eliminate the impact of the risk. The first step is to define the context, i.e., the blood centre's objectives and strategies, and other factors both internal (organizational structure, culture, and capabilities) and external (BTS trends, regulatory requirements, and expectations of key external stakeholders).

   The risk management process typically involves four key stages:

   A. Analyze the blood centre processes, identify potential risk and its impact using process mapping "Failure Mode and Effects Analysis" (FEMA), brainstorming, or similar tools.
   B. Evaluate risks: Assess them based on probability and severity/impact.
   C. Control risks: Mitigate significant risks by changing the process to remove the source, avoiding it altogether; address new risks if any introduced by these changes.
   D. Monitor risks: Choose indicators to monitor the outcome. Bring the risk down to the point where constraints such as available technology or budget prevent further risk resolution.

3. Acceptable risks:
    - A blood centre may deem a low-frequency risk with few negative consequences as acceptable.
    - In the context of a strategic planning exercise, some risks and their consequences may be accepted to further the blood centre's objectives and growth.
    - While the analysis and evaluation can be done by the staff responsible, the final decision will be with the management.

2.12. Facilities, work environment and safety:

Provision of appropriate accommodation and a working environment is a significant aspect of both licensing and quality implementation of quality systems in the blood centre. The fact that space must be hygienic and away from potential sources of contamination and infections is to be understood – not only from a minimum compliance standpoint, but from a good manufacturing practice (GMP) perspective. The prepared components are intended to be infused into human patients, and hence handling must be with care to prevent any opportunity for contamination.

Beyond fulfilling the mere space and number of designated rooms as required by the rules and regulations, an effort must be made during the design to ensure that quality and workflow standards are also fulfilled. The separation of the donor area from the testing and processing of the collected blood is essential. The blood centre design should also ensure that patient services are provided to ensure easy and convenient access to clinicians and staff with defined pathways for routine and emergencies. Efforts must be made to avoid unnecessary and inefficient workflow pathways that will result in excessive and wasteful exertion for staff. Various principles of production systems such as "lean" may be consulted before the design is finalized so that ergonomic workspaces are available. "Design Guidelines for Blood centres" (WHO, 2010)[2] has systematically addressed many of the issues, and such references may be consulted for more details.

Appropriate building materials must be utilized to ensure that surfaces can sustain repeated regular cleaning with antiseptic or detergent as required and will be resistant to corrosion.

Workplace safety, including electrical, chemical, and biosafety for all the stakeholders, must be addressed, and clear signage and well-lit emergency exits must be as per standard requirements. Where required, air conditioning must be provided to ensure that materials (reagents, blood, components), persons (donors and staff), as well as procedures are maintained as per stated requirements. Planning must ensure the anticipated growth and infrastructure designed to allow for adequate expansion without compromising the production processes. The building must take into consideration the management of waste that is generated and ensure that adequate space is provided.

Regular audits and inspections should also accompany this to ensure that the building is maintained in a manner that does not contaminate the environment and is compliant with the prevailing local and national regulations for control of pollution.

Blood centres that are housed within healthcare facilities may share some of the infrastructure services. However, it is the responsibility of the management of the blood centre to ensure that it is compliant and maintain appropriate documentation to be submitted to regulatory authorities on request.

Key Points

- A quality management system in blood transfusion services is essential for providing safe blood to all patients.
- The triumph of the quality management system depends on key objectives and policy-making capability, and the commitment of top management personnels.
- Facilities to understand customer expectations and satisfaction is necessary for continuous quality improvement.
- The streamlined policies for personnel selection, orientations, training, and competency evaluation are necessary.

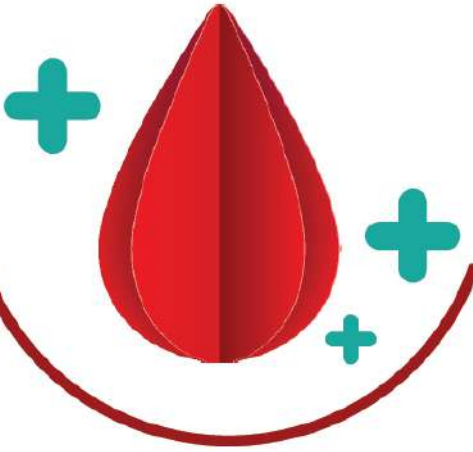
- Equipment management is the niche of the quality management system because blood transfusion services are entirely dependent on equipment. Periodical calibration of equipment is also necessary.
- The selection of well-qualified suppliers is vital for the smooth operation of the blood centres.
- New processes or procedures must be duly validated before they are implemented in routine practices.
- All activities in blood centres need to be properly documented and saved as confidential records in accordance with the requirements of the regulatory authorities.
- Identification, corrections, and prevention of non-conforming events in blood centres are necessary for continuous quality improvement.
- Internal or external quality assessment and/or proficiency testing by external services at periodical intervals is also essential in a quality management system.
- The organization will be required to provide sufficient space with appropriate ventilation, lighting, and drainage systems to get desirable quality work from the staff.

Reference

1. https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability
2. https://www.who.int/bloodsafety/publications/DesignGuideBloodCentres.pdf. 2010

Section 3

Blood Collection



1. Introduction

Blood transfusion is an indispensable life-saving measure for the treatment of patients around the world. Important considerations to ensure the availability of safe blood and blood components are the recruitment of voluntary non-remunerated blood donors, standardized screening and testing, assessment and selection of a healthy blood donor, appropriate and adequate blood collection, along with retention of these safe donors.

Recruitment of a safe donor begins with donor information, education, and counselling for a comprehensive understanding of the blood donation process through proper communication and exchange of information. Donor selection is a process undertaken to evaluate whether the donor is healthy enough to undergo blood donation without any significant complications and ensure that the donor is free from any transfusion transmissible disease that may be transmitted to the recipients.

It is the responsibility of the blood transfusion service to ensure that the act of blood donation is safe and causes no harm to the donor. A pleasant environment in the blood collection centre ensuring polite and effective communication between the blood centre staff and donors are a few of the factors that improve the blood donor experience at the blood centre and assist in the retention of blood donors.

2. Prerequisites for the continuous supply of safe blood

The essential part of providing a continuous supply of safe blood and blood components is to have strategies for motivation, recruitment, and retention of voluntary non-remunerated blood donors on a regular basis. The basic steps in the implementation of these strategies include[1]

- Estimate requirement of blood
- Motivate and recruit voluntary blood donors
- Identify low-risk donors and encourage self-exclusion by donors with high-risk behaviour
- Develop and maintain an effective donor selection procedure
- Provide a high standard of comprehensive donor care
- Maintain efficient donor records
- Develop systems to retain voluntary non-remunerated donors

The main aim of these strategies is to encourage regular blood donation based on altruism by voluntary non-remunerated blood donors for safe blood.

3. Estimating blood requirement

The national blood requirement is determined by the capacity of the country's health care system and its coverage of the population. The assessment of total blood requirement for a country is an important factor to be considered while planning National Blood Programmes. This is aimed at optimizing donor recruitment,

blood collection and component preparation to avoid excess/shortage of blood. The total blood and blood components required for a region/country depend on the population distribution, available health care facilities, the prevalence of disease requiring regular transfusions, specialized treatments such as dialysis, transplant, open heart surgery and oncology. In addition, clinicians' judicious use of blood components based on current patient blood management protocols is the need of the hour. The blood requirement is assessed by the following:

a) Total population

As per World Health Organization (WHO) recommendations, if 2% of the population donates blood, it should be sufficient to meet the need for blood requirements of a developing nation. One of the recent studies conducted by the National AIDS Control Organization (NACO) India showed that 32.2% of the total Indian population is eligible for blood donation as per the recent blood donation criteria.[2]

b) Medical facilities available in the area

Based on a number of hospitals in the vicinity of a blood centre to which it caters.

c) Acute hospital beds

The need for blood varies from 7-15 units per acute bed per year, depending on the type of medical care available. In primary health care set up, the demand can be as low as 5-7 units/bed/year, whereas, in medical care centre's providing specialized treatments, the blood demand can be as high as 25-30 units/bed/year. The demand may be more in institutions that support super specialities such as liver and bone marrow transplants. This method may not be accurate as there is a growing trend of providing transfusion as an OPD treatment in day-care settings.

d) Annual blood usage

Calculation of annual blood usage can be done by estimating the number of requests received and units issued by the blood centre throughout minimum 3 months or annually in a defined geographical area or population. This is one of the most practical methods for a constant supply of blood and blood components.

4. Blood donors/ donations[3]

The term "blood donor" includes donors of whole blood, red blood cells, platelets, plasma, and other blood components, donated as whole blood and/ or through apheresis. The following are the different categories of blood donors/ donations:

4.1 Voluntary non-remunerated blood donor (VNRBD)

A donor who donates blood, plasma, or other blood components of his/her own free will and receives no payment for it, either in the form of cash or in-kind, could be considered a substitute for money. This includes time off work, other than reasonably needed for donation and travel. Small tokens, refreshments and reimbursement of the direct travel costs are compatible with voluntary, non-remunerated blood donation.

Categories of voluntary blood donors are:

a) New voluntary donor: A voluntary non-remunerated blood donor who is a first-time donor and has never donated blood before.

b) Regular voluntary donor: A voluntary non-remunerated blood donor who donates blood regularly without any break for a long duration between two donations. A regular voluntary non-remunerated blood donor has donated at least three times, the last donation being within the previous year, and continues to donate regularly at least once per year.

c) Lapsed voluntary donor: A voluntary non-remunerated blood donor who has given blood in the past but does not fulfil the criteria for a regular donor.

4.2 Family/Replacement blood donor

A family/replacement donor donates blood when it is required by a member of the donor's family or community. This may involve a hidden paid donation system in which the patient's family pays the donor's. The donor may

or may not be a family member or friend of the patient. The donated blood may or may not be transfused to the patient as it is taken in replacement for the blood already issued to the patient.

4.3 Paid / Professional blood donor

A professional donor donates blood in exchange for money or any other form of payment. Professional blood donation is banned in our country w.e.f. 1st January 1998.

4.4 Forced blood donor

A person who is unwilling to donate blood on his/her wish but donates on being forced by his/her superior or employer. There is always a fear of losing one's job or promotion if he does not donate.

4.5 Autologous blood donor

A patient who donates his/ her blood to be stored and re-infused to him /herself at a later date, if needed, is referred to as an autologous blood donor. For details, refer to section 12.

4.6 Directed blood donor

A donor who donates blood for a patient and the donated blood is exclusively directed to the same patient. These donors are usually the relatives or friends of the patient. This may be recommended in certain situations, such as for patients with rare blood groups, multiple alloantibodies, infants with neonatal alloimmune thrombocytopenia, etc.

4.7 Apheresis blood donor

These are donors who donate the specific blood component required for a patient through cell separation on an apheresis machine. These donations may be either voluntary or replacement in nature.

5. Donor motivation

The term "Motivation" is derived from the Latin word "movere" meaning "to move". Motivation simply means "something that moves one to action". Thus, the process of blood donor motivation can be defined as a force or a process, which causes non-donors to donate blood at their own desire without any compulsion. Donor motivation programmes and campaigns form one of the major aspects of maintaining a regular voluntary non-remunerated blood donors pool.

Donor motivation starts with donor education, which is an important bridge between awareness and recruitment. Donor motivation encourages and stimulates possible donors as well as provides logic or reason to donate blood voluntarily. The motivation campaigns inspire the donors with a feeling or desire to solve a social problem as well as instil a feeling of pride in helping someone unknown that needs blood. In various voluntary blood donation programs, the selfless act of giving blood for community welfare has been the centrepiece of all donor recruitment initiatives.

Donor motivation can be either attitude-based or incentive-based. The motivation strategies should be mainly attitude-based as incentive-based motivation can never assure safe blood and blood components.

5.1 Understand and identify the target population

Donor education should be based on the type of target population, their myths, and beliefs for eliminating the misconceptions around blood donation. Each sector of the population shows different beliefs and attitudes towards blood donation. Understanding their beliefs provides an approach and direction towards educating and motivating them.

5.2 Channels of communication

Information and education that only safe blood, which is readily available, helps to save lives should be stressed through passive, active and reactive communication. Donor education and information materials

as posters, brochures, handouts, and donor questionnaires in simple language and translated to regional languages are passive ways of communication for donor motivation. Pictorial representation of information used to educate donors who cannot read goes a long way in donor education.

Verbal information and dissemination of information online through emails, social media, mobile apps and websites are active communication strategies and participative means for the response of donors to donor motivation.

Reactive communication through motivational talk is an easy, instant, and most effective way of donor motivation. Blood services now use digital platforms as the main channel of communication as the youth are the potential donors who are internet savvy and proficient at using the apps.

Basic research tools such as knowledge, attitude, and practice (KAP) studies provide great insights in identifying the target donor population's specific characteristics, value, and behaviour towards blood donation and developing appropriate motivational programmes.

5.3 Sociological and psychological theories of blood donation

Numerous sociological and psychological factors play an important role in motivating and recruiting blood donors. Multiple theories such as opponent-process theory have been postulated to understand these complex aspects, which directly influence the probability of non-donors becoming blood donor.[4]

6. Donor recruitment

The recruitment of blood donors is one of the most challenging tasks for any blood centre. It is their responsibility to regularly recruit an adequate number of donors to meet the patient's needs. Various strategies to recruit blood donors have been described in the following text.

6.1 Donor recruitment strategies

Different strategies to recruit voluntary blood donors regularly include:

- **Pure voluntary based recruitment** strategy based on a sense of altruism or community service. A voluntary donor donates the blood at his/her own free will without distinction of caste, creed, religion, colour, and status of the recipient and does not expect any monetary benefits in return. These donors should be given more attention, and all efforts should be made by the blood collection facility to encourage and retain these types of donors.
- **Social persuasion-based recruitment** strategy associated with persuasion of friends or colleagues, religious organizations, and political leaders to donate blood. Such persuasion is often done during the outdoor blood donation drives at colleges, political or religious units. In another form of the same strategy, a replacement donor donates for a patient, who is a relative or a close friend, without any monetary benefit. These donors donate out of their expression of care and concern for the patient or their family members. The blood collection facility needs to focus on and counsel these donors to become regular voluntary donors.
- **A remuneration-based recruitment strategy is used** by donors who donate blood to seek some monetary benefit, in any form, from the blood collection facility or patient's relatives. This kind of strategy is banned in India and should be discouraged.

6.2 Role of social media in donor recruitment

Regular recruitment of whole blood donors is an ever-evolving challenge for any blood transfusion service. The strategies to recruit have evolved from personal communication (donor or donor motivator recruiting donors) and printed advertisements to digital media. Online posts have recently evolved as a strong tool for blood donor recruitment, especially for young first-time donors. About eighty-six per cent of first-time donors and 85.7% of repeat donors use the internet for updating general information on a variety of topics.[5] The younger population is attracted to interactive websites and apps on many social media platforms, which plays a vital role in recruiting new donors and encouraging them to become repeat donors.[6]

Social media platforms can be optimally utilized to create positive awareness and importance of blood donations and remove the myths or misconceptions around blood donations. Regular updates by donors with various pictures of them donating can reinforce the fact that blood donation is a safe procedure. These platforms can also be utilized to book the appointment for donors and provide real-time location of mobile blood collection units. With endless information on the fingertips and convergence of the whole world on these social media platforms, their use for recruiting donors for blood transfusion has become essential.

6.3 Positive impacts of blood donation

Blood donation is a noble, selfless act of service to mankind. One of the main benefits of blood donation is the sense of satisfaction of giving a "gift of life" to someone in need. Another advantage of blood donation is an opportunity to undergo a general medical check-up by a qualified physician, which may also help in the diagnosis of any suspected health ailment. Few studies in the past have shown more tangible benefits, especially for middle-aged men, as blood donation is reported to be associated with lowering the risk of cardiovascular diseases in these donors.[7,8] The "iron-hypothesis" was the main basis of these proposed theories, as surplus iron in the body is proven to be a hallmark for heart failures.[9] More iron in circulation results in the generation of more free radicals, which further induce oxidation of lipids. However, the John Hopkins Hospital autopsy register showed less coronary artery disease in patients with haemochromatosis and haemosiderosis when matched with age and sex controls.[10] Though the hypothesis still remains that reduction of the body iron load with regular blood donation may help in reducing the risk of developing diseases like cancer, heart attack and fatty liver disease. The altruistic reason/nature associated with regular donation has also shown numerous psychological benefits and positively impacted the donor's mental health.

7. Blood donor counselling

The World Health Organization defines blood donor counselling as "a confidential dialogue between a blood donor and a trained counsellor about issues related to the donor's health and the blood donation process".[11] This definition focuses on two main points - the blood donation process (what is going to happen) and the donor's health to accomplish donation so that the safety of both the donor and the potential recipient is assured. Blood donor counselling takes place before, during and after blood donation. The counselling should match the donor's specific needs regarding the blood donation process in the context of language and education level. The main aim of donor counselling is

- To clarify myths and misconceptions of blood donors
- Prevent donation by potentially ineligible and unsafe donors
- Provide an opportunity to the donor to self-exclude from donation if they are aware of having been exposed to any risk of transfusion-transmitted infections (TTI)/known health problem/taken some treatment making them unsuitable for donation[12]
- Understand the consequences of TTI infections
- Give them their right to know their health status and plan behaviour modifications
- Improve donor perception of blood transfusion services (BTS) and increase the likelihood of donors to return for future donations/become VNRBD from replacement donors[13, 14]

7.1 Stages of counselling for blood donation

There are four stages of counselling for prospective blood donors:

a) Pre-donation information
b) Pre-donation counselling
c) Counselling during blood donation
d) Post-donation counselling

A trained blood donor counsellor who must maintain the privacy and confidentiality of the donor should provide counselling. All blood centres should train their donor organizers/paramedical staff/medical officers to undertake counselling in case dedicated manpower is not available.

7.1.1 Pre-donation information

Pre-donation information is usually the process of educating and increasing awareness of the potential donors about the blood or component donation. It can be provided by written or verbal information given to blood donors before donation by a counsellor or by the paramedical staff at the blood collection facility or during an outreach lecture or seminar addressing all pre-donation queries of the donors. Pre-donation information sessions provide an opportunity for a prospective donor to ask the following questions and clear the doubts about the blood donation process.

Pre-donation information should also be legibly displayed at the waiting or the reception area of the blood collection centres and includes

- Donor's rights and responsibilities
- Importance of voluntary blood donation
- Donor safety
- Eligibility criteria for blood donation and rationale of donor questionnaire
- Overview of the donation process
- Basic information about the common TTIs, including mode of transmission and window period
- Option for self-deferral

7.1.2 Pre-donation counselling

This is a more focused "one to one" counselling session in privacy before blood donation for all the registered and potential blood donors to review donor's understanding of blood donation and assess the personal risk history of TTI. It includes a detailed explanation of the blood donor questionnaire and the importance of providing correct answers. It aims to familiarize and increase donor awareness on

- Process of blood donation
- TTIs, their route of transmission and prevention
- Testing of their blood for TTI
- Implications and possible consequences of test results
- Availability of post-donation counselling, testing and support agencies
- Secure and obtain informed consent from the donor for donation and testing.
- It also provides an opportunity for the donor to self-deferral and discourages blood donation by people with test-seeking behaviour.[15, 16]

7.1.3 Counselling during blood donation

Counselling during blood donation is provided to make them comfortable at the time of donation. During the blood donation, the donor's experience shapes the overall perception and their willingness to donate blood again. The fear of needle is the most common cause of anxiety and fear during this stage. The discussion during this stage should be focused mainly to reduce the anxiety as well as reducing the chances of any adverse donor reaction during or post-donation. Post-donation advice and care for the venipuncture site should also be explained at this stage. The option for confidential unit exclusion (CUE) should also be discussed and made aware to the donor.

7.1.4 Post-donation counselling

Post-donation counselling is generally given based on need. Immediately after the donation, blood donors are given some brief instructions for self-care in the next 48 hours and information about what to do in case of adverse after-effects of donation. In addition to this, post-donation counselling may need to be provided to those donors who are found to be reactive for one of the TTI markers or show some unusual red cell serology. The main objectives of post-donation counselling in such cases include

- To explain the test results, the need for confirmation of the results, the health implications for the donor and the donated blood (discarded) and the suitability of the donor for future blood donation

- To encourage donors to provide all relevant information, including the possible source of infection.
- To alleviate donors' anxiety and to clarify his doubts or concerns
- To provide information on precautions for preventing the transmission of infection to others
- Provide information and refer donors for further investigation, management, treatment and care, if necessary

Donors with any unusual red cell serology are informed about the details, their importance for future donations/ receipt of blood.

8. Donor selection criteria

The donor selection criteria should ensure the safety of the donor and recipient**.** Each blood centre should prepare its standard operative procedures (SOP) for donor selection based on WHO guidelines, national standards, standards laid by regulatory authorities and accreditation bodies.[17,18] The donor selection criteria define conditions of acceptance and deferral for each criterion and are meant to ensure the safety of the donor as well as the recipient while maintaining the quality of the product. The main aims are:

- Protection of the health of the donor by blood collection only from healthy donors
- Protect the donor from any adverse reaction during or post-donation
- Ensuring patient safety by blood collection from donors whose blood donation is safe for recipients
- Reduce unnecessary donor deferrals
- Ensure quality of blood and blood products

For donor selection criteria for autologous blood donation, please refer to the section on autologous blood donation. Similarly, for donor selection criteria for double unit red cell collection, refer to the section on apheresis.

9. Donor selection process

Donors' selection is critical to the success of the supply of safe blood and its products. The donor selection shall be based on donor selection criteria in the blood transfusion service and at the outdoor blood donation camps.

A qualified medical officer, considering the medical history, limited physical examination, and simple laboratory tests, should carry out the donor selection process according to the SOP. The different steps involved are:

- Donor registration
- Pre-donation information and completion of donor questionnaire
- Donor interview and pre-donation counselling
- Donor health check-up and pre-donation haemoglobin estimation
- Informed consent
- Donor deferral

9.1 Donor registration

A donor questionnaire or registration form is used for the registration of all prospective donors. The questionnaire should be in English and the local language, which would enable easy understanding by the donor. Basic information such as donor name, demographic details, address, etc., from the intended blood donor, is obtained in a written format. The blood centre maintains the record.

Assistance should be provided to illiterate donors by the blood centre staff to fill up the donor's full name and demographic details with the date and time of donor selection. Efforts should be made to obtain the correct contact details of the donor so that the blood centre can contact him/her in future. The blood centre may ask for a photo identity, but it is not mandatory for blood donation. A unique number shall be allotted at the time of donor registration.

9.2 Pre-donation information and donor questionnaire

After registration, the donor should be provided with pre-donation information and asked to fill the donor questionnaire to provide information regarding the donor selection criteria defined by national standards. All potential and existing donors should be asked to adhere to the blood donor selection criteria by providing accurate information and answers to all questions asked to protect their health and that of patients who receive a transfusion.

9.3 Donor interview and pre-donation counselling

The donor questionnaire is reviewed in a face-to-face donor interview in the hope of ensuring a complete understanding of medical terminology and honesty in answering the questions. Pre-donation counselling forms an integral part of the donor interview and ascertains donors' understanding and response to all the questions. Answers to the donor history questionnaire in a confidential setting provide an opportunity to obtain information about potential risk factors in the blood donor. Compliance with all donor selection criteria is crucial to ensure a safe blood donation process and outcome. The donor should be allowed to self-defer after pre-donation counselling and donor interview.

9.4 Donor health check-up and pre-donation haemoglobin estimation

The medical officer subjects the donor to a basic health check-up through history taking, limited physical examination as blood pressure, pulse rate, temperature and haemoglobin (Hb) testing to determine their eligibility and suitability to donate blood. The donor is to be accepted only after being declared fit to donate.

Pre-donation haemoglobin estimation is an essential donor screening test performed before the process of blood donation. Haemoglobin estimation, before donation, tests the donor for anaemia as well as ensures the quality of blood components, which can be prepared from the whole blood donation. As per Drugs and Cosmetic Act of India, the minimum Hb required for whole blood donation is 12.5 g/dL or 38% haematocrit (HCT) for both males and females.[18] Haemoglobin estimation must be done using any of the available quantitative methods. Portable haemoglobinometers using blood from a finger prick is the most common method used by the blood centres for rapid estimation.

9.5 Informed consent

Once the prospective donor is certified to be eligible for donation as per national regulations, written informed consent should be taken before blood donation with the donor's signature or thumb impression. Informed consent signifies that the donor has understood the questionnaire and is willing to donate blood or blood components. The consent should include that his/her blood will be screened for Human Immunodeficiency Virus (HIV), Hepatitis B & C (HBV & HCV), Malaria, Syphilis and his willingness for post-donation notification as well as counselling in case of any abnormal results. The donor's consent signifies that:

- The donor has received, reviewed the educational materials, and understood the hazards and risks of the donation procedure.
- The donor agrees not to donate if the donation could potentially risk the safety, purity, or potency of the blood supply.
- A sample of the donor's blood will be tested for specified relevant transfusion-transmitted infections. If results are found to be reactive for any of the TTI markers, the collected blood will be discarded.
- The collected blood will be processed into blood components and may be further provided to a plasma fractionator for further processing.

9.6 Donor deferral

Donors who do not meet the selection criteria should be deferred on a temporary or permanent basis. In such cases, the donors may be informed in a warm and supportive manner of their unsuitability to donate blood. They must be informed whether the deferral is to safeguard their health or that of the recipient.

Enumerate the cause of deferred donations due to donor selection (anaemia, underweight/underage, medical/surgical cause or high-risk history) or as a result of reactive test results among male and female donors separately.[19]

In case of a temporary deferral, the donor must be informed of when they will be able to donate blood in the future. Those who need to be referred for further management should be guided suitably to a physician. It is important to give positive health messages and encourage the donors to return to donate after temporary deferral.

10. Whole blood collection

Whole blood collection is a critical process that determines the safety of the donor as well as the quality of the donated blood. The standards laid down by the regulatory authorities for infrastructure and premises for blood collection should be adhered to, besides other factors such as manpower, materials, machinery, and motivation of the staff, which also influences the quality of collected blood.

10.1 Infrastructure and premises

The premises of blood collection should be clean, comfortable, and conveniently approachable. It is mandatory to have air-conditioned rooms for blood collection to prevent the risk of adverse donor reaction.

a) **In-house blood collection centre:** These are blood collection facilities permanently located within a blood centre. The blood centre may be a standalone or located within a hospital and should have easy access to the donors. The facility should be well ventilated and lighted with non-slippery flooring. Educational and motivational materials should be displayed as well as readily available to the donors in the waiting and refreshment area. The donation room should be clean and hygienic so that there is minimal risk of contamination or exposure by microbes to the collected blood as well as equipment related to blood collection. The collection area should have free, uninterrupted unidirectional movement of staff with the possibility of maximum donor observation. The refreshment area should be separate from the collection area.

b) **Mobile blood collection (blood donation camps):** Mobile blood donation can be at a prefixed campsite with an organization or in a mobile bus or coach that is taken to a site accessible to blood donors. The mobile site for blood donation should be well ventilated and lighted, which is suitable for blood donation. Additional areas for rest and refreshment should be separate from the collection area. The area should have sufficient size to allow proper operations as well as ensure donor privacy. There should be a provision of handwashing and separate space for blood storage.

10.2 Manpower

Only trained personnel working under the direction of a qualified licensed physician should collect blood from the donor. A qualified phlebotomist or doctor should perform the venepuncture after verifying the donor screening details, unit number on the bag and preparation of the donor site. All personnel in the donor collection area should be vaccinated for the hepatitis B vaccine. The physician should be present on the premises when blood is being collected.

10.3 Material used in blood collection

Materials and equipment required for phlebotomy should be readily available for blood collection. A critical inventory of stock should be maintained. All materials should be inspected for any deviation from the standards and expiry date before being put into use. Table 1 provides the list of essential equipment required for blood collection.

Table1: Essential equipment and material required for blood collection

Equipment	Material
• Weighing scale • Thermometer • Haemoglobinometer • Donor couch or chair • Blood collection monitor • Tube stripper • Tube sealer • Sphygmomanometer • Cell Separator (Apheresis Machine, in case collecting apheresis) • Biomedical waste containers (colour coded) • Oxygen cylinder with accessories	• Lancet • Blood collection bag • Emergency medicines • Gloves • Spirit/ Iodine/ Isopropyl Alcohol • Sterile cotton swabs • Vacutainers (plain and EDTA) • Adhesive tape • Spillage kit

a) The blood donor chair or couch should be reclinable with adjustable arms rests for comfortable positioning of the donor. The upholstered surface should be easily washable for quick cleaning and disinfection.

b) The blood collection monitor determines the rate of flow and volume of blood being donated. They also adequately mix the contents of the blood bag with the anticoagulant solution during blood collection to prevent any clot formation. The monitor should have side-to-side movement of 16 cycles/min and should be periodically calibrated for accurate weight, the volume of blood collected and a number of side-to-side movements per minute. The monitor should have an audio-visual alarm for the desired volume of blood collection.

c) Tube stripper is used to strip blood in the blood bag tubing after collection for adequate mixing of blood with anticoagulant before sampling.

d) Tube sealer is used to seal the blood bag tubing post sampling and make tube segments which are later used for compatibility testing and quality control testing

e) Blood collection bag (blood container)

Blood bags are made of polyvinyl chloride (PVC) plastic that contains di-(2-ethylhexyl) phthalate (DEHP) to make the containers pliable. The bag is flexible, tough, kink resistant and scratch-resistant. The container's material should be able to withstand sterilization by gamma irradiation, ethylene oxide, electron beam or all of them. The plastic should allow adequate gas exchange for oxygen and carbon dioxide yet prevent water evaporation from the blood component bags. Bags should be pyrogen-free and sterile. Besides a statutory license for manufacturing blood bags, quality checks for standards by government-approved testing laboratories are mandatory before blood bags are put to use. Test reports should be ascertained before acceptance of the bags in the blood centre inventory.

The different configurations of blood bags are single, double, triple, quadruple or quintuple bags. A blood bag with its tubing and venepuncture needle is a closed system. More recently, additional configurations for blood containers include in-line filters for removal of leukocytes from the whole blood (WB) or Red Blood Cell (RBC) units. For removing contaminants from skin flora, a diversion pouch is attached with the collection tube, the blood from which can be used for test samples.[20] Blood collection bag design also includes safety devices such as the sliding sheath needle guard to prevent accidental needle-stick injuries. These devices allow retraction of the needle into a safety guard after blood collection.

Blood bags are supplied in pouches and should be used within the shelf life prescribed by the manufacturer. The bags should be checked for a batch number, lot number and date of manufacture and expiry on the bag. Blood bags should be transported and stored between 20-24^{0}C at all times before blood collection to maintain the integrity and sterility of the preservative solution. The preservative solution in blood bags contains an

anticoagulant (AC) to prevent donor blood from clotting. The preservative solution also contains nutrients for the blood cells and an additive solution for red cells' long-term storage. Table 2 discusses the details of the ingredients of an anticoagulant and its functions. The approved anticoagulant-preservative solutions used in the blood bag are as follows

- Acid-citrate-dextrose solution (ACD)
- Citrate-phosphate-dextrose solution (CPD)
- Citrate-phosphate-dextrose-dextrose solution (CP2D)
- Citrate-phosphate-dextrose-adenine solution (CPDA-1).

Table 2: Constituents of common anticoagulants (AC) and their functions

Solutions	Function
Sodium Citrate (C)	Binds with calcium ions and interferes with calcium-dependent steps in the clotting cascade
Dextrose (D)	Supports generation of ATP by glycolysis and supports red cell membrane to increase shelf life
Citric Acid	Prevents caramelisation of glucose during autoclaving
Phosphate (P)	It supports the metabolism of the red cells during storage to ensure they release oxygen readily at the tissue level
Adenine (A)	Provides the source of energy
AS1 (SAGM)	Saline (S)- Provides fluid for the suspension to maintain flow rate Glucose (G)- Provides basic nutrients for glycolysis Adenine (A) & Mannitol (M)- Provides ATP and Stabilizes RBC wall

Additive solutions (AS) are used to enhance the shelf life of RBC to 42 days. The three forms of additive solution (AS) used are AS-1, AS-3 and AS-5. The most commonly used additive solution is SAGM which contains Saline, Adenine, Glucose and Mannitol. Additive solutions maintain the adenosine triphosphate (ATP) levels necessary for red cell viability during storage and the 2, 3-biphosphoglycerate (BPG) levels, which affects the oxygen exchange capacity of red cells. These additive solutions should be pyrogen-free. Table 3 discusses the details of the different type of anticoagulants.

Table 3: Composition, pH, and approved shelf life of RBCs with a different type of anticoagulant solutions

Composition	CPD	CP2D	CPDA-1	ACD-A	ACD-B
pH	5.3-5.9	5.3-5.9	5.3-5.9	4.5-5.5	4.5-5.5
Ratio (AC ml: whole blood ml)	1.4:10	1.4:10	1.4:10	1.5:10	2.5:10
Approved Shelf Life	21 days	21 days	35 days	Automated component collection	Automated component collection

10.4 Blood donation process

The blood donation process is one of the most important steps for quality assurance of whole blood collection and preparation of components. The process is designed to maintain the vein-to-vein traceability of the donated blood and maintain donor confidentiality. Each blood donor is assigned a unique donor identification number which is most critical in ensuring the traceability of blood in the blood transfusion chain from donor to the recipient. The blood component identification process uniformly uses either a bar-code or an eye-readable unique number that is assigned to the electronic/manual records of the donation, donor registration form, blood bag, sample tubes, component bags and donor card.

Once the donor is verified, appropriate labels are used for labelling the blood bags. Sticker labels are pasted on the blood collection bag, its satellite bags, sample tubes and donor records. Separate labels are required for autologous donation and therapeutic phlebotomy bags to differentiate them from the routine inventory as they are for designated use or discarded, respectively. After the bag is ready, identify the appropriate vein for phlebotomy. Inspect both the donor's arms to select one arm on the basis of the presence of a prominent, large, firm vein in the ante-cubital fossa, which ensures a single, readily accessible phlebotomy site. The three choices in order of preference for selection of a vein for phlebotomy are: median vein, which is well anchored and centrally located in the antecubital fossa; a cephalic vein which is often superficial and laterally located (shoulder side) and the basilic vein, which is not well anchored and may roll during phlebotomy, lies on the underside of the ante-cubital fossa. The venepuncture site should also be inspected for any evidence of drug abuse or skin infection

The phlebotomy site is then disinfected to ensure local antisepsis. In the presence of a scarred phlebotomy site, surface cultures may be negative, while blood culture tests are positive. Organisms that are not part of the normal skin flora can also colonize on the skin and result in a septic transfusion reaction.

During phlebotomy, a small plug of donor skin can enter the needle and can flow into the collected blood. Bacteria residing deep within skin layers are not accessible to disinfectant; therefore, it can result in contamination if the skin plug enters the collection bag. Whether human skin fragments or epidermal cells are carried into the bag during routine blood donation has not been studied in detail. In order to avoid it, a bag, with a diversion pouch may be used, wherein the first few volumes of blood collected from the donor are diverted into the diversion pouch before filling the bag.[20,21]

The volume of blood collected during routine phlebotomy is typically 350/450 ml ± 10%. The volume of blood collected can be assessed in terms of gram weight by multiplying the volume collected by the specific gravity of blood (1.053). For example, the 450-ml collection weighs from 427 to 521 g. Blood bags indicate the amount of blood collected depending on the amount of anticoagulant present in the bag.

Close monitoring of the donor, needle and blood bag is required throughout the donation process. Observe the donor for any sign of sweating, pallor or complaint of feeling dizzy that may precede actual fainting. Advise the donor to squeeze the hand roller to improve blood flow gently. The phlebotomist must talk to the donor to make him/her comfortable. Never leave the donor alone or unattended while the process of donation is going on. On completion of the collection, withdraw the needle from the vein and apply firm pressure with sterile cotton over the point of entry of the needle into the vein. Instruct the donor to continue the application of pressure for several minutes and make the donor recline on the donor chair for a few minutes under close observation of the staff.

After the donation is complete, collect the blood samples in plain and EDTA vials for TTI screening and blood group confirmation. Label the tubes with a unique donation number. Make the donor sit up under observation prior to release to the refreshment area, where the staff monitors the donors and attends to donor reactions, if at all any. Re-inspect the blood bag for any defects or deficiencies. The donor numbers on the blood bag, sampling tubes, donation record and retention segment should be ascertained. The phlebotomist signs or initials the donor record, even if the phlebotomy does not result in the collection of a full unit. If more than one skin puncture is required due to any reasons, use a new blood bag set.

Place the blood bags at the appropriate temperature before processing. Whole blood from which platelet concentrate are not to be prepared should be placed at 1 to 6^{0}C after collection until it is transported from the collection site to the processing laboratory. If platelets are to be prepared, blood is stored in a manner intended to reach a temperature of 20 to 24^{0}C until platelets are separated. Platelets must be separated within 6 hours after collection of the unit of whole blood or within the time frame specified in the directions for the use of the blood collecting, processing, and storage system.

The whole process of blood collection should be documented. Donor records should include unique donation number, date of bleeding, name, address, the signature of donors with other particulars like age, weight, haemoglobin, blood grouping, blood pressure, medical examination, bag number, tubing number and category of donation (VD/RD). Records of ACD/CPD/CPD-A/SAGM/ADSOL bags giving details of manufacturer, batch number, date of supply, and testing results should be maintained. Additional donation history of each donor

should also be captured in the form, such as a number of previous donations, donation dates and any history of adverse reactions.

10.5 Post-donation care

It includes post-donation observation of the donor and giving the donor instructions about the post-phlebotomy period. Offer the donor something to eat and drink before he/she is released from the donor site. All personnel on duty throughout the donor area should be able to identify signs of a donor reaction such as lack of concentration, pallor, rapid breathing, and excessive perspiration and attend immediately. Ask the donor to drink more fluids than usual in the next 4 hours and avoid alcohol consumption until something is eaten. Advise the donor not to smoke for 30 minutes.

If there is bleeding from the phlebotomy site, advise the donor to raise the arm with the application of pressure to the site. In case of fainting or dizziness, instruct the donor to lie down or sit with the head between the knees. If any symptoms persist, advise to contact either telephonically or return to the blood centre or see a doctor. All normal activities can be resumed if the donor is asymptomatic. Donors who work in certain occupations (e.g., construction workers, operators of machinery) or persons working at heights are cautioned that dizziness or faintness may occur if they return to work immediately after giving blood. Thank the donor immensely for an important contribution and encourage them to repeat blood donation after the proper interval.

Donor room personnel should be competent to interpret instructions, answer questions, and accept responsibility for releasing the donor in good condition. A telephone number is provided on the donor card so the donor can report if he or she feels that the donated unit should not be used (Confidential Unit Exclusion[22]), has any reactions, or experiences any signs or symptoms of infection**.** Any adverse reactions that occurred are noted on the donor record.

10.6 Confidential unit exclusion (CUE):

This is a system that permits the donors an opportunity to inform the blood centre immediately after donation or subsequently if they consider that their blood may be unsuitable for transfusion. This is useful if donors have been persuaded or coerced to donate. Where CUE is used, donors should be given information to contact the blood centre and communicate that their blood should not be used for transfusion. Emphasis should be laid on giving donors proper contact details of the blood centre in case of a blood donation drive especially off-site.

11. Donor complications and management

Before, during or after blood donation, an untoward feeling by the blood donor is known as an adverse donor reaction. There is a psychological element to most reactions, so a friendly, cheerful atmosphere can reduce donor anxiety and perhaps prevent any adverse reaction. Untoward feelings by a blood donor before, during or after blood donation is known as a donor reaction. The adverse donor reactions can be broadly classified[23] as Table 4:

Table 4: Definition, presentation and management of adverse donor reactions

S.No.	Adverse Reaction	Definition and presentation	Management
A	**Local Symptoms**		
A1	**Blood Outside Vessel** a. Haematoma (bruise) b. Arterial Puncture c. Delayed bleeding	a. Accumulation of blood in the tissue b. Puncture of the brachial artery or its branch c. Re-bleeding after initial bleeding has stopped	a. Apply cold compresses intermittently for 12-24 hours, then use warm compresses thereafter. b. Discontinue the procedure immediately. Apply constant firm pressure for at least 10 minutes. Check for radial pulses and good circulation after bleeding has stopped. c. If bleeding resumes, apply firm, direct pressure and elevate the arm directly above the head.
A2	**Pain in the Arm** a. Nerve injury/ irritation b. Another painful arm	a. Injury or irritation of a nerve b. Pain without characteristics of nerve irritation, large Haematoma	a. Discontinue procedure immediately. b. Apply constant, firm pressure until bleeding stops. Apply cold compresses.
A3	**Localized infection/ inflammation of vein or soft tissue**	Inflammation along the course of a vein, which may progress to localized infection; there may be clotting. These can be due to thrombophlebitis or cellulitis	Apply the local medication to reduce inflammation
A4	**Other major blood vessel injury** a. Deep venous thrombosis b. Arteriovenous fistula c. Compartment syndrome d. Brachial artery pseudoaneurysm	a. Thrombosis of a deep vein in phlebotomy arm b. The acquired connection between vein and artery c. Increased intra-compartmental pressure leading to necrosis d. Collection of blood outside an artery, contained by adventitia or the surrounding tissues alone	Reassess and get an expert opinion for further management
B	**Generalized Symptoms**		
B1	**Vasovagal Reactions (VVR)**	General feeling of discomfort and weakness with anxiety, dizziness, and nausea, which may progress to loss of consciousness (faint) which may last for a long period. This may also result in fall or accident-causing injury.	

S.No.	Adverse Reaction	Definition and presentation	Management
		a. **Mild vasovagal Reaction with no loss of consciousness (LOC):** Cold extremities/chills, feeling of warmth, hypotension, light-headedness/dizziness, nausea/vomiting, pallor (pale skin or lips), slow or rapid pulse, sweating, twitching/weakness	a. Tilt donor bed back or make the donor lie down, elevate feet, apply cold towel, and offer fluids. Monitor, observe and reassure donor. If nauseous, turn towards the side and provide an emesis basin
		b. **Moderate vasovagal Reaction with LOC (< 60 sec) uncomplicated:** Signs and symptoms of mild with LOC < 60 sec	b. Discontinue procedure. Tilt donor bed back or make the donor lie down, elevate feet, and apply cold towels and offer fluids. Monitor, observe and reassure donor. If nauseous, turn towards the side and provide an emesis basin
		c. **Severe vasovagal Reaction with LOC (>60 sec) complicated:** Moderate signs and symptoms PLUS convulsions, LOC >60 seconds, loss of bowel/bladder control	c. Discontinue procedure. Tilt donor bed back or make the donor lie down, elevate feet, apply cold towels and offer fluids. Monitor, observe and reassure donor. If nauseous, turn towards the side and provide an emesis basin
		d. **Severe vasovagal Reaction with an injury:** Severe signs and symptoms PLUS injury/fall	d. Discontinue procedure. Tilt donor bed back or make the donor lie down, elevate feet, apply cold towels and offer fluids. Monitor, observe and reassure donor. If nauseous, turn towards the side and provide emesis basin. Treat injury. If symptoms resume, ask the donor to sit down immediately, do not operate heavy machinery.
B2	**Hyperventilation**	Rapid breathing with or without a tingling of lips, fingers and hands, perspiration, the possible feeling of suffocation	Discontinue procedure. Make donor breathe into a paper bag for 1-3 minutes. Provide routine post-donation instructions. Educate and reassure donor.

S.No.	Adverse Reaction	Definition and presentation	Management
C	**Related to Apheresis Donations**		
	a. Citrate reaction	a. Neuromuscular hyperactivity related to reduced Ca2+. **Mild:** Tingling around the mouth in the face and/or hands and feet, lethargy, feeling a sense of "vibration" cramps in hands/feet.	**Mild:** Pause procedure, offer oral calcium, monitor donor and press "continue" when ready. If s/s persists: Decrease ACD-A rate. Monitor donor for 10 minutes, decrease ACD-A again if s/s still persists. Discontinue procedure if symptoms are intolerable – give rinse back if possible.
		Severe: Confusion/disorientation carpal –pedal spasms, tetany – muscle tightness, chills/shivering, circumoral paraesthesia, nausea/ vomiting, pallor, rapid pulse	**Severe:** Discontinue procedure. Do not give rinse back, call for support from the emergency services, Comfort donor, monitor vitals. Till the emergency services arrive, start IV calcium (1 or 2 g of calcium gluconate, equivalent to 90 or 180 mg elemental calcium, in 50 mL of 5% dextrose [D5W] or normal saline [NS]) to be infused over 10 to 20 minutes)
	b. Haemolysis	b. Damaged donor red cells, releasing haemoglobin causing pinkish to cherry red fluid in the collection line	b. Discontinue procedure. Do not give rinse back, call for support from the emergency services, comfort donor, monitor vitals
	c. Air Embolism	c. Air bubble introduced into the donor's circulation	c. Discontinue procedure. Do not give rinse back, call for support from the emergency services, comfort donor, monitor vitals
	d. Infiltration	d. Intravenous solute (saline solution) enters tissues. Swelling, donor discomfort at phlebotomy site, IV fluid leaking into tissues or outside the vein.	d. Discontinue procedure. Do not give rinse back. Apply constant, firm pressure to the site until bleeding has stopped. Apply a cold compress to site.
	e. Allergy (local)	e. Red or irritated skin at the venepuncture site, itching/hives, skin irritation, rashes	e. Continue procedure with donor consent. If symptoms worsen, seek medical attention. A cold towel may be applied to the irritated area.

S.No.	Adverse Reaction	Definition and presentation	Management
	f. Generalized allergic reaction (Anaphylactic reactions)	f. Anaphylactic reactions may begin soon after starting the procedure and progress rapidly. Diagnosis is clinical. The most common sign and symptoms are cutaneous (e.g., sudden onset of generalized urticaria, angioedema, flushing, pruritus). Danger signs: Rapid progression of symptoms, respiratory distress (e.g., stridor, wheezing, dyspnoea, increased work of breathing, persistent cough, cyanosis), vomiting, abdominal pain, hypotension, dysrhythmia, chest pain, collapse.	f. Discontinue procedure, call for support from the emergency services, comfort donor, monitor vitals. Promptly and simultaneously, give IM epinephrine (1 mg/mL preparation): Give epinephrine 0.3 to 0.5 mg intramuscularly, preferably in the mid-outer thigh. Can repeat every 5 to 15 minutes (or more frequently), as needed. Place the patient in a recumbent position, if tolerated, and elevate lower extremities. Oxygen: give 8 to 10 L/minute via facemask or up to 100% oxygen, as needed. Normal saline rapid bolus: treat hypotension with rapid infusion of 1 to 2 litres IV. Repeat as needed. Shift the patient to the care of emergency services as soon as possible as the patient may require intubation or may be resistant to epinephrine.
D	**Other Serious complications** a. Acute cardiac symptoms b. Myocardial infarction c. Cardiac Arrest d. Transient ischemic attack e. Cerebrovascular accident f. Death	Must be medically diagnosed, imputability assessed	Discontinue procedure, call for support from the emergency services, comfort donor, monitor vitals

11.1 Donor haemovigilance

Donor haemovigilance is a continuous process of data collection and analysis of adverse donor events and reactions before, during or after whole blood or apheresis donations. Donor haemovigilance data should be investigated for their possible causes and should be utilized for clinical and public health decision making to prevent their occurrence and to promote safe donation. All adverse events and reactions in donors should be identified, documented and reported uniformly. The Indian National Blood Donor Vigilance Programme (NBDVP) was launched on June 14, 2015, on the World Blood Donor Day at Science City, Kolkata, West

Bengal, India. The centres enrolled under the NBDVP collect data regarding adverse reactions associated with blood donation and report on an online platform. This data is then validated and analyzed to identify trends, recommend best practices and interventions required to improve donor safety. These recommendations are forwarded to the Drug Controller General (India), Central Drugs Standard Control Organization, to formulate safety-related regulatory decisions to improve donor safety and satisfaction.

12. Recall and referral of Sero-reactive blood donors

The donor who has consented to be contacted by the blood collection centre in case of any abnormal test results should be recalled so that they can be informed about the initial seroreactivity of transfusion-transmitted infection screening tests. This consent is part of the pre-donation counselling and should be well documented. The blood centre should repeat the test using the same technique using the pilot tube/ sample from the blood bag prior to labelling the donor as initial sero-reactive and recalling for a referral. The blood collection centre should make appropriate efforts to contact (by phone or letter) the sero-reactive donor for recall, and the process should be documented on record. The screening results should not be informed on the phone.

All recalled donors should be offered post-donation counselling and referred to appropriate facilities for further counselling, confirmation, and management. The donor should be counselled not to donate till the results of his diagnostic tests are clear. Suppose the initial sero-reactive donor does not return to the blood centre despite three consecutive weekly attempts. In that case, the list of HIV initial sero-reactive blood donors should be shared with the linked ICTC confidentially under guidance from the State AIDS Control Society. All blood donors found to be sero-reactive for HBV, HCV, syphilis, and malaria shall be referred to appropriate clinical specialities for retesting, confirmation and further management. Confidentiality should be maintained at all steps in donor recall and referral. Result seeking blood donor, even if non-reactive, should also be informed of their transfusion-transmitted infection screening status with reiterated counselling to remain negative and continue to donate blood.

13. Therapeutic phlebotomy

Therapeutic phlebotomy or venesection (bloodletting) is a procedure that has been performed throughout history for various indications. In present clinical practice, therapeutic phlebotomy is an evidence-based preventive or therapeutic intervention performed under the supervision of a doctor for disorders such as polycythaemia vera and haemochromatosis. A prescription for therapeutic phlebotomy is required from the primary treating physician. It should include diagnosis, indication for therapeutic phlebotomy, haematocrit levels, volume to be removed and frequency of phlebotomy procedures.

The blood collection centre should define guidelines for patient safety during phlebotomy procedure as well as disposal of the collection bag as per biomedical waste guidelines. The blood collected cannot be used for allogeneic transfusion purposes as it is not taken from a healthy donor and hence need not be screened for transfusion-transmitted infections. The centre should educate the patient regarding intra and post-phlebotomy symptoms and obtain consent for the procedure. The patients should be encouraged to drink more fluids before and after the procedure. The staff at the blood centre should be adequately trained for the process of phlebotomy, patient safety as well as managing any adverse reaction, including cardiopulmonary resuscitation. Replacement fluid should be considered for patients who cannot tolerate the rapid loss of blood; hence the prescription should ideally include type of replacement fluid (if required), amount as well as infusion rate both intra and post phlebotomy. Not more than 2 units should be removed during a 24-hour period. The blood collection centre should document the pre-and post-procedure vitals (blood pressure, pulse rate, respiration rate and temperature), volume removed, and access used (peripheral/ through a catheter). The therapeutic phlebotomy can also be done using a cell separator (erythrocytapheresis) which can result in rapid red blood cell removal with limited plasma loss.

14. Donor records

Donor records about each blood donation activity are important and should be stored as per regulatory requirements ranging from 5 years to life long. A signed donor questionnaire with informed consent also forms part of donor records. Records should be confidential and allow traceability from each donation to the recipient and vice versa. The key records to be maintained include:

- Donor registration and information
- Donor questionnaire with informed consent
- Donor interview and assessment
- Donor deferral records
- Unique donation number assigned to each donation
- Donor counselling records
- Adverse donor reactions

Confidentiality is critical in the management of blood donor. All donor information is privileged and must be kept confidential. Records must be kept secure at all times, and only designated staff should have access to records. These records should be reviewed and analyzed for monitoring the effectiveness of donor selection and reasons for donor deferral.

15. Donor feedback

Donor feedback should be welcomed, acknowledged and responsive to donors, where appropriate. It is important to deal with positive and negative feedback evenly. All complaints ranging from serious to trivial about any area of BTS should be taken up seriously. It is important to investigate donor complaints to ensure that identified errors/ problems are corrected and prevented from recurring.

16. Deterrents to blood donation

There are many deterrents or potential barriers to blood donation among the general public. These deterrents can be due to numerous factors either associated with the blood collection centre or associated with the blood donors.

16.1 Deterrents associated with the blood collection centre

a. Location of the donor centre

Convenience to donate has also been identified as an important factor in the retention of blood donors. Easily approachable location with appropriate public transport connection and provision for vehicle parking at the blood collection centre are factors that often play an important role. The blood centres should also understand the value of adapting to the mobile blood donation sites, in other words going close to the community to recruit and collect blood.

b. Cleanliness and staff

A clean and ambient atmosphere at the collection centre encourages and gives confidence to the blood donor to come forward and donate. The unfriendly staff has also been reported to be one of the main deterrents for donors to come back as repeat donors at the blood centre. Negative experience during blood donation also influences the return of donors for repeat donation. Hence, all efforts should be made to provide an overall pleasant experience of blood donation at the blood donation centre.

c. Inconvenient operating hours

Usually, the blood collection centres operate on weekdays during office hours (9 am to 5 pm). Extending the collection hours to the evening and on weekends enables more donors to come forward and donate. Blood donation drives during night hours can be dangerous for the donors as a donor is exhausted from the day and good night sleep is one of the requirements for the donation.

d. Ineffective incentive

Elimination of paid and professional donors from the donor pool is one of the main concerns of any blood collection centre; hence use of appropriate incentive, not money or any incentive, which can be converted to cash, should be avoided. Incentives to encourage the feeling of altruism should be provided to the donors, such as badges, certificates, mementoes as well as recognition at public forums.

e. Modes of communication

Traditionally, phone calls and letters were used as a mode of direct communication of blood centre with donors compared to a more efficient and cost-effective mode such as email, text messages, and social media. The blood collection centre should adapt to the newer and more innovative communication mode, especially with the youth.

16.2 Deterrents associated with blood donors

a. Personal values
b. Negative attitude (blood donation is not important)
c. Lack of knowledge/ concern about the negative effect on health
d. Fear of needle and discomfort during donation/ fear of the sight of blood
e. Time constraints (too busy and/or the process takes too long/ extending timing as well as open on weekends)
f. Medical disqualification

17. Quality management of blood donation

Both donor and donation are a priority for the blood centre. The quality chain starts from the blood donor and has implications on the quality of blood transfused to the recipient. The quality management system should be in place with defined responsibilities, policies, SOP and identification of critical control points to be monitored for continual quality improvement. Training of personnel at donor reception in interpersonal skills with a high standard of personal hygiene for donor care should be part of quality management.

Quality Indicators are important tools for a quality management system to monitor and control the efficiency of the key systems. The critical control points and quality indicators of importance during pre-and post-blood collection are as listed in Table 5.

Table 5: Critical control points and quality indicators during blood collection

Critical control points	Quality indicators
Defective supplies of blood bags	Percentage of wrong / defective blood bag supplied
Donor re-identification	Donor identification failure rate
Calibrated blood collection equipment	The inadequate volume of blood collection
Donor phlebotomy	Rate of bacterial contamination due to inadequate arm cleansing
	Donor phlebotomy failure rate
Post donation events	Number of labelling error of donor bag/ sample
Donor reaction	The adverse donor reaction rate
Donor feedback analysis	Donor satisfaction rate

Quality indicators provide proof of the level of quality of performance, and the information gained is used to seek improvement in the quality of performance. Adhering to quality management systems for critical processes as recruitment of donors, donor counselling, stringent donor selection, and blood collection determines the safety and quality of blood for patients.

It also ensures increased confidence and satisfaction of donors in the BTS. The satisfied donors are more likely to become regular donors and more likely to promote voluntary non-remunerated blood donation. Any act of carelessness or lack of professionalism by staff during or after donation can be detrimental to the donors coming back again to donate blood.

Key points

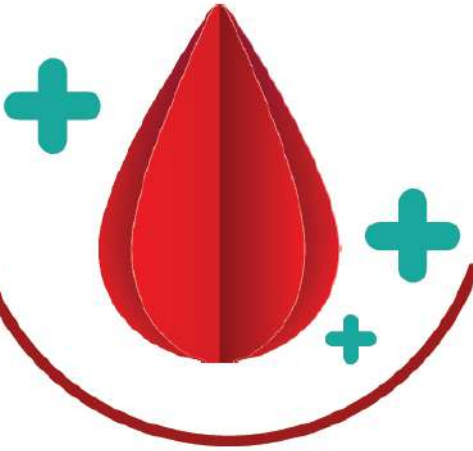
- Sustainable blood supply can be achieved by augmentation of blood collection through regular voluntary non-remunerated blood donation. Therefore, it is important to create awareness and impart knowledge through information, education, and communication (IEC) to the target population and motivate people for voluntary blood donation.
- Blood collection and processing is a multifactorial process that involves many variables as critical control points for quality in blood collection.
- Blood donor counselling focuses on two main points - the blood donation process and the donor's health to accomplish donation so that the safety of both the donor and the potential recipient is assured.
- The donor selection criteria define conditions of acceptance and deferral for each criterion. They are meant to ensure the safety of the donor and the recipient while maintaining the quality of the product.
- The minimum Hb required for whole blood donation is 12.5 g/dL or 38% haematocrit for both males and females
- Additive solutions enhance the shelf life of RBCs to 42 days.
- Implementation of quality management in BTS is essential for donor recruitment, selection, blood collection, staff training and documentation to ensure quality in blood.
- Trained blood centre staff plays a vital role in providing professional efficiency in donor recruitment, donor selection, donor counselling, blood collection, donor care to enhance donor confidence and donor retention.
- Donor haemovigilance is a continuous process of data collection and analysis of adverse donor events and reactions before, during or after whole blood or apheresis donations.

References

1. Global Database on Blood Safety. Summary Report, WHO, 1998-1999. Available from: https://www.who.int/bloodsafety/global_database/SumRepGDBS_En1998-1999.pdf?ua=1, accessed on 31st August 2020
2. National Estimation of Blood Requirement in India. National AIDS Control Organization (NACO), India; U.S. Centres for Disease Control and Prevention (CDC), New Delhi; National Institute of Medical Statistics, India; Christian Medical College, Vellore and Christian Medical Association of India (CMAI), India. 2018. Available from http://naco.gov.in/sites/default/files/Final%20Estimation%20Report%20of%20Blood%20Requirement%20in%20India%20%281%29.pdf, accessed on 31st August 2020
3. Voluntary Blood Donation Programme- An Operational Guide. [Internet] National AIDS Control Organization (NACO), Ministry of Health and Family Welfare, Government of India. 2007. Available from: http://www.naco.gov.in/sites/default/files/voluntary%20blood%20donation.pdf accessed on 31st August 2020.
4. Piliavin, J. A., Callero, P. L., & Evans, D. E. Addiction to altruism? Opponent-process theory and habitual blood donation. Journal of Personality and Social Psychology 1982;43: 1200–13.
5. Moog R, Fourné K. Recruitment of prospective donors: what do they expect from a homepage of a blood transfusion service? Transfus Med 2007; 17:279-84.
6. Sumnig A, Feig M, Greinacher A, Thiele T. The role of social media for blood donor motivation and recruitment. Transfusion 2018; 58; 2257-59.
7. Meyer DG, Strickland D, Maloley PA, Seburg JK, Wilson JE, McManus BF. Possible association of a reduction in cardiovascular events with blood donation. Heart 1997;78:188-193.

8. Salonen JT, Tomainen TP, Salonen R, Lakka TA, Nyyssonen K. Donation of blood associated with reduced risk of myocardial infarction. The Kuopoi Ischemic Heart Disease Study. Am J Epidemiol 1998; 148: 445-51.
9. Sullivan JL. Iron and the sex difference in heart disease risk. Lancet 1981; 1:1293-4.
10. Miller M, Hutchins GMM. Hemochromatosis, multiorgan hemosiderosis and coronary artery disease. JAMA 1994; 272: 231-3.
11. Blood Donor Counselling: Implementation Guidelines. World Health Organization 2014.
12. Basavaraju SV, Mwangi J, Nyamongo J, Zeh C, Kimani D, Shiraishi RW et al. Reduced risk of transfusion-transmitted HIV in Kenya through centrally coordinated blood centres, stringent donor selection and effective p24 antigen-HIV antibody screening. Vox Sang 2010; 99: 212–9.
13. Reiss RF. Blood donor well-being: a primary responsibility of blood collection agencies. Ann Clin Lab Sci.2011; 41: 3–7.
14. Guo N, Wang J, Ness P, Yao F, Dong X, Bi X, et al. for the NHLBI Retrovirus Epidemiology Donor Study-II (REDSII), International Component. Analysis of Chinese donors' return behavior. Transfusion 2011; 51: 523–30.
15. Goncalez T, Sabino E, Sales N, Chen YH, Chamone D, Busch M et al. Human immunodeficiency virus test-seeking blood donors in a large blood bank in São Paulo, Brazil. Transfusion 2010; 50:1806–14.
16. Testing positive. Counselling blood donors. AIDS Action 1996; 34: 6.
17. Blood donor selection. Guidelines on assessing donor suitability for blood donation. Annex 3. Geneva, World Health Organization; 2012. Available athttp://www.who.int/bloodsafety/publications/bts_guideline1/en/index.html, accessed on 4th March2015.
18. Regulatory requirements of blood and/or its components including blood products. Central drugs standard control Organization, Director General of Health Services, Ministry of Health and Family Welfare, Government of India. Available at http://www. cdsco.nic.in, accessed on 2 March2015.
19. Rehman S, Arif SH, Mehdi G, Mirza S, Saeed N, Yusuf F. The evaluation of blood donor deferral causes: atertiary care centre-based study. J Blood Disorders Transf. 2012; 3: 131.
20. McDonald CP, Roy A, Mahajan P, Smith R, Charlett A, Barbara JA. Relative values of the interventions of diversion and improved donor-arm disinfection to reduce the bacterial risk from blood transfusion. Vox Sang. 2004; 86: 178-82.
21. Lee CK, Wong HK, Ho PL, Tsoi WC, Lee KY, Tsui GT et al. Significant bacterial contamination risk reduction with the use of diversion pouch. Transfus Med. 2012; 22: 404-8.
22. Sümnig A, Konerding U, Kohlmann T, Greinacher A. Factors influencing confidential unit exclusions in blood donors. Vox Sang 2010; 98: e231-40.
23. Goldman M, Land K, Robillard P, Wiersum-Osselton J. Development of standard definitions for surveillance of complications related to blood donation. Vox Sang. 2016; 110:185-8.

Section 4



Blood Component Preparation and Storage

1. Introduction

Blood component therapy is an integral part of modern-day healthcare practice. The introduction of plastic blood bags, satellite bags, and hermetically sealed integrally attached tubing helped the transfusion services to move from whole blood towards blood component therapy.

2. Type of blood components

Various blood components available are listed in Table1.

Table 1: Various blood components available

Cellular blood components	Acellular blood components
➢ Packed red blood cells ➢ Packed red blood cells in additive solutions ➢ Modified packed red blood cells • Saline washed red cells • Leucodepleted red cells • Irradiated red cells • Frozen packed red blood cells • Packed red cell aliquot	➢ Fresh frozen plasma (FFP) ➢ Concentrate of anti-haemophiliac factor ➢ Cryo poor plasma ➢ Liquid plasma ➢ Thawed plasma ➢ Recovered plasma
➢ Random donor platelet concentrates ➢ Pooled platelets concentrate ➢ Modified platelets concentrate • Leucodepleted platelet concentrate • Irradiated platelets concentrate • Washed platelet concentrate • Platelets suspended in additive solution • Cryo preserved platelet concentrate ➢ Single donor apheresis platelets	
➢ Granulocyte concentrates • Pooled buffy coat derived • Apheresis derived	

3. Advantages of blood component preparation

The advantages of blood component therapy over whole blood transfusion are:

- Rational use of the scarce resource as more than one patient can be benefitted from a single donation
- Transfusion of the desired component
- Improved quality and functional capacity of each blood component
- Optimization of storage of blood components by appropriate choice of additive solution, temperature and type of bag
- Reduced risk of transfusion reactions

4. Whole blood collection for component preparation

Blood should be collected from blood donors who meet the eligibility criteria as per Drugs and Cosmetics Act 1940 & Rules, 1945 (DCA) and amendments thereafter in March 2020.[1] Whole blood should be collected into an approved container with a single clean non-traumatic venepuncture that allows rapid flow. Blood should be periodically mixed with the anticoagulant during collection either manually or using blood collection monitors. The average draw time should vary between 5-10 minutes. However, the unit collected with a draw time beyond 10 and 12 minutes is not suitable for preparing platelet concentrates from 350 and 450 ml blood bags, respectively, and if the collection time exceeds 13 and 15 minutes it is not suitable for preparing FFP from 350 and 450 ml of blood bags respectively.[2]

4.1. Volume of blood units for component preparation

The ideal volume of blood units for component preparation should be 350±10% (315-385 ml) and 450±10% (405-495 ml) for 350 ml and 450 ml blood bags, respectively, to ensure the correct anticoagulant to whole blood ratio. Blood units containing blood volume beyond 10% of the specified limit should be discarded as over-collected units. However, PRBCs can be prepared from blood bags containing 66-89% of 350/450 ml collected blood (Table 2). These PRBCs are labelled as low volume units and can be used for transfusion, while platelets and plasma from such units should be discarded as these components will have high citrate concentration.[1]

Table 2: Volume of blood units to be collected

	350 ml blood bag	450 ml blood bag
Ideal volume (total volume ± 10%)	315-385 ml	405-495 ml
Low volume (up to 35% lower than total vol.)	233-314 ml	300-404 ml
Under collected (>35% lower than total vol.)	<233 ml	<299 ml
Over collected (> 10% of total vol.)	>385 ml	>495 ml

5. Blood component preparation and processing

5.1 Whole blood handling before component processing

Whole blood collected either in an outdoor blood donation camp or indoor blood donation complex should be transported to the component preparation laboratory as soon as possible after collection. Platelet concentrates, and FFP should be separated within 6 hours of collection of the whole blood unit.[3] However, The Council of Europe guidelines recommend that for platelet preparation, whole blood (WB) units that are rapidly cooled to room temperature (RT) can be held before processing for up to 24 hours in conditions validated to maintain a temperature between 20- 24ºC.[2]

Requirements for cooling and transportation of whole blood depend upon the blood components to be prepared. As per DCA, if platelets are to be prepared, blood units should be held and transported at temperatures between 20 to 24^{0}C. Special cooling plates containing 1,4-butanediol wax are available, which brings down the temperature of collected blood to 20^{0}C within 2 hours.[4] On the other hand, blood units subjected to fresh frozen plasma or cryoprecipitate preparation should be transported in blood transport containers with ice or gel packs maintaining the temperature below 10^{0}C. At no point during transport, ice should be allowed to come in direct contact with the blood as the red cells nearest to the ice may freeze and haemolyze. In case of long-distance outdoor blood donation camps or during high environmental temperatures, the quantity of ice should at least be equal to that of the blood.[5]The temperatures of blood transport containers should be monitored. Portable temperature monitors are available nowadays to record the temperature continuously during transport.

5.2 Differential centrifugation

The first step in the preparation of blood components (red cells, platelets, and plasma) is differential centrifugation of whole blood (WB), as these blood constituents differ in size and density and sediment at different rates when centrifugal force is applied. The red cells settle at the bottom of the blood bag owing to their highest density, followed by white blood cells, platelets leaving clear plasma plus anticoagulant at the top.[6](Table 3).

Table 3: Specific gravity of blood components[1]

Blood component	Specific gravity
Whole blood	1.053
Packed red cells without an additive solution	1.08
Packed red cells with additive solution	1.06
Platelet concentrates	1.03
Plasma	1.02

The refrigerated centrifuge used for the preparation of blood components should be regularly maintained and calibrated. The refrigerated centrifuge should be calibrated before initial use, after major repair or adjustment, and annually/every 6 months depending upon hospital policy. The critical variables that affect the separation of cells from WB by differential centrifugation are rotor size, centrifuge speed, duration of centrifugation, and acceleration/deceleration protocol. For a given centrifuge, the rotor size is fixed. Therefore, the other variables, i.e., centrifuge speed, duration of centrifugation and acceleration/ deceleration protocols, temperature can be altered in a stepwise fashion to achieve the optimal conditions for the preparation of blood components.[7]

5.3 Blood component separation

Following centrifugation, components must be carefully separated using either manual plasma expressors or automated/semi-automated extractor devices. Automated expression allows standardization and consistency in the preparation of blood components.

5.4 Labelling and storage of blood components

Each blood component prepared should be labelled as per DCA. The blood component must bear a unique identification number that can be traced back to the donor. Other minimum information like name, address, and license number of the blood centre, name of the product, date of collection, date of expiry, blood group, and volume of the product, along with results of the test for transfusion-transmitted infections, should be included in the component label. As per National AIDS Control Organization Standards for Blood Banks and Blood Transfusion Services 2007, the date of expiry of blood components is calculated considering the day of collection as day zero. Special information regarding the storage and use of blood components should also be mentioned on the labels. Blood components should be stored at temperatures as per the requirements. Failure to follow correct storage requirements may result in decreased transfusion efficacy and/or potential

harm to the patient.

6. Factors affecting the quality of blood components

The quality of the blood component depends on various factors enlisted in Table 4.

Table 4: Factors affecting the quality of blood components

Factors		Property affected
Donor Related Factors	Pre-donation platelet count Blood Group Medications Donor infection	Platelet yield Level of coagulation factors in cryoprecipitate Platelet function
Blood bag composition	Different plasticizers with different gaseous exchange properties	pH of platelets
Type of anticoagulant and additive solutions	Primary anticoagulant-preservative: CPD, CPDA-1 and additive solutions	Shelf life of red cell concentrates
Phlebotomy	• Venepuncture: clean atraumatic • Adequate blood flow withdrawal time: not more than 15 minutes • Adequate mixing of blood with anticoagulant	• Platelet count and level of coagulation factors
The volume of blood collected	Adequate volume of blood to be collected: to maintain the ideal ratio of anticoagulant to blood (1:7)	Ratio of anticoagulant to blood leading to clots in blood bag
Temporary storage and transport of whole blood before centrifugation	For platelet preparation, whole blood to be held at room temperature (20-24^{o}C) For FFP and Cryo preparation, whole blood to be held at 1-10^{o}C	• Platelet count • Levels of labile coagulation factors
Centrifugation requirements	Rotor size, temperature, duration of centrifugation, maximum g-force achieved, balancing of centrifuge cups, degree of braking	• The volume of all components • Haematocrit of RBCs • Platelet yield • Red cell contamination of platelet/ plasma components
Method of separation of cellular and plasma components	• Platelet-rich plasma method or buffy-coat method: Buffy-coat removal allows a reduction in leucocytes in cellular components. • Automation: decreases operator-dependent variability and prevents contamination of plasma and platelets by red cells.	• Leucocyte content in cellular components • Consistent, high-quality components with higher yields
Storage conditions and temperature	• Blast freezing of plasma • Continuous gentle agitation of platelet concentrates during storage	• Recovery of coagulation Factor VIII • pH and quality of platelets

7. Blood components and modifications

7.1. Whole blood

Whole blood (WB) is the source material for blood component preparation. WB is rarely used for transfusion where a component separation facility is not available. Fresh WB may also be used to resuscitate severe traumatic haemorrhage when platelets are not available in a military setting.[8] WB units collected in blood bags containing anticoagulant Citrate Phosphate Dextrose (CPD) have a shelf life of 21 days when stored at 2-6^{0}C while in Citrate Phosphate Dextrose Adenine (CPDA-1) solution, the shelf life is 35 days. WB should have a minimum haematocrit between 30-40%. Labile coagulation factors deteriorate rapidly, and platelets also get activated, lose functionality and viability during WB storage at 2-6^{o}C.[6]

7.2. Packed red blood cells

Packed red blood cells (PRBCs) are prepared by separating plasma from centrifuged whole blood. PRBCs in anticoagulant preservative CPD have a shelf life of 21 days, while in CPDA-1 solution, the shelf life of PRBCs is 35 days when stored at 2-6^{0}C. PRBCs should have a haematocrit between 65-70%.[1]

Total haemoglobin (Hb) content of the PRBC unit is affected by blood donor's pre-donation haemoglobin and haemoglobin loss during processing such as in the buffy coat preparation method, and leucodepletion.[9] There are no recommendations regarding the total haemoglobin content of PRBC in India or in the United States. However, the European Union has set a lower limit of 45 g per unit for PRBCs.[10]

7.2.1. Buffy coat reduced AS red blood cells

Buffy coat (BC) reduced RBCs suspended in additive solution (AS) are prepared from either top and top or top and bottom quadruple blood bags. These blood bags consist of a primary collection bag containing an anticoagulant preservative, either CPD or CP2D, with three integrally attached satellite bags with one containing an AS. AS from different suppliers may contain sodium chloride, adenine, glucose, mannitol, carbonate, citrate, phosphate, or guanosine in varying combinations and amounts.[1]AS can be referred to by their brand names (e.g. AS-1 or Adsol, AS-3 or Nutricel, AS-5 or Optisol, AS-7 or SOLX) or simply by their combination of constituents (e.g. SAGM). The volume of AS varies depending upon the volume of whole blood collected from a blood donor, e.g.,100 ml AS for 450 ml collection bag and 80 ml for 350 ml bag.

BC reduced RBCs are derived by removing plasma and BC following centrifugation of whole blood with the subsequent resuspension of the red cells in an additive solution. Removal of BC reduces the leucocyte content of the RBCs to less than 5 X 10^8 (1 Log reduction), thereby reducing febrile reactions. Moreover, removing the WBCs also improves RBC storage by removing a high energy requirement cell population.[11]The addition of AS increases the shelf life of RBCs to 42 days. The minimum haematocrit requirement for AS RBCs as per national regulatory authorities is 50-60%.[1] Lower haematocrit facilitates good infusion flow rates and easy administration.

7.2.2. Leucoreduced RBCs

Leucoreduced RBCs are red cell components from which WBCs are removed through various methods. Leucoreduced RBCs prevent febrile non-haemolytic transfusion reactions (FNHTR), human leucocyte antigen (HLA) alloimmunization in multi-transfused patients, and transmission of leucotropic viruses, especially cytomegalovirus (CMV). The Indian DCA and United State Federal Drug Association (US-FDA) recommend leucocyte content in leucoreduced RBC should be less than 5x10^6/unit.[1,3] However, the Council of Europe recommends that the residual leucocyte count should be less than 1 X 10^6/unit.[2] Leucoreduced RBCs can be prepared by several methods depending on the available resources and requirements of the patients. Leucoreduction using filters is the preferred method and can be performed either pre-storage, post-storage before the issue, or at the bedside.

7.2.2.1. Pre-storage leucoreduction

Pre-storage leucoreduction is generally performed soon after or within five days of whole blood collection using either inline filter blood bags or additional filters attached to the tubing using a sterile connecting device.[1] Inline leucocyte filtration can be performed either on whole blood before component preparation, or RBCs after removing the plasma and buffy coat**.** Whole blood filtration is not in use commonly because of the chances of platelet loss. Leucoreduced RBCs should contain at least 85% of the original red cell content as per US FDA.[3]

However, the Council of Europe standards require a minimum of 40 g of haemoglobin to be present in each leucoreduced unit.[2]

Advantages

- Pre-storage filtration removes leucocytes before the disintegration and release of cytokines into the plasma. These cytokines can cause immune modulation and transfusion reactions in the recipient.
- Leucoreduction improves in vivo 24 hr red cell recovery as it removes highly metabolically active cells. It also prevents red cell damage by enzymes released by leucocytes after fragmentation
- Good laboratory practice (GLP) can be applied, as the procedure is performed by trained personnel
- Quality control of product can be performed

7.2.2.2. Post-storage leucoreduction - before issuing from the blood centre

Post-storage filtration can be performed either before issuing from the blood centre or at the patient's bedside.

Advantages of post-storage-before issue leucofiltration

- Easy to standardize
- It can be easily incorporated into laboratory procedures

Disadvantage of post storage leucofiltration

- Cytokines that accumulate due to the disintegration of leucocytes during storage cannot be removed

7.2.2.3. Bedside filtration

Bedside filtration is achieved using a transfusion set with an inline leucocyte reduction filter.[6]

Disadvantages of bedside leucofiltration

- Quality control is not possible
- Good laboratory practices cannot be applied
- It has been associated with hypotensive reactions in patients taking angiotensin-converting enzyme (ACE) inhibitors
- Cytokines that accumulate due to the disintegration of leucocytes during storage cannot be removed

7.2.3. Saline washed red cells:

Saline washed red cells are prepared for patients with a history of severe allergic reactions to transfused blood products, and antibodies against immune globulin A (IgA) in an IgA-deficient recipient when IgA-deficient RBCs are not available.[12] Red cells are washed with 1-2 litre sterile normal saline (0.9% NaCl) by centrifugation at 4^0C. Automated cell washers are now available. Washing removes electrolytes and 99% of plasma proteins. Up to 20% of the red cell mass may also be lost depending on the protocol used for washing RBCs. Washing removes 85% of the leucocytes from PRBCs leading to 1-2 log reduction but not enough to prevent alloimmunization. Red cells washed in an open system should be used within 24 hours post washing due to the theoretically increased risk for bacterial contamination. However, RBCs washed in a closed system have an expiration time of 14 days.[13]

7.2.4. Irradiated red blood cells

Red blood cells are irradiated to prevent transfusion-associated graft versus host disease (TAGVHD). Irradiation leads to the inactivation of T lymphocytes and can be done either using gamma rays (from caesium 137/ cobalt 60) or X rays (from linear accelerators/standalone units). Standard radiation dose to the centre of the irradiation field may vary from 25 Gray (Gy) - 50 Gy, while a minimum of 15-25 Gy dose should be delivered to any portion of the blood component depending upon the regulatory authorities. Irradiation sensitive labels should be used to ensure the desired dose is delivered to the component.

Blood units can be irradiated up to the end of their storage shelf life as per US guidelines and up to 28 days post collection as per European guidelines.[2,3] Irradiation of RBCs leads to efflux of potassium from red cells leading to a two-fold rise in potassium levels. Therefore, irradiated RBCs can be stored for 28 days post-irradiation or till the original expiration date whichever is earlier as per US guidelines. However, as per European guidelines, irradiated RBCs may not be stored for longer than 14 days after irradiation or 28 days after collection.

7.2.5. Frozen/Cryopreserved RBCs

Red cells can be frozen and are stable for prolonged periods in a frozen state. Freezing of RBCs is mainly done to preserve units of blood with rare phenotypes or to build blood inventory for emergency use in disasters. Red cells should always be frozen with cryoprotective agents. Damage to RBCs can occur via two mechanisms if frozen without cryoprotective agents[6]:

- **Red cell dehydration:** When extracellular water freezes before intracellular water, red cells collapse due to an osmotic pressure difference created between red cells and their surroundings, resulting in the diffusion of intracellular water out of the red cells.
- **Intracellular ice:** When intracellular water freezes before extracellular water, red cells rupture owing to an increase in intracellular salt concentration drawing in more water and expanding the cell.

Glycerol is the most commonly used cryoprotective agent because of its cost and safety. Glycerol is a small molecule that can penetrate the red cell membrane. After entering the red cell, it provides an osmotic force that will stop water migration from the red cell due to extracellular ice formation. In addition, high glycerol concentration also prevents the formation of intracellular ice crystals, thereby preventing cell membrane damage. Glycerol solution must be added slowly with constant mixing to allow equilibration of solution and cells. Failure to equilibrate during addition can lead to red cell damage which is seen as excessive haemolysis and poor red cell recovery when the unit is thawed. Usually, large polyolefin plastic bags are used to freeze RBCs as they are less brittle and are less likely to break during transportation and storage than polyvinyl chloride (PVC) bags.

Glycerol is added in either low (20%) or high (40%) concentration to RBCs (Table 5) within 6 days of the collection after removing plasma and/or additive solution. In the low glycerol concentration method, glycerol is added at 20% concentration, and RBCs are rapidly cooled by plunge freezing in liquid nitrogen while high glycerol concentration uses glycerol of about 40% concentration, and slow cooling is done in -80ºC freezers. A high concentration of glycerol (40%) is the most commonly used method.[1]

Table 5. Differences in red cell cryopreservation methods

Parameters	High-Concentration Glycerol (40%)	Low-Concentration Glycerol (20%)
Glycerol concentration	40%	20%
Freezing rate	Slow	Rapid
Requirement of controlled freezing rate	No	Yes
Initial freezing temperature	–80ºC	-196ºC
Type of freezer	Mechanical	Liquid nitrogen
Maximum Storage temperature	–65ºC	–120ºC
Thawing and Refreezing	Possible	Critical
Storage Bag	Polyvinyl chloride, polyolefin	Polyolefin
Shipping	Dry ice	Liquid nitrogen
Special deglycerolizing equipment required	Yes	No
Deglycerolizing time	20-40 minutes	30 minutes

Frozen RBCs can be stored for 10 years. Rare frozen units may be used beyond the expiration date as literature reports satisfactory cell recovery and viability from units stored for up to 21 years. However, this has to be done only after medical review and approval based upon the patient's needs and availability of other rare compatible units and should be documented.

When frozen RBCs are to be recovered (deglycerolized), the unit should be thawed at 37°C with gentle agitation taking about 10 minutes for complete thawing. Glycerol must be removed gradually from thawed RBCs by washing with sterile saline solutions of decreasing osmolarity to avoid red cell haemolysis. The

cells are finally suspended in 0.9% sodium chloride and 0.2% dextrose solution. Addition and removal of glycerol (deglycerolization) is usually performed in an open system with the shelf life of thawed and deglycerolized RBCs of only 24 hours when stored at 1-6°C. Automated devices are also available nowadays for the addition and removal of glycerol from RBCs in a closed system. Thawed RBCs prepared in such a manner are suspended in Additive solution formula -3 (AS-3) and have a shelf life of 14 days at 4 °C.[14]Whatever technique is used, it should be properly validated, and the final product should be free of a cryoprotective agent with minimal signs of haemolysis and should yield at least 80% of the RBCs originally frozen.[6]

7.2.6. Packed red cells aliquots

Packed red cells can be aliquoted for small volume transfusions especially for paediatric patients. The advantage of using aliquoted units include limited donor exposure, decreased blood wastage, and prevention of circulatory overload. These aliquots can be prepared using either mulltiple pack systems or a sterile connecting device. In multiple pack units, whole blood is drawn in the primary bag which has integrally attached smaller bags. The plasma is separated and diverted to one of the bags while the remaining red cells are divided into the other smaller bags. The expiration date of the smaller are RBC units is the same as the original unit as a closed system has been maintained. If the blood centre has a sterile connecting device, there are many options by which aliquots can be prepared. e.g. by using transfer bags or tubing with integrally attached syringes. The expiration date of the aliquoted RBC unit here also is the same as the original unit as a closed system has been maintained.

7.3. Plasma

Plasma can be prepared either from whole blood collections following differential centrifugation or by apheresis technology using various automated cell separators (source plasma). Plasma preparations are defined based on differences in collection methods, secondary processing, timing and storage after thawing.

Blood collection time for whole blood donations intended to be processed for plasma should not exceed 13 to 15 minutes for 350- and 450-ml blood bags, respectively, as poor flow leads to the consumption of coagulation factors. Once collected, such whole blood units should be transported in ice or gel packs, maintaining a temperature below 10°C. Plasma must be frozen rapidly to maintain coagulation factor activity especially labile coagulation factors such as FVIII and FV, which deteriorate rapidly if stored at 2-6°C.[6]

Plasma contains proteins such as coagulation factors, albumin and immunoglobulins and is a starting material for the preparation of specific plasma derivatives.

7.3.1. Fresh frozen plasma

Fresh frozen plasma (FFP) is frozen within 6 hours after blood collection. Plasma must be rapidly frozen either using a snap freezer or deep freezer maintaining a temperature between minus 75°C to minus 80°C. Once frozen, FFP should be stored in a deep freezer, maintaining the temperature between minus 30°C to minus 40°C. Ideally, the time taken to freeze the plasma to a core temperature less than -30°C should not exceed 1 hour from the time freezing is commenced. Core temperature refers to the temperature in the centre of the unit – the warmest part of the plasma pack during the freezing process.[6]

FFP contains normal amounts of all coagulation factors, antithrombin, and ADAMTS13, and it has a shelf life of 1 year when stored at a temperature less than -30°C. PVC bags used for storing plasma have a glass transition temperature of about -25°C to -30°C. Hence, the containers should be handled with care to prevent breakage during handling and transport as they are brittle at such low temperatures.

As per US-FDA guidelines, plasma should be thawed at 37°C in a water bath or plasma thawing baths.[12] FFP bags should be placed in protective plastic overwraps when a water bath is used for thawing FFP. Once thawed, FFP can be stored for 24 hrs at 1-6°C.

To reduce the risk of transfusion-related acute lung injury (TRALI), it is recommended to prepare plasma from only male donors and avoid preparing from multiparous female donors.[15]

7.3.2. Pathogen inactivated plasma

Plasma can be treated to inactivate microbial agents by pathogen inactivation. Methods available for pathogen inactivation and approved by the Council of Europe (CE) include methylene blue, psoralen (amotosalen/

ultraviolet A), riboflavin, and solvent/detergent (SD). Of these, two methods - solvent/detergent (SD) and psoralen (amotosalen/ultraviolet A) have also been cleared by US-FDA for pathogen reduction of plasma.

Methylene blue (MB) is added to thawed FFP, followed by its activation using visible light. MB intercalates with nucleic acids or binds to lipids, and following activation results in nucleic acid strand breakage or lipid peroxidation, with subsequent modification of surrounding membrane proteins. Plasma is refrozen after the removal of MB-using the filter. MB treated plasma contains 15-20% less factor VIII and fibrinogen than untreated plasma.

Amotosalen can be added to plasma prepared from whole blood or by apheresis, followed by its activation using UV-A light. Treated plasma is frozen for storage after removing amotosalen using an adsorption device. Treatment with amotosalen/UV-A light does not affect the activity levels of coagulation and antithrombotic factors.

Single unit plasma prepared either from whole blood or using apheresis can be treated with riboflavin (vitamin B2), followed by UV light illumination (Mirasol system) for 6-10 minutes. Riboflavin is a naturally occurring vitamin and does not require removal. Hence, immediately after illumination, plasma can be released or frozen for storage. Coagulation and anticoagulation proteins are found to be well preserved in treated plasma.[16]

SD plasma is prepared using an industrial scale process rather than in a blood centre. SD plasma is prepared from a pool of plasma from many donors (630-1520) that is additionally tested for non-enveloped viruses – parvovirus B19 DNA and hepatitis E virus RNA. The plasma pool undergoes treatment with 1% tri-n-butyl phosphate and 1% Triton X-100. SD treatment inactivates lipid enveloped viruses by disrupting and destroying the lipid bilayer membranes required for cell adhesion and receptor binding to initiate an infection. Most coagulation factor levels are decreased by approximately 10% except for factor VIII levels which are reduced by 20%.[17]

7.3.4. Plasma frozen within 24 hours after phlebotomy

Plasma frozen within 24 hours of collection is labelled as plasma frozen within 24 hours (PF24) of phlebotomy. Once thawed, PF24 have a shelf life of 24 hrs at 1-6°C. Thawed PF24 held longer than 24 hours must be relabelled as Thawed Plasma and can be stored for additional 4 days at 1-6°C.

7.3.5. Plasma frozen within 24 hours after phlebotomy held at room temperature up to 24 hours after phlebotomy

Plasma collected either by manual or automated methods and held up for 24 hours after collection at room temperature before freezing is labelled as plasma frozen within 24 hours after phlebotomy held at room temperature up to 24 hours after phlebotomy (PF24RT24) by FDA. Once thawed, PF24RT24 also has a shelf life of 24 hrs at 1-6°C.

7.3.6. Liquid plasma

In the United States, liquid plasma can be separated from whole blood at any point of time during storage. It is stored at 1-6°C for up to 5 days beyond the whole blood's expiration date.

7.3.7. Thawed plasma

FFP that has been thawed and held at 1-6°C for >24 hours is relabelled as thawed plasma. Thawed Plasma can be stored for additional 4 days at 1-6°C. Decreased levels of labile coagulation factors - factor V (>60%) and factor VIII (>40%) have been observed in thawed plasma.[18] However, ADAMTS 13 levels have been found to be well maintained in the thawed plasma. Currently, thawed plasma is still not licensed by Indian DCA.

7.3.8. Cryoprecipitate

Cryoprecipitate or Cryoprecipitated antihaemophilic factor (AHF) is prepared from FFP stored at a temperature less than -30°C. It is a cold insoluble protein that precipitates when FFP is thawed at 1-6°C overnight in a refrigerator or circulating cryo bath. The precipitate is concentrated in the bottom of the plasma bag following hard spin centrifugation at a cold setting. The cryoprecipitate-poor ('cryo-poor') supernatant plasma is then

transferred into a satellite bag leaving only about 10-20 ml of plasma for resuspension of the cryoprecipitate. Cryoprecipitate is then refrozen within an hour of removal from the refrigerated centrifuge and should be stored at a temperature less than -30°C for a period of 1 year from the date of collection.

Cryoprecipitate contains factor VIII, factor XIII, von Willebrand factor (vWF), fibrinogen, and fibronectin. The Indian DCA requires that anti-haemophiliac activity (factor VIII) in the final product should not be less than 80 units per bag. Rapid freezing of FFP has been found to increase the factor VIII yield in cryoprecipitate.[19] Higher levels of factor VIII have been found in cryoprecipitate prepared from blood group A and B blood donors compared to O blood group blood donors (120 vs 80 per bag, respectively).[20]Combined amount of anti-A and anti-B antibodies present in cryoprecipitate is only 1.15% of the total amount of these antibodies in a single unit of plasma.[21]

Thawed cryoprecipitate should not be refrozen. Once thawed, a single unit or a pool of cryoprecipitate should be used within 6 hours if prepared as a closed system using a sterile connecting device and 4 hours if a pool is prepared using an open system. Approximately 10%, 20%, and 30% mean decline in factor VIII levels is seen at room temperature at 2, 4, and 6 hours respectively.[22]

Cryoprecipitate is indicated for the treatment of bleeding conditions such as disseminated intravascular coagulation (DIC). Cryoprecipitate contains concentrated factor VIII, hence, also used in Haemophilia A patients when factor VIII concentrates are not available. It can also be used as a source of factor XIII and vWF.

7.3.9. Cryoprecipitate poor plasma

Cryoprecipitate (cryo) poor plasma is a by-product of cryoprecipitate preparation. Once prepared, cryo poor plasma should be refrozen within an hour of removal from the refrigerated centrifuge and should be stored at a temperature less than -30°C for a period of 1 year from the date of collection.

Levels of factor VIII, factor XIII, and vWF activity are decreased in cryo poor plasma. However, the component contains normal levels of factor V (85%), factor I, factor VII, factor X, antiplasmin, antithrombin, protein C, and protein S.[23] The component still has a fibrinogen level of about 200 mg/dl.[24]

7.4. Platelet concentrates

Platelet concentrates (PC) can be prepared either from whole blood donations (random donor/recovered platelets, RDP) or they may be obtained by platelet apheresis from a single donor (single donor apheresis platelets, SDP) using automated cell separator machines.

Prerequisites for making a PC

- Apart from meeting all criteria required for whole blood donation, the donor should not have taken antiplatelet medications that irreversibly inhibit platelet function (72 hours for aspirin and 14 days for clopidogrel and ticlopidine).[25]
- Blood draw time should not exceed 10-12 minutes
- The temperature during transportation and storage of whole blood intended to make platelets should be between 20-24°C
- Platelet shall be separated within 6 hours after the time of collection of the unit of whole blood

Platelets from whole blood can be prepared by two methods

- Platelet rich plasma (PRP) method
- Buffy coat (BC) method

PRP method involves a soft spin followed by a hard spin, while in the BC method, PCs are prepared by a hard spin followed by a soft spin. BC method causes less platelet activation during processing as PCs are centrifuged against the cellular elements of whole blood compared to the PRP preparation method, where platelets are centrifuged against the platelet bag.

PCs are usually suspended in 50-70 ml of plasma. However, the volume of plasma for re-suspension of platelets is determined by the maintenance of pH of not less than 6 during the storage period. Once prepared,

PCs should be stored between 20-24ºC with continuous gentle agitation throughout storage for a period of 5 days.

7.4.1. Pooled platelet concentrates

Pooled platelet concentrates may be prepared by pooling of 6 units of ABO and Rh (D) blood group matched random donor platelets prepared either by buffy coat method or by platelet-rich plasma method in a closed system using specialized pooling sets. The expiration date of the pooled product is the shortest expiration date of the pooled units. Each pool should be labelled with total volume, ABO and Rh (D) type (Rh-positive unit in a pool of Rh-negative units is labelled as Rh-positive) and a number of units in the pool. A record of the unique donation numbers of each individual unit in a pool should be maintained. Pre-storage pooling of BC-PC can be preserved in platelet additive solution or in plasma from one of the individual units of the pool.[28] Automated instruments for pooling are also available nowadays.

7.4.2. Leucoreduced platelet concentrates

Inline filters or filters attached to the tubing by a sterile connecting device to remove leucocytes from whole blood and/or platelets are available. WB filters sparing platelets are also available. Ninety-five percent of leucoreduced PC should have less than $8.3x10^5$ leucocytes by AABB standards[27]while European standards require less than $0.2 x10^6$ leucocytes per unit of platelets from WB.[28]

7.4.3. Washed platelet concentrates

Washing of the PC is done to remove the plasma proteins. Indications for washing include severe allergic reactions to plasma containing components, antibodies to IgA in an IgA deficient recipient. One to two-litre of sterile saline may be required, and 33% of platelet yield is lost during washing.[12] The washed platelets should rest at room temperature without agitation between centrifugation and resuspension with saline. The shelf life of washed PC is 4 hours after the start of washing.

7.4.4. Irradiated platelet concentrates

Platelet concentrates can be irradiated to prevent transfusion-associated graft vs host disease (TA-GvHD). The irradiation dose is the same as for PRBCs. PC can be irradiated till their expiration date, and post-irradiation, the expiration date remains the same as the original date. PCs are not damaged by an irradiation dose as high as 50Gy.[29]

7.4.5. Platelet concentrates in additive solutions

The addition of platelet additive solution (PAS) containing acetate, potassium, magnesium, and phosphate would result in maximal plasma recovery and benefit clinically by decreasing allergic and febrile reactions after platelet transfusions. With 40-50% plasma carryover, a crystalloid solution would suffice to store platelets but with lower carryover, supplementation with sodium acetate or citrate is required. The addition of potassium and magnesium improves in vitro storage quality of platelets.

7.4.6. Platelet concentrates with volume reduction

Volume reduced platelets are prepared by centrifugation of platelet concentrates and reducing the final volume of platelet concentrates to between 35-40 ml with a high platelet yield. Platelet concentrate volume can be even reduced to 10-15 ml/unit just before transfusion. In vitro recovery rate of platelets and platelet properties have been found to be maintained with volume reduction.[30] Shelf life of volume reduced platelets is 4 hours if an open system is used. However, the shelf life has not been established for closed systems. Volume reduced platelets may be required to prevent cardiac overload and intrauterine transfusions and minimize ABO antibody infusions in ABO-incompatible platelet transfusions.

7.4.7. Pathogen reduced platelet concentrates

Platelet concentrates can also be treated to inactivate microbial agents by pathogen inactivation. Two photochemical methods (amotosalen and UVA light; riboflavin and UV light) and one light alone method (UV

light) are available for pathogen inactivation, which are Council of Europe (CE) marked in Europe. US FDA has recently cleared Amotosalen and UVA light for pathogen inactivation of apheresis platelets suspended in platelet additive solution. All these methods target the nucleic acid of viruses, bacteria, and parasites, preventing their replication. Although platelets do not contain genomic nucleic acid and intact mitochondrial DNA is not required for platelet storage, but still, small losses of platelet in vitro properties and function have been observed.

7.4.8. Apheresis platelets/single donor platelet concentrates (SDPC)

The main advantage of SDPC is an adult therapeutic dose of platelets obtained from a single donor and which limits the risks of transfusion-transmitted infections and alloimmunization to HLA antigens. Its use is limited by the high cost involved. Various apheresis instruments are now available in the market, which allows the collection of a large number of leucoreduced platelets from a single donor, providing a potent product with fewer donor exposures to the patient. Apheresis platelets can be volume reduced. Newer technology has resulted in higher yields in the product, which can then be split into multiple units. Platelet additive solutions may also be used, resulting in a lower amount of plasma with its benefits. For more details, refer to the section on Apheresis

7.5. Granulocytes

Granulocyte transfusion is required for neutropenic patients with infections not responding to antibiotics. Unlike other blood components, granulocytes can be prepared either by pooling multiple units of buffy coat or by apheresis technology. Collection by apheresis technology involves multiple steps, including screening and counselling donors, infectious disease and serology testing, and donor stimulation with steroids alone or with Granulocyte-Colony Stimulating Factor (G-CSF). The donor has to undergo an approximately 2-hour procedure where citrate is used as an anticoagulant. At the same time, the preparation of the final product requires a sedimentation agent such as hydroxyethyl starch to facilitate the separation between red blood cells and granulocytes.

On the other hand, buffy coat product is derived from the buffy coat layer between red cells and plasma in centrifuged whole blood donations. Granulocytes must be irradiated and can be stored at 20-24ºC without agitation and used within a maximum period of 24 hours. However, they should be administered as soon as possible after collection as they rapidly undergo apoptosis. The density of granulocytes is only slightly lower than that of red blood cells. The separation between granulocyte and red blood cell layers is quite difficult, and granulocyte collection is often contaminated with red blood cells. Hence the unit should be ABO compatible with the recipient's plasma. Microaggregate filters or leucocyte reduction filters are contraindicated while transfusing granulocytes as they remove the collected granulocytes.

8. Automation of blood component preparation

Automation of blood components from whole blood is widely implemented. Automated processing decreases operator-dependent variability, controls the volume to be collected from the main blood bag into the satellite bag, and prevents contamination of plasma and platelets by red cells. Thus, it produces consistent, high-quality products with higher yields. It helps to optimize workflow and reinforce Good manufacturing practices. Automation in blood component processing became possible in the 1980s with the development of first-generation devices i.e., semiautomated component extractors. These devices use optical sensors that determine the layers of centrifuged whole blood. The different components are expressed and transferred to satellite bags by automated clamping and sealing off tubes between the main bag and the satellite bag. Subsequently, second-generation instruments were available, which led to the automation of pooling of buffy coat platelets, centrifugation, and filtration, leading to the production of leucoreduced platelet concentrate. Nowadays, third-generation automated devices are available, which automate and integrate all manual steps from start to finish of whole blood processing, i.e., balancing, centrifugation, expression, sealing, and volume and platelet content determination.

Key points

- For component preparation, blood should be periodically mixed with the anticoagulant during collection either manually or using blood collection monitors. The required volume should be collected within 5-10 minutes.
- The ideal volume of blood units for component preparation should be 350±10% (315-385 ml) and 450±10% (405-495 ml) for 350 ml and 450 ml blood bags, respectively, to ensure correct anticoagulant to whole blood ratio.
- The blood components should be separated within 6 hours of collection of the whole blood unit.
- The blood components (red cells, platelets, and plasma) are prepared by differential centrifugation of whole blood (WB). The blood constituents are different in size and density, and sediment at different rates when centrifugal force is applied.
- The critical variables that affect the recovery of cells from whole blood by differential centrifugation are rotor size, centrifuge speed, duration of centrifugation, and acceleration/deceleration protocol.
- PRBCs should have a haematocrit between 65-70% and can be stored for 21days or 35 days in CPD and CPDA1 solutions, respectively.
- Buffy coat reduced Additive solution RBCs have a shelf life of 42 days and should have a minimum haematocrit of 50-60% as per national regulatory authorities.
- Red blood cells are irradiated to prevent transfusion-associated graft versus host disease (TAGVHD). Irradiated RBCs must be given a radiation dose of at least 25 Gy to the centre of the irradiation field and 15 Gy to any portion of the blood component.
- Blood units can be irradiated up to the end of their storage shelf life as per US guidelines and up to 28 days post collection as per European guidelines. The expiration date of irradiated RBCs changes to 28 days from irradiation or maintains the original outdate, whichever comes earlier as per US guidelines. However, as per European guidelines, irradiated RBCs may not be stored for longer than 14 days after irradiation or 28 days after collection.
- Leucoreduced RBCs should have absolute leucocyte content less than $5x10^6$. Leucoreduction prevents alloimmunization in multi-transfused patients and transmission of leucotropic viruses, especially cytomegalovirus (CMV).
- Cryopreservation of red cells is done in cases of red cells with rare phenotypes and to build blood inventory for emergency use in disasters. Glycerol is the most commonly used cryoprotective agent. Cryopreserved RBCs must be stored at a temperature less than -65°C, usually for 10 years.
- FFP must be prepared within 6 hours of blood collection and has a shelf life of 1 year when stored at a temperature less than -30°C
- Cryoprecipitate or Cryoprecipitated antihaemophilic factor (AHF) is prepared from FFP stored at a temperature less than -30°C.
- Cryoprecipitate contains at least 80 units of anti-haemophilic factor VIII, factor XIII, von Willebrand factor (vWF),150 to 250 mg of fibrinogen and fibronectin.
- Random-donor platelets must contain at least 5.5×10^{10} platelets; single-donor platelets must contain at least 3×10^{11} platelets; each carries a shelf-life of 5 days.

References

1. Drugs and Cosmetic Act 1940& Rule 1945, amended on 18th March 2020 (accessed on 12/07/2020) https://cdsco.gov.in/opencms/opencms/en/Notifications/Gazette-Notifications/
2. The Guide to the preparation, use and quality assurance of blood components. European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM), 19th Edition (2017), Council of Europe publishing, Strasbourg, France.

3. Dumont LJ, Papari M, Aronson CA, Dumont DF. Whole-Blood Collection and Component Processing. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff C, editors. AABB Technical Manual. 18th ed. Bethesda MD: AABB Press; 2017. p. 135-66
4. Food and Drug Administration. Guidance for industry: Recommendations for collecting Red Blood Cells by automated apheresis methods-technical correction February 2001. (February 13, 2001) Silver Spring, MD: CBER Office of Communication, Outreach, and Development, 2012. [Available at http:// www.fda.gov/ Biologics Blood Vaccines/ Guidance Compliance Regulatory Information/ Guidances/ Blood/ uvcm076756.htm (accessed November 12, 20…)
5. Storage and transportation of blood and blood components. World Health Organization Manual on the management, maintenance and use of blood cold chain equipment, (Head of title Safe blood and blood products) WHO Geneva pg. 5-14.
6. Hardwick J. Blood processing. ISBT Science Series 2008; 3: 148-176.
7. Reiss RF, Katz AJ. Optimizing recovery of platelets in platelet-rich plasma by the Simplex strategy. Transfusion 1976; 16: 370-4.
8. Spinella PC, Cap AP. Whole Blood: back to the future. Current Opinion Haematology 2016; 23: 536-42.
9. Agnihotri N, Pal L, Thakur M, Kumar P. The need to label red blood cell units with their Haemoglobin content: a single centre study on Haemoglobin variations due to donor related factors. Blood Transfus 2014; 12: 520-6
10. European Union. Commission Directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/ EC of the European Parliament and of the council as regards certain technical requirements for blood and blood components 30.3.2001. UR-Lex 2004; 91; 25-39. [Available at http;//eur-lex.europa.eu/lexUriServ/ LexUriServ.do? uri=OJ: L: 2004: 091: 0025: 0039: EN: pdf).
11. Heaton WA, Holme S, Smith K, Brecher ME, Pineda A, AuBuchon JP, et al. Effects of 3–5 log10 pre-storage leucocyte depletion on red cell storage and metabolism. British Journal of Haematology 1994;87: 363–8.
12. Dunbar NM. Hospital storage, monitoring, pretransfusion processing, distribution and inventory management of blood components. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff C, editors. AABB Technical Manual. 18th ed. Bethesda MD: AABB Press; 2017. p. 135-66
13. Schmidt A, Reffaai M, Kirkley S, Blumberg N. Proven and potential clinical benefits of washing red blood cells before transfusion: current perspectives. *Int. J. Clin*. Transfus. Med;4:79-88.
14. Valeri CR, Ragno G, Pivacek LE, Srey R, Hess JR, Lippert LE, et al. A multicentre study of in vitro and in vivo values in human RBCs frozen with 40-percent (wt/vol) glycerol and stored after deglycerolization for 15 days at 40C in As-3: Assessment of RBC processing in the ACP 215. Transfusion 2001; 41: 933-9.
15. AABB. TRALI risk mitigation for plasma and whole blood for transfusion. Association Bulletin#14-02. Bethesda, MD, AABB, 2014.
16. Rock G. A comparison of methods of pathogen inactivation of FFP. Vox Sang 2011; 100: 169-78.
17. Sharma AD, Sreeram G, erb T, Grocott HP. Solvent-detergent-treated fresh frozen plasma: A superior alternative to standard fresh frozen plasma? Journal of Cardiothoracic Vascular Anaesthesia 2000; 14: 712-17.
18. Noordin SS, Karim FA, Mohammad WMZBW, Hussein AR. Coagulation Factor Activities Changes Over 5 Days in Thawed Fresh Frozen Plasma Stored at Different Initial Storage Temperatures. Indian J Haematol Blood Transfus. 2018 Jul;34(3):510-516
19. Farrugia A, Prowse C. Studies on the procurement of blood coagulation factor VIII: Effects of plasma freezing rate and storage conditions on cryoprecipitate quality. Journal of Clinical Pathology 1985; 122: 686-92.
20. Hoffman M, Koepke JA, Widmann FK. Fibrinogen content of low volume cryoprecipitate. Transfusion 1987; 27: 356-8.

21. Smith JK, Bowell PJ, Bidwell E, Gunson HH. Anti A Haemagglutinins in factor VIII concentrates. Journal of Clinical Pathology 1980; 33: 954-7.
22. Pesquera-Lepatan LM, Hernandaz FG, Lim RD, Chua MN. Thawed cryoprecipitate stored for 6 h at room temperature: A potential alternative to factor VIII concentrate for continuous infusion. Haemophilia 2004; 10: 684-8.
23. Yarraton H, Lawrie AS, Mackie IJ, Pinkoski L, Corash L, Machin SJ. Coagulation factor levels in cryosupernatant prepared from plasma treated with amotosalen hydrochloride (S-59) and ultraviolet A light. Transfusion 2005; 45: 1453-8.
24. SmakGregoor PJH, Harvey MS, Briet E, Brand A. Coagulation parameters of CPD fresh frozen plasma and CPD cryoprecipitate poor plasma after storage at 4 C for 28 days. Transfusion 1999; 33: 735-8.
25. Wagner SJ. Whole Blood and Apheresis Collection for Blood Components Intended for Transfusion. In: Fung MK, Eder A, Spitalnik S, Westhoff C, editors. AABB Technical Manual. 19th ed. Bethesda MD: AABB Press; 2017. p. 125-60.
26. Van der Meer P, de Korte D. The buffy-coat method. In; Blajchman M, Cid J, Lozano M, editors. Blood component preparation from benchtop to bedside. Bethesda, MD: AABB Press 2011. p. 55-81.
27. Levitt J, editor. Standards for blood banks and transfusion services. 29th ed. Bethesda, MD: AABB, 2014.
28. European Directorate for the Quality of Medicines and HealthCare. Guide to the preparation, use and quality assurance of blood components. 17th ed. Strasburg, France: Council of Europe Publishing, 2013.
29. Chapman J, Finney RD, Forman K, Kelsey P, Knowles SM, Napier JAF, et al. Guidelines on gamma irradiation of blood components for the prevention of transfusion associated graft-versus-host disease. Transfusion Medicine 1996; 6: 261-71.
30. Moroff G, Friedman A, Robkin-Kline L, Gautier G, Luban NL. Reduction of the volume of stored platelet concentrates for use in neonatal patients. Transfusion 1984; 24: 144-6.

Section 5

Transfusion Transmissible Infections

1. Introduction

Transfusion transmitted infections (TTI) are still considered serious adverse reactions of transfusion despite a decrease in the rate of post-transfusion infections from 30% in the 1960s to only 3 infections in 2018 Serious Hazards of Transfusion (SHOT) report of United Kingdom.[1] This decrease in incidence might be due to improved donor education, donor screening criteria, testing facilities to reduce window period etc. But still, there are many emerging pathogens that are a potential risk to transfusion recipients.

A pathogen should have the following characteristics for labelling as a transfusion transmissible infectious agent.

1. It should be asymptomatic or cause subacute infection in the donor.
2. It should have a long incubation period for the development of symptoms.
3. It should be able to live, persist or recur in the donor and recipient population.
4. It should be stable in stored blood and components at various temperatures.
5. It can have a latent phase via microbial nucleic acid incorporation into the blood cells of the infected host. The microbial nucleic acid can reactivate to initiate infection in the recipient when transfused with blood from an infected donor.

The common pathogens transmitted through transfusion of blood or blood components are shown in Table 1.

Table 1: Common pathogens which can be transmitted through transfusion of blood or blood components

S. No.	Pathogens	
1.	Viruses	Human Immuno-deficiency Virus 1 & 2 (HIV 1& 2)
		Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Hepatitis G virus
		Human T-cell lymphotropic viruses Types I/II (HTLV-I/II), Torque teno virus, Dengue virus, Chikungunya virus, Ebola virus, Parvo virus, Influenza, West Nile virus, Zika virus, Nipah virus, Lymphatic choriomeningitis virus (LCMV), Epstein Barr virus (EBV), Human Herpes viruses 6, 7 and 8 (HHV), etc.
2.	Parasites	Plasmodium, *Babesia microti*, *Trypanosoma cruzi*, Leishmania, Toxoplasma gondii
3.	Bacteria	*Treponema pallidum*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus cereus*, Streptococcus, *Propionibacterium acnes*
		Enterococcus faecalis, *Escherichia coli, Serratia, Enterobacter, Klebsiella, Yersinia enterocolitica, Morganella morganii*, Acinetobacter, Proteus, Pseudomonas

2. Transfusion transmitted viral infections

2.1. Retrovirus:

The four main retroviruses include human immunodeficiency viruses type 1 and 2 (HIV-1 and HIV-2) of the lentivirus group and human T-cell lymphotropic viruses (HTLV-I and HTLV-II) of oncona group.

2.1.1 Human immunodeficiency virus (HIV)

Introduction: HIV-1 was first isolated from the cells of an infected patient in 1983, and HIV-2 was identified in 1986. The virus was subsequently identified as the causative agent of Acquired Immuno-Deficiency Syndrome (AIDS). It comes under the Lentivirus group of the Retroviridae family.

Currently, HIV-1 is classified into four groups with a total of 12 known subtypes:

- Group M (major): subtype A-K
- Group O (outlier): subtype 1, 2
- Group N (new)
- Group P (pending the identification of further human cases)

Prevalence in India: The HIV 1 subtype C is the most commonly transmitted subtype in India and southern African countries. As per the National AIDS Control Organization (NACO) report 2017, the prevalence of HIV in the adult population and blood donors was 0.22% (0.16% – 0.30%) and 0.12%, respectively.[2]

Structure: HIV is a 100nm spherical shaped retrovirus consisting of an envelope of glycoproteins, core proteins and an inner core of viral RNA and reverse transcriptase. The outer envelope of HIV consists of a lipid bilayer with spikes of transmembrane gp41 (glycoproteins), which is loosely bound with gp120. Inside this envelope is a nucleocapsid matrix protein (p17), which surrounds a central core of protein or core antigen, p24. Within this core are two copies of the ss RNA, proteins p7 and p9, and a reverse transcriptase (RT) enzyme.

The genome of HIV: HIV has a total of nine genes, of which three are structural genes *gag* (group-specific core antigens), *pol* (polymerase and integrase enzymes) and *env* (envelope), two regulatory genes *tat* and *rev*, and four are accessory genes named *vif*, *vpu, nef* and *vpr*. Unlike HIV 1, HIV 2 lack *vpu* but have alternative gene *vpx*.

Modes of transmission: HIV has been isolated from most of the body fluids like blood, semen, cervical secretions, cerebrospinal fluid, tears, saliva, urine, and breast milk. The modes of transmission of HIV are sexual contact, childbirth, breastfeeding, and parenteral exposure to blood. Higher risk is associated with men who had sex with other men, needle-sharing drug users, and recipients of blood transfusions.

Transfusion significance: A seropositive unit can transmit HIV infection at a 90% rate to recipients. In a unit of blood collected from asymptomatic and symptomatic donors, the estimated tissue culture infective dose of HIV is 1.5×10^4 and 1.75×10^6 respectively.[3] The virus is well preserved in refrigerated and frozen blood but washed, leucoreduced or cold-stored blood for several weeks diminish the amount of virus, reducing the likelihood of transfusion transmission.[4] Albumin, immunoglobulins, antithrombin III, hepatitis B vaccine, and plasma fractionated by the cold-ethanol process has not been associated with HIV infection.[5]

Clinical features: Common reported symptoms include flu-like manifestations with myalgias, skin rashes, headache, anorexia, and diarrhoea or associated with the presence of opportunistic infections like candidiasis, herpes zoster, cryptococcosis, and Pneumocystis jiroveci pneumonia and secondary cancers like Kaposi's sarcoma and non-Hodgkins lymphoma. Acquired immunodeficiency syndrome (AIDS) is the outcome of chronic HIV infection and consequent depletion of CD4 cells up to <200 cells/μL. The general natural history of HIV-2 infection is similar to that of HIV-1 infection. However, it is characterized by lower levels of plasma virus, a slower decline in the CD4 cell count, and a long asymptomatic period of chronic infection.

Window period: After getting the HIV infection, viral RNA is detected within 3-10 days on different platforms of nucleic acid amplification test (NAT). Around 95% of donors show the presence of p24 antigens within

1-8 weeks, which is detected using enzyme-linked immunosorbent assay (ELISA) and rapid testing. Seroconversion is seen within 4 weeks in 95% of the individuals. HIV antibodies can be detected by 20-28 days with 4th generation ELISA or rapid test, and more than 99% of individuals are completely positive for HIV antibodies by 3 months.

Leucoreduction efficacy: 2 to 6 log reduction of the HIV infected cells in donor blood by leucoreduction is reported.

Pathogen reduction efficacy: HIV is an enveloped virus that is destroyed by almost all pathogen reduction techniques.

Laboratory diagnosis

a. **Serological tests:** Mostly, serological tests are used for blood donor screening of HIV. Serological tests detect either viral antigen, antibody, or both.
   i. **Rapid testing:** The most commonly employed rapid anti-HIV tests are based on the principle of Immuno-concentration/dot blot immunoassay (vertical flow), Immunochromatographic (lateral flow), particle agglutination (e.g., gelatine or latex) and dipstick, and comb assay based on ELISA. NACO recommends the use of rapid test kits, which detect >99.5% of all HIV-infected individuals and have false-positive results in <2% of those who were tested. A study by Sudha et al found 99.5% sensitivity and 99.9% specificity of the Tri-dot rapid test.[6]
   ii. **Enzyme-linked immunosorbent assay (ELISA) testing:** ELISA is the gold standard test for TTI screening in India. It has more sensitivity and specificity compared to rapid test. The sensitivity and specificity increase with the generation of ELISA test kits, and fourth-generation ELISA is almost 100% sensitive and specific.
   iii. **Chemiluminescence or Electro-chemiluminescence Testing:** Similar to ELISA in principle, sensitivity and specificity except that the chromogenic substrate is replaced by a chemiluminescent compound that generates light during a chemical reaction. The advantage over ELISA testing is that it has a short turnover time and a facility for spot testing is present.
b. **Nucleic acid amplification test:** Polymerase chain reaction in mini-pool testing and transcription-mediated amplification tests in the individual donor (ID) testing is used for detecting viral RNA in HIV infection in India.
c. **Confirmatory testing [Western Blot (WB) test]**: In the WB test, the various HIV specific recombinant or synthetic antigens are adsorbed onto a nitrocellulose paper. WB is a highly specific conformational test. NACO is presently providing it at the National Reference Laboratory level for resolving indeterminate results.

2.1.2 Human T-cell lymphotropic viruses type I/II (HTLV-I/II)

Introduction: HTLV-I and HTLV-II are delta type retroviruses. HTLV infects various cells like T-cells, B cells, dendritic cells, and fibroblasts, etc. Once the RNA has been transcribed into DNA, it is integrated randomly into the host cell's genome.

Prevalence in India: India is considered a non-endemic zone for HTLV infections.

Structure: HTLV is an enveloped virus with a diameter of 80-100nm and contains two covalently bound genomic RNA strands. The virus has reverse transcriptase (RT; with associated RNAse H activity), integrase, protease enzymes, and capsid proteins.

Genome: These viruses have the essential structural genes *gag* (group antigen), *pol* (reverse transcriptase) and *env* (envelope) in addition to regulatory genes.

Mode of transmission: HTLV is mainly transmitted by blood transfusion, sexual contact, sharing of infected needles and breastfeeding.

Transfusion significance: Transmission of HTLV I and II is by cellular blood products mainly through non-filtered cellular blood components stored for less than three weeks. Transmission is not seen following

transfusion of cell-free plasma or plasma derivatives.

Window period: Infectious window period of transfusion-transmitted HTLV is around 51 days (range 36–72 days).

Leucoreduction efficacy: Leucofiltration of cellular blood products may reduce the viral load of HTLV-I by 2 to 6 logs.[7]

Pathogen reduction efficacy: HTLV I & II is destroyed by most of the pathogen reduction techniques.

Laboratory Diagnosis: In the acute phase, IgM antibodies were detected while IgG antibodies persisted for a longer period. Tests for HTLV antibodies are ELISA and gelatin particle assays. Viral genome can be detected by NAT testing. Confirmatory tests are western blot and radioimmunoprecipitation assays (RIPAs). In India, screening for HTLV I and II is not mandatory.

2.2. Hepatitis viruses

2.2.1. Hepatitis A virus (HAV)

Introduction: Hepatitis A virus (HAV) infection, previously known as infective hepatitis, account for approximately 40 % of all acute viral hepatitis worldwide. HAV is classified within the hepatovirus genus of the Picornaviridae family.

Structure: HAV is a non-enveloped and icosahedral-shaped virus of approximately 27-30 nm size. Due to the absence of protein shell, it is resistant to detergent, acid (pH 1), solvents (such as ether and chloroform), drying, and temperatures up to 60ºC. It can survive for months in fresh and saltwater.

Genome: Genetic material of HAV consists of single-stranded RNA of positive polarity packed in a protein shell. The single-stranded RNA genome is approximately 7500 nucleotides long and contains a single, long open reading frame. The encoded polyprotein includes structural proteins for the 27 to 28nm diameter capsid, non-structural proteins with protease or polymerase activities and other proteins with functions that have not been fully determined. There is only one serotype of the virus, but multiple genotypes exist.

Modes of transmission:

- The faeco-oral route is the major route of transmission. It may occur by direct (person-to-person) contact or indirectly through contaminated water, food, or milk.
- Parenteral transmission of HAV can occur via transfusion of blood and blood products or by skin penetration through contaminated needles.
- Sexual transmission of hepatitis A can occur mainly among homosexual men because of oral-anal contact.

Transfusion significance: HAV can also be transmitted through the parenteral route via transfusion of blood and blood products.

Incubation period: Average incubation period of 28 days (range 15 to 50 days) and signs or symptoms persist for less than 2 months. Acute hepatitis A is often self-limiting with a very short duration of viremia and no chronic carrier state.

Pathogen reduction efficacy: HAV virus is quite resistant to many pathogen inactivation procedures. As a result, outbreaks of HAV were reported in the 1990s following transfusion of clotting-factor concentrates treated with the solvent/detergent pathogen-reduction process. Thereafter, plasma for further manufacture is routinely screened for HAV RNA by pooled NAT.

Laboratory diagnosis: Most blood donors with an acute HAV infection are symptomatic, and testing for HAV in donors is not performed routinely. However, if screening for HAV infection is required in certain scenarios, it can be done by serological or NAT testing.

a. **Serological testing**: Testing for anti-HAV IgM and IgG antibodies can be performed by immunological assay like a rapid test, ELISA etc. IgM antibodies to HAV regularly appear after infection and persist for 6-12 months after infection (Figure 1). When anti-HAV IgM is used for blood donor screening, very few infectious donors will be identified. IgG antibodies also appear after a few days of infection. Both IgG and

IgM antibodies have neutralizing activity. IgG antibodies usually persist for life and provide immunity to subsequent infections.

b. NAT test: Testing of HAV RNA by NAT assays. NAT is the preferred method as HAV rapidly disappears from the blood when anti-HAV appears.

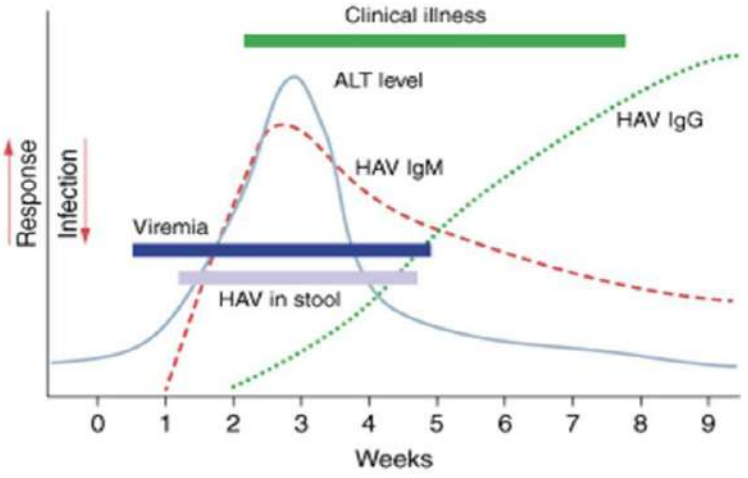


Figure 1: Expression of HAV antigen and antibodies and clinical illness

2.2.2. Hepatitis B virus (HBV)

Introduction: It is the causative agent of hepatitis B infection, resulting in both acute and chronic hepatitis infections. HBV infection, previously also known as serum hepatitis, is a major cause of post-transfusion hepatitis. Implementation of screening for HBsAg in blood components resulted in a significant decline in the incidence of post-transfusion hepatitis. HBV is a partially double-stranded DNA virus that belongs to the Hepadnaviridae family which is in the Orthohepadnavirus genus.

Structure: The infectious virion, also known as the Dane particle, is 42 nm in diameter, with an inner icosahedral nucleocapsid core of 28 nm size. The outer coat or the envelope comprises virus-encoded polypeptides, HBsAg (originally known as Australia antigen), and host-derived lipid components. The HBV envelope protein also occurs free in the serum as 22 nm spheres and 22 nm to 200 nm filaments. These spherical or tubular sub-viral particles are highly immunogenic yet non-infectious as they do not contain DNA. Additionally, the virion contains two more proteins associated with the capsid core of the virion; hepatitis B core antigen (HBcAg) and "e" antigen (HBeAg). The inner core of HBV contains the viral genome and the viral DNA polymerase. HBV can survive outside the body for at least 7 days. During this time, the virus can still cause infection. HBV virions can be inactivated following incubation with formaldehyde at a concentration of 1:2000 at 37°C for 72 hours, heat treatment at 98°C for 2 minutes or treatment with 2% glutaraldehyde at room temperature for 5 minutes.

Genome: The genome of HBV is circular, partially double-stranded DNA, with a complete negative DNA strand and an incomplete positive DNA strand. Based on the replication cycles, the DNA virus is close to the retroviridae, thus, sometimes also known as DNA retrovirus. The genomic information of the virus present on the long strand of the DNA comprises four highly overlapping (67%) open reading frames (referred to as S, C, P, and X genes), encoding the envelope (pre-S/S), core (pre-core/core), polymerase, and X proteins, respectively. HBV is mainly classified into eight genotypes (A to H). These HBV genotypes influence the clinical outcomes, seroconversion rates, mutational patterns in the pre-core, core promoter regions, and response to antiviral therapy.

Modes of transmission: Transmission of HBV is mainly by a parental route which involves direct contact with body fluids. The most common routes of infection are:

- Contact with infected blood, either by exposure of wounds to infected blood or to contaminated needles and syringes used in injecting drugs, tattooing, ear-piercing or acupuncture.

- Sexual contact
- Neonatal or perinatal transmission

Transfusion significance: HBV can be transmited through transfusion of infected blood or blood products.

Incubation period: The average incubation period for HBV infection is 60-90 days but it can range from 24-180 days.

Pathogen reduction efficacy: Pathogen reduction methods possibly have a role in reducing the risk of transfusion-transmitted HBV. The use of solvent/detergent treatment of pooled plasma is known to destroy lipid enveloped viruses, including HBV. Additionally, PUVA treatment has been shown to inactivate HBV in plasma and platelet components. However, its utility for red blood cells is still under evaluation.

Laboratory diagnosis

Serological testing:

- **HBsAg:** HBsAg can be identified in an infected donor's serum or plasma by EIA, chemiluminescence, radioimmunoassay or other immunological based assays. However, in countries where blood donor centres have limited resources, rapid and less expensive immunofiltration, latex-based or immunochromatographic methods are also used.
- **Anti-HBc testing**: It is used to detect some HBV infected, HBsAg negative donors [occult HBV infection (OBI)]. Anti-HBc testing is often not recommended due to high false positivity rates.[8] Nevertheless, in low prevalence countries, HBV DNA by NAT and anti-HBc testing may be superior to HBV DNA by NAT and HBsAg testing in a moderate or high prevalence zone.
- **Serological findings:** When HBV infects an individual, characteristic changes in the serum levels of hepatitis B antigens, antibodies and HBV DNA are seen, as mentioned in Table 2 and Figure 2.

Table 2: Changes in the level of HBV markers according to the clinical status.

S.No.	Markers	Clinical Status
1.	HBV DNA	Acute Infection
2.	HBsAg	Active infection, acute or chronic
3.	Anti-HBs	Clinical recovery, infection resolved, immunity develops
4.	Anti- HBc (IgM)	Early acute infection
5.	Anti- HBc (IgG)	Active or past infection (carrier state)
6.	HBeAg	Acute or serious chronic infection
7.	Anti- HBe	Resolution of acute infection may signal late sequelae

Occult hepatitis B infection: This subset is known as OBI, which is defined as being HBsAg negative with persistent HBV DNA detectable by PCR, in the presence (80%) or absence (20%) of anti-HBc with or without anti-HBs. Most of these patients have very low or only intermittently detectable serum HBV DNA levels, although HBV DNA is usually present in the liver. Donors with OBI may transmit HBV via blood transfusion, but the frequency of such infection is low and seems to be absent if anti-HBs is present.

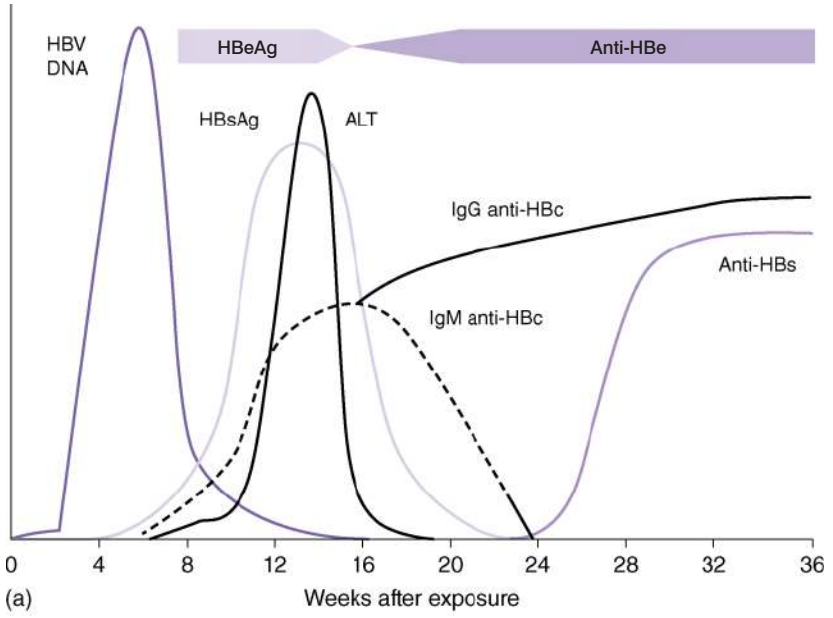


Figure 2: Expression of HBV antigens and antibody markers

NAT test: Detection of HBV DNA reduces the window period for detection of HBV infection. HBV DNA detection is performed by nucleic acid testing (NAT) by either mini-pool (6-16) or individual NAT testing.

Confirmatory testing: Confirmation can be done by neutralization testing.

2.2.3. Hepatitis C virus

Introduction: Hepatitis C is classified under the family *Flaviviridae* and the genus 'hepacivirus' virus.

Prevalence in India: The prevalence of HCV infection in India is 0.9 to 1.9%, which varies across different regions of the country. According to the NACO 2017 report, the prevalence of HCV in blood donors in India was 0.30%. HCV genotypes 3 and 1 are common in India, with approximately 60 % and 30%.

Structure: The diameter of the virus is around 40-70 mm. The virus is composed of a lipid envelope, two envelope proteins E1 and E2, and an icosahedral capsid containing a positive-sense, single-stranded RNA genome.

Genome: Six virus genotypes have been identified. Viral genome contains approximately 9500 bases coding for approximately 3000 amino acids. (Table 3)

Table 3: Hepatitis C virus proteins and their main functions

HCV proteins	Main known functions
Structural proteins	
Core	**Capsid protein**
E1	Envelope glycoprotein — the fusion of the virus with cellular membranes
E2	Envelope glycoprotein — attachment to the cell
Non-structural proteins	
p7	Formation of ion channel in endoplasmic reticulum membrane
NS2	Protease — cleavage at the NS2-3 site
NS3	Protease — cleavage at the NS3/4A, NS4A/B, NS4B/5A, and NS5A/B sites

HCV proteins	Main known functions
NS4A	Helicase — role in the viral RNA replication process
NS4B	Cofactor of NS3
NS5A	Formation of membranous structures essential for viral replication
NS5B	Regulation of viral replication RNA-dependent-RNA polymerase — replication of the hepatitis C virus genome

Modes of transmission: Hepatitis C is transmitted mainly by exposure to contaminated blood through transfusion, needle stick, haemodialysis, human bite, transplant, acupuncture, tattooing, body piercing and IV drug use. The virus is also known to be transmitted through sexual contact and from mother to infant perinatally.

Transfusion significance: HCV is mainly transmitted by blood components and incompletely inactivated plasma fractionation products including IVIG, anti-D Ig for intravenous use and factor VIII concentrate and has never been transmitted by albumin concentrates or anti-D for intramuscular use.[9, 10]

Clinical features: During the acute phase of HCV infection, the individual is usually asymptomatic or may experience mild symptoms such as fatigue, flu-like symptoms, dyspepsia, or jaundice for 2–12 weeks following infection. HCV RNA will be detectable 1–3 weeks after HCV infection, whereas seroconversion may take place up to 4–10 weeks after exposure.

Incubation period: The incubation period is 2 to 26 weeks. The average incubation period is 7 to 8 weeks, followed by seroconversion occurring in 8 to 9 weeks.

Leucoreduction efficacy: Not effective

Pathogen reduction efficacy: Pathogen inactivation can destroy viral lipid coat. All kinds of pathogen reduction technologies can result in 4-5 log reduction of viral load in platelets, plasma and red cells.

Laboratory diagnosis

Serology testing: The diagnosis of HCV infection is essentially based on detecting antibodies against the core protein and the non-structural proteins NS3 to NS5. This can be done by rapid test, ELISA, chemiluminescence test etc. In ELISA or chemiluminescence tests, the first-generation test detects only anti-HCV antibodies against the NS4 (c100-3) antigen, which has a window period of around 12 to 26 weeks. The second-generation test detects antibodies against two antigens, one core antigen and the second NS3 antigen. It reduces the window period up to 10 to 24 weeks. The third-generation test detects an additional antibody against NS5 antigen and further reduces the window period by 1 week. The recent fourth-generation test detects both HCV antigen and antibody, it increases the sensitivity and specificity of the test and the average window period is 26.8 days.

NAT test for HCV RNA: NAT testing reduced the window period to 4-6 days. Due to the high doubling time in the ramp-up phase of about 11 hours, the diagnostic window for HCV differs only about one to two days between blood donor screening in mini pool or individual NAT.

Occult hepatitis C infection: Castillo et al[11] defined occult hepatitis C virus (HCV) infection (OCI) as the presence of HCV RNA in hepatocytes or peripheral blood mononuclear cells (PBMCs) but not in the plasma/serum. The OCI can be divided into two types: seronegative OCI in which both anti-HCV antibodies and HCV RNA is absent in serum, and seropositive OCI in which anti-HCV antibodies are present in serum, but HCV RNA is absent. The proposed hypothesis of OCI is the mutation in the virus encapsulation capacity or the formation and release of virions into blood circulation, which leads to low levels of undetectable viremia.[12] Screening of blood donors for anti-HCV antibodies, HCV RNA, and even measuring aminotransferase concentrations will not exclude HCV infection because these enzyme concentrations can transiently normalise in some patients with occult HCV infection. So, the past history of HCV infection or jaundice is the only preventable strategy in blood donors.

2.2.4. Other Hepatitis Viruses:

Other hepatitis viruses like hepatitis D, hepatitis E, hepatitis G and Torque teno virus (TTV) complex is also reported to be transmitted by transfusion. Details of these viruses are mentioned in Table 4.

Table 4: Other hepatitis viruses and significance of their transmission by transfusion

SN	Name	Family	Structure	Genome	Mode of transmission	Transmission by transfusion	Incubation period	Laboratory testing
1	Hepatitis D virus (HDV)	Deltaviridae	36 ± 4 nm, Enveloped	ssRNA	HDV follows the same parenteral route of transmission of the helper HBV	Yes, with Hepatitis B	3-7 weeks	During the acute phase of infection HDV RNA and HDAg is detected. Anti-HDAg-IgM in acute infection and in chronic infections, both IgM and IgG type antibodies coexist. HDV RNA by NAT can be used in cases of acute HDV infection or in occult hepatitis B infections that are negative for HBsAg antigens.
2	Hepatitis E virus (HEV)	*Hepeviridae* Genus: *Hepevirus*	30-34 nm Non-enveloped	ssRNA. Positive sense	Faeco-oral route Vertical transmission from a pregnant woman to her fetus.	Yes	40 days (3-8 weeks)	Donor screening not recommended routinely, But it can be performed using anti-HEV IgM and IgG antibodies and NAT.
3	Hepatitis G virus/ GBV-C	*Flaviviridae* Genus: *Pegivirus*	50-100 nm Enveloped	ss RNA Positive sense,	Sexual transmission	Yes	2 days to 2 weeks	Donor screening is not recommended. But it can be screened for anti-E2 by ELISA or by detecting viral RNA using NAT.
4.	Torque Teno Virus (TTV) Complex	*Anelloviridae* Genus: *Alphatorquevirus*	18-50 nm Non-enveloped	ss DNA Negative sense	Spread by saliva droplets. Mother to the fetus or sexual routes.	Yes	4-7 days	No FDA-licensed blood donor screening test exists. Virus is detected by NAT.

2.3. Transfusion transmitted herpes virus infection

2.3.1. Cytomegalovirus

Introduction: Family: Herpesviridae Subfamily: Beta Herpesviridae; Genus cytomegalovirus or human herpesvirus 5.

Prevalence in India: CMV is found in all geographic locations, with a higher prevalence in developing countries and lower socioeconomic conditions. In India, almost 100% of blood donors are positive for IgG anti-CMV antibodies, while it is only 40% in developed countries like America, Europe, and Australia.

Structure: Cytomegalovirus (CMV) is a large 150 – 200nm sized virus covered by an icosahedral capsid envelop.

Genome: Consists of double-stranded DNA with approximately 230,000bp.

Modes of transmission: CMV is mainly transmitted from mother to fetus, sexual contact, transplantation, and blood transfusion. The target cells of CMV are monocytes and macrophages.

Transfusion significance: CMV has been isolated from the mononuclear and polymorphonuclear cells of patients with acute infections. The risk of CMV disease in recipients of infected blood transfusion is as per Table 5. CMV transmission can occur through all cellular blood components (whole blood, red blood cells, platelets, and granulocytes) except fresh frozen plasma and frozen deglycerolized red cells. As per Paloheimo et al,[13] fresh blood (containing many viable WBCs) may be more infectious than stored blood. CMV transmission rates depend on the number of transfusions, age of blood, time of year and immunocompetence of the recipient.[14]

Table 5: CMV-seronegative patient populations at risk of CMV disease following transfusion of CMV infected blood/blood components

Highest Risk	Allogeneic marrow transplant
	Fetuses receiving an intrauterine transfusion
Moderate Risk	Solid-organ transplant
	Patients who may need allogeneic or autologous bone marrow transplantation in the future
	A person with HIV infection.
Low Risk	Autologous marrow transplant
	Low birth weight neonates

Window period: 4-8 weeks

Clinical Features: The acute infection of the virus is an asymptomatic or mild mononucleosis-like syndrome. Severe infection of CMV causes neutropenia, cellular immunity depression, and inversion of T-cell ratios.

Leucoreduction efficacy: Previous studies reveal that leucoreduction by filtration may fail to prevent CMV transmission because 10^5 to 10^6 WBCs may still be transfused, and an estimated 1 in 1000 to 1 in 10 000 WBCs are infected by CMV during latency.[15]A study by Bowden et al[16] has shown that leucocyte removal is as safe as CMV-negative donors because the exact number of residual leucocytes for no risk of CMV transmission is unknown. Leucoreduced components can be termed as 'CMV-safe' in contrast to 'CMV-negative'. Cytokines released by WBC-containing cellular blood components during storage may cause reactivation of latent CMV in donor cells by triggering cellular differentiation that promotes viral reactivation. Pre-storage leucoreduction can remove both donor WBC and prevent accumulation of WBC-derived cytokines during storage, so pre-storage leucoreduction is more effective than post-storage.[14]

Pathogen reduction efficacy: CMV virus is destroyed by almost all types of pathogen reduction techniques.

Laboratory Diagnosis

Serological testing

a. CMV antibody test: Two types of CMV antibody tests are commercially available today:
   - Particle or latex agglutination assay: It has a sensitivity of 98% and specificity of 100% in comparison to ELISA.
   - Enzyme immunoassay: More widely used test in indirect and capture ELISA format.

b. Complement fixation test.

c. CMV p65 antigen detection: 'Gold standard' for the initiation of antiviral therapy.

NAT Test: PCR-NAT

3. **Transfusion transmitted parasite infections**

2.1 Malaria

Introduction: There are four different species of malarial parasites i.e., *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale,* and *Plasmodium malariae*, which transmit infection in humans. Order: Haemosporida; Family: Plasmodiidae.

Prevalence in India: India has the 3rd largest burden of malaria, and about 89% of the cases reported from the South-East Asian regions are from India.

Life cycle: This parasite requires two different hosts to complete their life cycle – an Anopheles mosquito (sexual stage) and humans (asexual stage).

Modes of transmission: Asexual sporozoites of the parasite are injected into humans by female anopheles' mosquitoes. Malaria can also be transmitted by blood transfusion, organ transplantation, sharing of intravenous drugs or needles, accidental laboratory exposure, congenitally or rarely in persons working at airports.

Transfusion significance: Asexual form of the malarial parasite present in erythrocytes (trophozoites) is responsible for transfusion-transmitted malaria. The minimum number of parasites for induction of transmission is not known but Bruce-Chwatt et al[17] found that 10 parasites/ml for P. vivax were enough in inducing experimental transmission. Though transmission occurs mainly through red cell components (whole blood or packed red cells), other red cell contaminated blood components (liquid or frozen plasma, platelets, and granulocyte concentrates) and cryopreserved red cell units can also transmit the parasite. Adenine present in red cell preservatives like CPDA (Citrate- phosphate-dextrose-adenine), SAGM (Saline-Adenine-Glucose-Mannitol) enhances the malarial parasite viability in stored blood. Plasma fractionated products like albumin, IV Ig and factor VIII do not transmit the parasite. At 4–6^{0}C storage, P. malariae is viable for less than five days while P. falciparum is viable for up to ten days.

- **Type of haemoglobin and red cell antigens**
  - Duffy blood group antigen is necessary for invasion by P. vivax.
  - P. falciparum malaria has a significantly lower incidence, parasite density and hospital admission rate in children with HbAS than HbAA in sickle cell anaemia.
  - Due to the persistence of haemoglobin F in thalassemia patients, which is relatively resistant to haemoglobin digestion by malarial haemoglobinase, the multiplication of the P. falciparum is significantly reduced.
  - Ovalocytosis, hereditary elliptocytosis and pyruvate kinase deficiency have also shown protection against malaria.

Incubation period: The incubation period varies depending upon infecting species, immune status of patient and number of parasites transmitted. For P. falciparum and P. vivax, incubation period may be 1 week to 1 month but may require several months for P. malariae.

Pathogen reduction efficacy: No specific data is available; in vitro studies evaluated that riboflavin + UV treatment reduces the parasite load in the whole blood.

Leucoreduction efficacy: As malaria is an intraerythrocytic parasite, leucoreduction is not effective for prevention of malaria.

Laboratory diagnosis:

Serological test: The detection of circulating antibodies or antigens of malaria parasite aims to screen blood donors. Antibody testing is mainly done by indirect immunofluorescence assay (IFAT) or ELISA. The window period for IFAT is usually 7–14 days for infection by P. falciparum. This method is highly recommended for screening donors in non-endemic countries. Pf HRP-2 antigen is a water-soluble, histidine-rich antigen present in immature P. falciparum gametocytes[18], and monoclonal antibodies (MoAb) against it have been the basis of detection of circulating antigens.

Microbiological examination: For detecting the parasite, a thick or thin smear of peripheral blood film is the most commonly used method, but it has a low sensitivity of around 50 parasites/ml or 0.001% infected RBC.

NAT test: PCR testing is 100 times more sensitive than peripheral blood film examination with a sensitivity of 1–4 parasites/50 ml for P. falciparum and 40–130 parasites/50 ml for P. vivax.

2.2 Other Parasite Infections:

Other parasitic infections like Babesia, Trypanosoma, Leishmania and toxoplasma also show transmission by transfusion of infected blood/blood components. Details of these are mentioned in Table 6.

4.Transfusion transmitted spirochaete infections

4.1 Treponema pallidum

Structure: Treponema pallidum is a thin-walled, motile, spiral, Gram-negative rod or spirochaete that cannot be visualized with Gram's stain and does not grow in bacteriologic media or cell culture.

Mode of transmission: It spreads by sexual contact, transfusion, percutaneous exposure, and transmission from mother to infant.

Transfusion significance: The organism can survive for 1 to 5 days at 4ºC in red blood cells. Platelets stored at 20 to 24ºC provide a more hospitable temperature for T. pallidum; but it does not survive for a longer time at high-oxygen tension in modern platelet storage bags. The chief reason for the decline of transfusion-transmitted syphilis seems to be the almost universal practice of storing blood at 4ºC before transfusion, universal donor testing and decline in the prevalence of syphilis in many countries since the advent of penicillin.

Incubation period: Donors at any stage of disease, including late, latent syphilis, can transmit the infection with a variable incubation period from 4 weeks to 4.5 months, averaging 9–10 weeks.

Leucoreduction efficacy: Not reported.

Pathogen reduction: Almost reduces by 6 logs.

Laboratory diagnosis

Serological testing: The serological tests are of two types:

Non-specific test or non-treponemal tests: Non-treponemal tests (also known as tests for reagin antibodies) are based on serum reactivity from infected patients to a cardiolipin-cholesterol-lecithin antigen. The most commonly used tests in blood donor screening are non-specific tests because they are simple, rapid and economical. Non-treponemal tests include the venereal disease research laboratory (VDRL) test and rapid plasma reagin (RPR) test.

Treponemal specific tests: These tests are based upon the detection of antibodies directed against specific treponemal antigens. Treponemal tests are qualitative only and are reported as "reactive" or "nonreactive". These tests are mainly confirmatory tests and have lengthy procedures. They are not suitable for routine screening of donor's blood. Treponemal tests includes Treponema pallidum haemagglutination test (TPHA),

Table 6: Other parasitic organisms and significance of their transmission by transfusion

SN	Name	Family	Structure	Mode of transmission (Vector)	Transfusion transmission	Survival in blood components	Incubation period	Laboratory testing
1	Babesia Microti: Babesiosis	Order: Piroplasmidora Family: Babesiidae	1-2.5 μm	Ixodes scapularis tick bite. Other: Organ/ tissue transplantation and transplacental route	Yes	Red cells at 4°C: 35 days Frozen red cells for years.	1–4 weeks after a tick bite 1 to 9 weeks after transfusion-associated infection.	• Microscopic examination • Immunofluorescence assays • Real-time PCR
2	Trypanosoma cruzi: Chagas Disease	Order: Kinetoplastida Family: Trypanosomatidae	16-20 μm (trypomastigotes) 1.5 x 4.0 μm-(amastigotes)	Triatomine or reduviid bugs' feces Other: Organ transplantation, transplacental route and breast-feeding	Yes	Whole blood & RBCs at 4°C: 18 days. Platelets: 5 days. Frozen plasma :24 hours or less	20-40 days	• ELISA testing (licensed by FDA) • Immunofluorescence assay • Chemiluminescence test • Western blot • Nucleic acid test
3	Leishmania spp. – Leishmaniasis	Genus Leishmania, Order: Kinetoplastida, Family: Trypanosomatidae	2.5 x 5 μm sized protozoan.	Phlebotomine sandflies: *Phlebotomus* genus (Old World) and *Lutzomyia* genus (New World).	Yes	Whole blood & RBCs at 40C: 25 days Platelets: 5 days Frozen red blood cell: 35 days	2 weeks to 8 months	• Microscopic Examination • Immunofluorescence assay • ELISA • Western blot • NAT for kinetoplast DNA (kDNA)
4.	Toxoplasma gondii; Toxoplasmosis	Order: Eucoccidiorida; Family: Sarcosystidae	2.5 x 5.0 μm	Cats (consumption of oocyst contaminated water, raw meat containing tissue cyst) Other: Transplacental route and organ transplantation.	Yes	Whole blood & RBCs: 42 days.	1-2 weeks	• Microscopic Examination • Bioassay (gold standard) • ELISA • Immunosorbent agglutination assay (ISAGA) • Indirect fluorescent antibody test (IFAT) • Indirect haemagglutination assays (IHA.) • NAT

Fluorescent treponemal antibody absorption test (FTA-ABS), Treponema pallidum immobilization test (TPI), Enzyme immuno assay (EIA) and Chemiluminescence assay (CLIA) etc.

5. Transfusion transmitted bacterial infections

Introduction: Currently, bacterial contamination of blood products is the major microbiologic cause of transfusion-associated morbidity and mortality. According to various reports, transfusion-associated sepsis resulting from bacterial contamination was the most frequently reported cause of mortality after haemolytic reactions, accounting for over 10% of transfusion fatalities.[19]

Incidence of bacterial transfusion reactions: The rate reported for bacterial contamination of blood components clearly exceed the incidence of bacterial reactions following transfusion. A contaminated blood component has the potential to cause a transfusion reaction, but this depends on a range of factors. These include the underlying condition of the patient, the type of antibiotic therapy is administered, type of blood component being transfused, the level of bacterial inoculum transfused as well as the nature of the particular organism (or even strain) transfused. The rate of bacterial contamination of RBC units ranges from 0-0.2%, while for platelets, the rate varies from 0-10 %. It is estimated that 1 in 1000-3000 platelet units are bacterially contaminated, of which 1 in 25,000 transfusions results in clinical sepsis, with a fatality rate of approximately 1 in 60,000 transfusions.[20] Bacterial transfusion reactions due to plasma component are rare as these components are frozen but can occur with significant contamination. *Pseudomonas cepacia* and *P. aeruginosa* have been cultured from cryoprecipitate and plasma thawed in contaminated water baths. On some occasions, albumin has been found to be contaminated with bacteria, typically Pseudomonas species resulting in endotoxic shock, transient bacteraemia, and febrile reactions in recipients. Contamination with *Enterobacter cloacae, Stenotrophomonas multophilia* and *Enterococcus gallinarum* have been reported due to cracks in the glass bottles of albumin. Stem cell contamination has also been reported, with incidence ranging from 0.23% to 4.5%, increasing by an additional 1% after cryopreservation.[21] Normal skin flora, including coagulase-negative *staphylococci, Propionibacterium acnes*, α-haemolytic *Streptococci*, *Staphylococcus aureus,* and *Enterobacter cloacae* have also been implicated. However, no adverse clinical sequelae has been reported because of the prophylactic antibiotic treatment at the time of the stem cell infusion.

Source of bacterial contamination: Source of the bacterial contamination could be donor-derived or could be related to contamination of the blood bags during the collection and processing steps. The possible sources of bacterial contamination are mentioned in Table 7.

Table 7: Sources of bacterial contamination and possible reasons

S. No	Source	Possible Reason
1.	Donor's arm derived	• Skin surface contamination • Inadequate disinfection before phlebotomy • Phlebotomy site not appropriate
2.	Donor bacteraemia	Asymptomatic bacteraemia
3.	Contaminated blood bags	• Defective blood bags • Improper storage
4.	Environmental	Poor hygiene in the blood processing area
5.	Blood processing	• Spiking • Not using sterile connecting devices

Organisms implicated in bacterial transfusion reactions: Organisms responsible for causing clinically apparent bacterial transfusion reactions are listed in Table 8. Gram-positive organisms accounted for more septic transfusion reactions as compared to Gram-negative organisms. Staphylococcus epidermidis, a skin commensal, is the most frequently transmitted organism, indicating the importance of donor arm disinfection.

Table 8: Organisms implicated in transfusion-transmitted bacterial infections by red cells and platelets

Red blood cells	Gram-positive	Gram-negative
	Staphylococcus epidermidis *Staphylococcus aureus* *Bacillus cereus* *Coagulase-negative* *Staphylococcus* *Streptococcus species* *Group B streptococcus* *Propionibacterium acnes* *Enterococcus faecalis*	*Escherichia coli* *Serratia species* *Enterobacter species* *Klebsiella species* *Yersinia enterocolitica* *Morganella morganii* *Acinetobacter species* *Proteus species*
Platelets	*Coagulase negative* *staphylococcus* *Staphylococcus aureus* *Bacillus cereus* *Staphylococcus epidermidis* *Streptococcus species* *Enterococcus faecalis* *Propionibacterium acnes*	*Acinetobacter species* *Serratia species* *Yersinia enterocolitica* *Pseudomonas species* *Escherichia coli* *Enterobacter species* *Klebsiella species* *Proteus species*

Strategies for minimizing transfusion-transmitted bacterial infection: There are various strategies which are helpful to prevent transfusion-related septic reactions. These are as follows:

- Improved donor selection
- Improved disinfection of venepuncture site
- Diversion
- Optimizing storage temperature and time
- Leucodepletion filters
- Overnight hold
- Optimizing transfusion indications and triggers
- Increased use of apheresis-derived platelet concentrates
- Bacterial screening testing
- Pathogen reduction

Bacterial screening testing methods: Screening for bacterial contamination of the blood components is paramount to prevent transfusion-transmitted bacterial infections. Ideal requirements for a bacterial screening test includes an easy and inexpensive method to perform screening with minimum requirements of specialized training, clear and easy interpretation of results, high sensitivity and specificity of the test method.

Screening for bacterial contamination of blood component poses a unique challenge for the following reasons:

1. A Broad range of bacteria that can contaminate blood components
2. The Initial inoculum of the bacterial organism may be extremely low and thus often undetectable during storage
3. Unpredictable bacterial growth kinetics during storage of blood components
4. Contamination can occur during the transport and storage of the blood components
5. Multiple blood components require screening due to different storage conditions
6. Rapid, sensitive, and specific tests are not yet available

In addition to the above challenges for bacterial detection, other issues that should be addressed during the

screening procedure includes screening, the amount/volume that needs to be tested, and which test method should be adopted. A bacterial screening test can be performed near the time of blood collection/separation (pre-storage testing) or just prior to issue (post-storage testing) of the blood component for transfusion (Table 9). Although pre-storage testing is operationally feasible (simultaneous viral screening and blood grouping, etc.) but are associated with a low bacterial count and thus require an extremely sensitive assay with larger volumes of sample for testing. Culture-based assays are most appropriate for a screening near the time of collection. However, screening immediately after blood collection would often detect bacteria that may not be able to survive in a product or organisms that do not enter into an exponential growth phase. Hence, screening at 24 hours post-donation or later is desirable as bacteria at even very low counts are able to grow in the blood component and potentially will have entered the exponential phase of growth. Secondly, several assays which are performed prior to the issue of the blood component allow use of less sensitive but rapid tests (1–2 hours) due to the larger number of bacterial organisms in the blood component compared with culture. Such tests will also require less volumes of sample for testing and thus are highly desired for bacterial screening. But testing before the issue can also lead to a delay in the issuing of blood.

Table 9: Details/description of various testing methods for transfusion-transmitted bacterial infection

Bacterial screening methods					
Method	**Principle**	**Sensitivity**	**Advantage**	**Disadvantage**	**Remarks**
A. Pre-storage period					
1. Plate culture	Detects bacterial growth patterns	1-10 CFU/ml	Highly sensitive	Skilled staff Labour intensive. Time taking procedure. Not useful for screening purposes	An incubation period of 8–24 hours is required for bacterial detection
2. Automated culture	**BacT/ALERT**: Bacterial growth in nutrient broth produces CO_2, which reacts with the water to produce carbonic acid & lowers pH. Results: Colour changes from yellow to green. **Pall eBDS**: Aerobic bacteria grow, consume oxygen, and decrease oxygen concentration in the system's culture pouch.	1-10 CFU/ mL	Highly sensitive	24 hours turnaround time A large volume of sample required (4-16 mL) High upfront cost	Near the time of blood collection
3. Scan system	The gram-positive and gram-negative bacteria's DNA labelled with a DNA-specific fluorescent marker. Based on the measurement of different fluorescence, size and shape, microorganisms can be identified.	10^3 CFU/mL	Rapid (<90 minutes)	Labour intensive. Requirement of skilled staff	Can be used for a pool size of 20 units RBC.

Method	Principle	Sensitivity	Advantage	Disadvantage	Remarks
4. Di-electrophoresis	Movement of cell in the non-uniform electric field as determined by their dielectric properties (conductivity and permittivity)	10^4 CFU/mL	Rapid, easy to use and less labour intensive	Detects both live and dead bacteria	
B. Point of transfusion					
1. Visual inspection	**RBC:** Discoloration or increased haemolysis **Platelets:** Decreased swirling or clumping	$1.8x10^4$ - $1.6x10^9$ CFU/ml	Prudent, cost-efficient	Sensitive but not specific	Swirling/ discolouration/ haemolysis can be seen due to other reasons
2. Microscopic examination	Gram stain: Staining of cell wall. Acridine orange: Staining of the nuclear material	10^5-10^6 CFU/ml	Inexpensive and easy to perform	Do not determine the viability of the organisms. Require skilled personnel	Able to detect only heavily contaminated units
3. Metabolic changes (pH and glucose levels)	Proliferating bacteria metabolize glucose and produce acid lowering the pH	10^5–10^8 CFU/ml	Rapid (glucose 30 seconds and pH 60 seconds)	In general, only high bacterial levels are detected, and specificity is questionable	Non-specific
4. Microvolume fluorimetry	An assay using antibiotic labelled probes which bind to bacteria and are detected by microvolume fluorimetry	10^5 cells/ml.	Rapid, Low cost	Detects both live and dead bacteria.	
5. Flow cytometry	Fluorescent dye thiazole orange is used to stain bacterial nucleic acid for the detection	10^4 cells/ml.	Rapid	Detects both live and dead bacteria.	
6. Bacterial cell wall detection	**Pan Genera Detection (PGD) assay:** Detect conserved class-specific cell wall bacterial antigens; lipoteichoic acid on Gram-positive and lipopolysaccharides on Gram-negative bacteria. **BacTx™**: Based on the binding of specific proteins and derived peptides to the peptidoglycan component of the bacterial cell wall	10^3- 10^4 CFU/ml	Small sample volume <1-2 mL Inexpensive Rapid turnaround time 20-30min Detects both Gram-positive and Gram-negative bacteria	Lower sensitivity when compared to culture-based systems. Single determination per sample	

Method	Principle	Sensitivity	Advantage	Disadvantage	Remarks
7. Endotoxin assay	Bacterial endotoxin can activate the enzymes present in the amoebocytes of the American horseshoe crab leading to clot formation	10-10^5 CFU/ml	Sensitive, Quantitative, Rapid	Detects only endotoxin producing organism	
8. Molecular techniques	Detection of bacterial 23S rRNA and HSP by RT-PCR assay. Detection of bacterial 16S rRNA with oligonucleotide probes	10-100 CFU/ml	Sensitive method High throughput	Risk of contamination of the reagents itself	

6. Emerging infections:

Emerging infections are defined as those whose incidence in humans has increased within the past 2 decades or threatens to increase in the near future. Emergence may be due to the evolution of an existing organism, the spread of a new agent, the recognition of an infection present in the population but has gone undetected, or the realization that an established disease has an infectious origin. Emergence may also be used to describe the reappearance of a known infection after a decline in its incidence.

There is increasing recognition of such new infectious agents and concern about the possibility of disease transmission through blood components due to the lack of available resources/ techniques for screening asymptomatic blood donors. In the event of the emergence of an infectious agent in the community, such infectious agents may threaten the available blood donor pool and decrease eligible blood donors. In the absence of knowledge of the natural history of the infectious agent, pre-donation screening of a donor with a history of disease, infection or exposure is also not known.

As a futuristic approach towards planning and policymaking for infection control, along with research and development of newer modalities of testing or treatment of infected units, different priority risk levels have been defined. The priority risk assessment is based on the available scientific/epidemiologic evidence, public perception and/or regulatory concern regarding blood safety and concern regarding the disease agent among the general public. These categories are enlisted in Table10, and details of some organisms are mentioned in Table 11.

Table 10: Risk categories of emerging infections

Risk levels	Red	Orange	Yellow	White
Criteria	Low to strong scientific /epidemiological evidence with the potential for severe clinical outcomes	Sufficient scientific / epidemiological evidence that might support their elevation to a higher priority in the future.	Absent to low scientific/ epidemiologic evidence	No higher priority appears warranted at this time and represents a watch list
Example	vCJD Dengue virus Babesia species	Chikungunya virus St. Louis encephalitis Leishmania species Plasmodium species Trypanosoma cruzi	Hepatitis A virus HHV-8 HIV variants Parvovirus B19 Influenza A (H5N1))	Hepatitis E virus (HEV) Anaplasma phagocytophilum

Table 11: Emerging infections and significance of their transmission by transfusion

SN	Name	Family	Structure	Genome	Mode of transmission	Transmission by transfusion	Incubation period	LR efficacy	PR efficacy	Laboratory testing
1.	Dengue virus (DENV) Four serotypes (DENV1,2,3& 4)	Flavi viridae	Enveloped Icosahedral ~ 50 nm	Linear, positive ssRNA	Mosquito borne (Aedes aegypti and Aedes albopictus)	Possible transmission	3-14 days	No data available	4 - 6 log reduction	Donor screening for NS1-antigen using ELISA/rapid tests. NAT for dengue virus RNA
2.	Chikungunya virus (CHIKV)	Toga viridae	Enveloped, Icosahedral 60-70 nm	Linear, positive ssRNA	Mosquito borne (Aedes aegypti and Aedes albopictus)	Not documented, but asymptomatic viremia could be a source for transmission	3-12 days	No data available	Up to 6 log reduction	NAT for CHIKV RNA
3.	Ebola virus	Filo viridae	Enveloped, 80 nm	Linear, negative ssRNA	Human-to-human contacts, droplets	Not documented Risk of parenteral transmission via blood from ill patients	5-10 days	No data available	Possible: No data available	ELISA & NAT assays.
4.	Influenza Three types: A, B, and C	Orthomyxo viridae	Enveloped, 80-120 nm	Linear, negative ssRNA	Droplet infection	Not documented Asymptomatic viremia present for a short time	1-5 days	No data available	Possible: No data available	Antibody screening and NAT assays are available.
5.	Lymphatic choriomeningitis (LCMV)	Arena viridae	Enveloped, 50-300 nm	Bisegmented, negative, ssRNA	Contacts with rodents, organ transplantation	Not documented Viremia present during the acute phase.	8-13 days	No data available	Possible: No data available	Plasma and serum may be tested for IgM and IgG antibodies.
6.	Parvovirus B19	Parvo viridae	Nonenveloped, Icosahedral 20–25 nm	Linear, negative ssDNA	Droplet infection	Viral transmission documented from plasma products. Screening required prior to the preparation of plasma products.	5-6 days	No data available	Possible: less efficient	NAT recommended for plasma product preparation. ELISA and haemagglutination for donor screening
7.	Epstein Bar virus (EBV)	Herpes viridae	Enveloped, icosahedral 120_220 nm	Linear, dsDNA	Human-to-human Contacts (oral)	Yes, Endemic: >90% of blood donors have anti-EBV antibodies. With increasing storage time, B-lymphocytes deteriorate, which gets cleared from recipient circulation after transfusion.	21–30 days	Yes	Up to 6 log reduction	ELISA Detection of IgM and IgG anti-VCA, IgG anti-early antigen (anti-EA) and IgG anti-Epstein Barr nuclear antigen (anti-EBNA).

SN	Name	Family	Structure	Genome	Mode of transmission	Transmission by transfusion	Incubation period	LR efficacy	PR efficacy	Laboratory testing
8.	West Nile virus (WNV)	Flavi viridae	Enveloped, Icosahedral 50 nm	Positive ssRNA	Mosquito borne (Culex and Aedes)	Yes	2-14 day	No data available	4 - 6 log reduction	NAT testing for donor screening ELISA for Anti-WNV IgM antibodies
9.	Zika virus (ZIKV)	Flavi viridae	Enveloped Spherical, 50 nm	Positive ssRNA	Mosquito borne (Culex and Aedes)	Zika virus transmission through platelet transfusions documented	3–14 days	No data available	4 - 6 log reduction	Blood donor screening using NAT assay
10.	Crimean-Congo Haemorrhagic Fever virus (CCHFV)	Bunya viridae	Enveloped spherical. 80-120 nm	Circular, segmented ssRNA	Ixodid (hard) ticks	Not documented. Theoretical of transmission by contact with body fluids or blood	3-7 days	No data available	4 - 6 log reduction	Donor screening can be by ELISA or NAT
11.	Nipah virus	Paramyxo viridae	Envelopedpleo-morphic 40-600 nm	Negative ssRNA	Direct contact, droplet infection	Not documented	4 - 14 days	No data available	Possible: Data not available	RT PCR from body fluids as well as antibody detection via ELISA
12	Herpes virus (except CMV, EBV)	Herpes viridae	Enveloped, icosahedral particle, 100-200 nm	Linear, dsDNA	Human-to-human Contacts (oral), Transfusion, Sexual (HHV-8)	HHV-6 or HHV-7 not reported to date. HHV-8 can transmit only up-to 4 days of storage	2-21 days	Effective	4 - 6 log reduction	Donor screening can be by ELISA or NAT
13.	SARS Coronavirus	Corona viridae	Enveloped spherical to pleomorphic, 100-130nm	Linear, Positive ssRNA	Direct, Indirect contact, Droplets	Not documented	2-10 days	No data available	Possible: Data not available	Blood donors are not screened for SARS
14.	SARS-CoV2 (Disease: COVID-19)	Corona viridae	Enveloped spherical to pleomorphic particles, 80 and 160 nm	Positive ssRNA	Direct, indirect contact, droplets	Not documented	1-14 days	No data available	Possible: Data not available	Donor screening can be performed for antibody using rapid tests or ELISA or chemiluminescence

*LR: Leukoreduction; PR: Pathogen Reduction

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7. TTI screening testing methods

7.1. Serological tests

Generation of serological tests: There is advancement in serological testing to improve the sensitivity and specificity of the test. It also helps to reduce the window period of testing. Currently, four generations of serological tests exist showing advancement as we go from the first to the fourth and are mentioned in Table 12.

Table 12: Generations of serological tests

Generation	Parameters tested
First	Antigens from virus lysates
Second	Recombinant proteins and/or synthetic peptides
Third	Recombinant proteins and/or synthetic peptides in an antigen sandwich configuration
Fourth	Detection of both virus antigen and both antibodies, IgG, and IgM

Types of Serological tests

1. Rapid Tests
2. ELISA Tests
3. Chemiluminescence Test

Rapid test: Rapid tests are technically simple to perform and point of care tests that do not require any special equipment. The sensitivity and specificity of rapid tests is comparable to ELISA. Recombinant or synthetic antigens are used for rapid test kits. Rapid test kits can be stored at an ambient temperature (20ºC to 25ºC).

Rapid tests are based on the following principles:

Immuno-concentration / Dot Blot immunoassay (vertical flow): In this, viral antigens are coated on a porous membrane. The test specimen & reagent pass through the membrane and are absorbed on the underlying pad. If antibodies are present in the test specimen, they binds to the coated antigens. This reaction produces a distinct coloured dot against a white background.

Immuno chromatography tests: In this, both the antigen and signal reagent are coated onto the nitrocellulose strip/cards. The test specimen migrates through the strip with the help of buffer solution and combines with the signal reagent. A positive reaction result comes as a visual line on the membrane where the viral antigen has been incorporated along with a control test line.

Particle agglutination tests: In this, antigen is coated on the carrier particle. When the test specimen is added, the antigen-antibody reaction is observed as clumps. These assays incorporate a variety of antigen-coated carriers, e.g., red cells, latex particles, gelatine particles and microbeads. This structure can be visualized macroscopically as per the directions in the kit insert.

Dipstick and comb assay: These tests incorporate antigens “spotted” on a solid support. They are based on typical EIA methods, with enzyme-substrate reactions occurring and resulting in the production of colour at the site of antigen spotting.

Enzyme-linked immune-sorbent assay (ELISA): The basic principle of ELISA is that either antigen or antibodies attach to a solid phase which is followed by a conjugate and substrate detection. The solid phase for ELISA testing can be wells of microplate or strips of nitrocellulose paper. The viral antigens may be viral lysates, and conjugates are antibodies (IgG or IgM) bound with enzymes (alkaline phosphatase or horseradish peroxidase) and fluorochromes. The substrates commonly used for ELISA are 4-nitrophenylphosphate (for alkaline phosphatase) and o-phenylenediamine dihydrochloride (OPD) and TMB (for horseradish peroxidase), which produces colour by being acted upon by the respective enzymes. The colour of ELISA reaction can be measured on an ELISA reader as optical density (OD) values. This can be performed either manually or by automated methods.

Types of ELISA: Four types on the basis of the principle of the test:

1. **Indirect ELISA**
2. **Competitive ELISA**
3. **Sandwich ELISA**
4. **Capture ELISA**

Indirect ELISA: In this, viral antigens are attached to the solid phase, allowing antibodies in the serum/plasma to bind with them. The bound antibodies are further detected by enzyme labelled anti-human immunoglobulins and substrate. If serum/plasma contains antibodies, a colour reaction will be observed. The change in colour of the reaction is directly proportional to the number of antibodies in serum/plasma.

Competitive ELISA: As the name suggests, competitive ELISA is the competition between two antibodies to bind with an antigen**.** In this, viral antibodies and antibodies in the conjugate are added at the same time. Then both antibodies compete to bind with the antigen coated on the solid phase. Anti-viral antibodies have higher affinity and form a stronger bond than conjugate antibodies. So, if viral antibodies are present in the serum/plasma, then they bind more and firmly to the antigen than conjugate antibodies. The reaction colour is observed only if conjugate antibodies bind to the antigen and no or less colour when viral antibodies bind to the antigen. So, in this ELISA the amount of the viral antibodies is inversely proportional to the amount of colour produced.

Antibody capture ELISA: In this, monoclonal anti-human globulin antibodies against viral antigens are bound to a solid phase and viral antigen added as a reagent. At the time of testing, serum/plasma is added to the well. If viral antibodies are present in the specimen, they will bind to the antigen on a solid surface, and the development of colour indicates a positive reaction. The capture ELISA is more specific than indirect ELISA and is useful for detecting lower concentrations of antibodies (e.g., urine and saliva) or a specific class of antibodies like IgG, IgM, or IgA.

Sandwich ELISA: In this, viral antibodies are sandwiched between two antigens. The antigens are coated on the solid phase on which the test plasma/ serum is added, and then again, enzyme labelled antigens are added. As antibodies have multiple binding sites, they will bind to both coated antigens, and enzyme labelled antigens, thereby forming a sandwich of antigen + antibody + enzyme labelled antigen complex. Colour develops after the addition of substrate if this sandwich complex is formed, which is measured by an ELISA reader.

ELISA performance variables: There is a requirement to strictly follow the standard operating procedures for performing the ELISA because any deviation from the process flow like the volume of reagent, washing and incubation time and temperature can affect the test results. For validating the test, kit controls like internal control and external controls must be included in each test run.

Factors affecting the ELISA results: It can be divided into three types-pre-analytical, analytical, and post-analytical, based on different test phases.

1. Preanalytical phase: Affected by haemolyzed, lipemic, contaminated or improperly stored samples. It can also be affected by contaminated, expired, or deteriorated reagents.
2. Analytical phase: Affected by improper pipetting of sample or reagents, wrong incubation time and temperature and improper washing procedure. It can also be affected by equipment failure, calculation errors and carry-over of the samples.
3. Post-analytical phase: Affected by transcriptional errors.

The common causes of false-positive results are autoimmune diseases, non-viral hepatitis, biliary cirrhosis, leprosy, and multiple pregnancies etc. while false negative results can be observed when an individual is in the window period or due to technical errors.

Chemiluminescence (CL) Assay: Luminescence is the emission of visible or near-visible (Wavelenth = 300–800 nm) light by a substance when it returns from an excited state to a ground state. Chemiluminescence is the emission of light as the result of a chemical reaction. A chemiluminescent reaction's sensitivity is dependent on the efficiency of the reaction to produce light photons. A single chemiluminescent compound

produces only one photon.

Chemiluminescence Immunoassay (CLIA): CLIA combines the chemiluminescence reaction and the immunoreactions. CLIA is a method to determine the concentration of samples according to the intensity of the luminescence that the chemical reaction emits. The principle of CLIA is similar to ELISA, except that chromogenic substrate is replaced by chemiluminescent compound, which generates light during a chemical reaction, and that light can be detected by a luminometer. The most popular CL substrates are luminol, isoluminol and their derivatives, acridinium ester derivative, peroxidase, and alkaline phosphatase (ALP). In the CLIA technique, labelling enzymes horseradish peroxidase (HRP) and ALP are routinely used to label the proteins.

Types of CLIA: Based on the difference between the mechanisms of light emission, CLIA can be divided into three different label systems:

1. Indirect CLIA using enzyme markers.
2. Direct CLIA using luminophore markers.
3. Electro CLIA

Indirect CLIA: In this process, the testing molecules are not directly involved in chemiluminescence reaction and so are not able to generate luminescence. The excess energy of the molecule transfers to a fluorophore that in turn is excited, releasing to its ground state with photon emission.[22] Technically, it is an enzyme-linked immunoassay that uses luminescent chemical as a substrate. Enzymes used in indirect CLIA are HRP and ALP, and substrates used are luminol and adamantyl 1, 2-dioxetane aryl phosphate (AMPPD).

Direct CLIA: The direct CLIA reaction uses two reagents, a substrate and an oxidant, with some cofactors. The substrate is the chemiluminescence precursor, which is converted into the electronically excited molecule after reacting with oxidant in the presence of a catalyst. It is responsible for light emission or acting as the energy (Figure 3). The most popular chemiluminescent substrates are luminol, isoluminol and their derivatives, acridinium ester derivatives, peroxidase, and alkaline phosphatase.

Advantages over indirect CLIA

- It has stronger chemiluminescence intensity due to direct reaction.
- It does not lose luminescence efficiency even after binding to an antigen or antibody.
- No enzyme is involved in this testing.

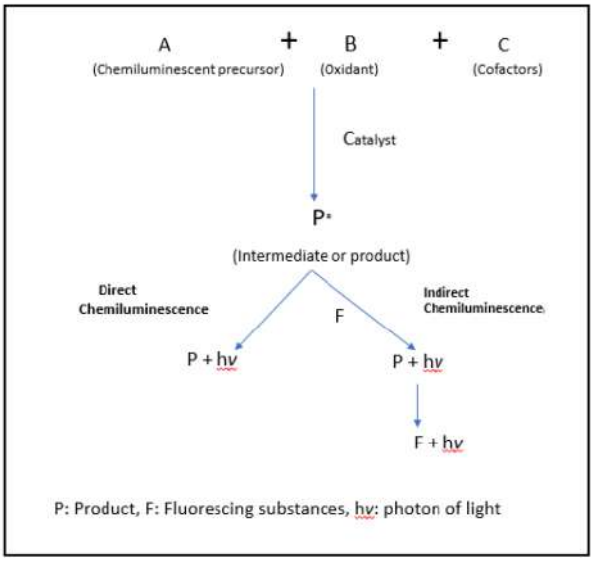


Figure 3: Direct and Indirect CLIA

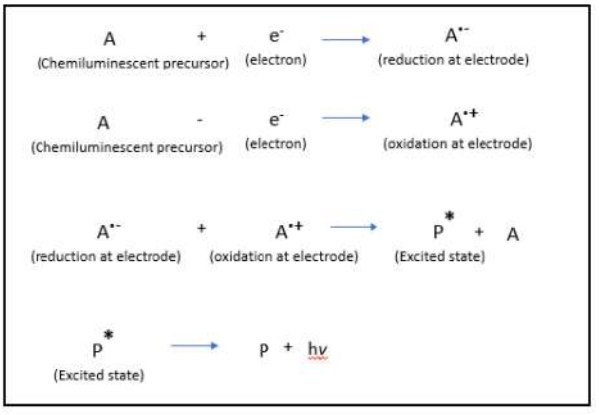


Figure 4: Electro CLIA

Electro chemiluminescence or electrogenerated chemiluminescence (ECL): Electrochemiluminescence means conversion of electrical energy into light or radiative energy. It involves the production of reactive intermediates from stable precursors at the surface of an electrode. ECL technology uses redox or reduction-oxidation reaction. In redox reactions, there is transfer of electrons between chemical substances from one chemical (the reducing agent) undergoing oxidation (losing electrons) to another chemical (the oxidising agent) which undergoes reduction (gains electrons) by the alternate pulsing of the electrode potential, which is also called annihilation (Figure 4). This reaction ultimately leads to the emission of light. This reaction utilizes ruthenium tris-bipyridine Ru(bpy) as the label.[23] An excited state of Ru(bpy)32+ is produced when stimulated by electricity which decays to the ground state by emitting light. Ruthenium is regenerated and can be recycled.

Advantages of chemiluminescence assay: The major advantage is that it is fully automated, requires less quantity of sample, has less turnaround time, can operate in random access mode, and has the capability to produce a short turnaround time. The disadvantages of CLIA are that it is costlier than ELISA or rapid testing, and a few false-positive cases are also reported.

CLIA for blood donor screening in India: CLIA testing for blood donor screening is available for HIV, HCV, HBV, and syphilis in India. CLIA is almost equally sensitive as ELISA for testing these pathogens. In India, nowadays, most of the transfusion centres are shifting from ELISA to CLIA due to their reduced turnaround time advantage. Roat et al[24] performed an evaluation study for the usage of chemiluminescence in their centre. It concluded that automated chemiluminescence has comparable results with ELISA and has a faster turnaround time, which can be used as a replacement for ELISA.

7.2. Nucleic acid testing (NAT):

Nucleic acid testing (NAT), also termed nucleic acid amplification technology (NAAT), is a molecular amplification technology that targets, amplifies, and detects the genetic material (RNA/DNA) of a pathogen (virus, bacteria, or parasite). NAT technique is highly sensitive and specific for viral nucleic acids. Despite thorough measures to select donors and sensitive ELISA testing of blood donations, some transmissions of the most relevant transfusion-transmitted viruses (HCV, HIV and HBV) occurred. NAT is based on the amplification of targeted regions of viral ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). NAT reduces the window period of HIV, HBV, and HCV infections by early detection of the viral genome. The main objective of NAT testing is to reduce the risk of transmission of the virus to recipients by detecting the minimum amount of viral copies (Cps) of all known genotypes and subgroups. NAT testing of blood donor screening is a qualitative assay that results in reactive and non-reactive results. The analytical sensitivity of NAT depends on the limit of detection (LOD), which is the minimum detectable concentration [minimum Cps/International Units (IU)] of the target. Pool size is inversely related to LOD; with the larger the pool size more significant the loss in sensitivity. Factors affecting analytical sensitivity can be categorized under three headings:

(a) Preparation of sample: It includes the volume of test sample used, virus disruption, nucleic acid extraction, and removal of unwanted substances

(b) Amplification of nucleic acid: It depends on the extracted volume of nucleic acid, copy number of the target region, number of regions amplified, the technology of amplification and the ability to tolerate interfering substances

(c) Detection of nucleic acid: It depends on the detection technology, the type and number of probes, and the ability to demarcate a signal from noise.

NAT for blood donor screening should fulfil the following major criteria:

- Highest sensitivity
- Highest specificity
- Highest robustness
- Highest validity
- Highest throughput
- Highest speed possible

Difference from serology: The most sensitive NAT (in individual format) shortens this window period to 2.9 days for HIV, 10.3 days for HBV and 1.3 days for HCV as compared to serological assays, which have a window period ranging from 14-28 days for HIV, 42-55 days for HBV and 50-80 days for HCV. NAT also benefits by resolving false reactive donations of the serological methods, which is very important for the donor notification and counselling. But NAT testing is not an alternative to serological testing because long term chronic TTIs have sufficient amount of antibodies with a low viral load which can be detected only on serological testing.

NAT testing methods for screening of blood donors: For blood screening, current in-house and commercial assays are extensively based on two techniques: Reverse transcription-polymerase chain reaction (RT PCR), and Transcription mediated amplification (TMA). The differences between these two technologies are mentioned in Table 13.

Reverse transcription PCR (RT PCR): RT PCR uses a Reverse Transcriptase enzyme that converts the target RNA into complementary DNA (cDNA). This cDNA is then amplified like in a standard PCR which involves three basic steps of target denaturation, annealing of primers and extension of the new strand (Figure 5 and 6). A PCR-based assay has many advantages: simplicity, flexibility, inexpensive and can amplify damaged DNA. The major disadvantages of PCR reaction are contamination risk, primer complexity, which can amplify rare species and multi-temperature requirements.

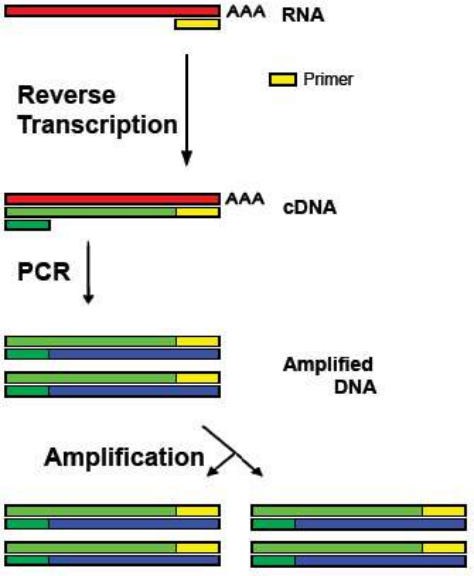


Figure 5: The basic flow of a RT PCR reaction

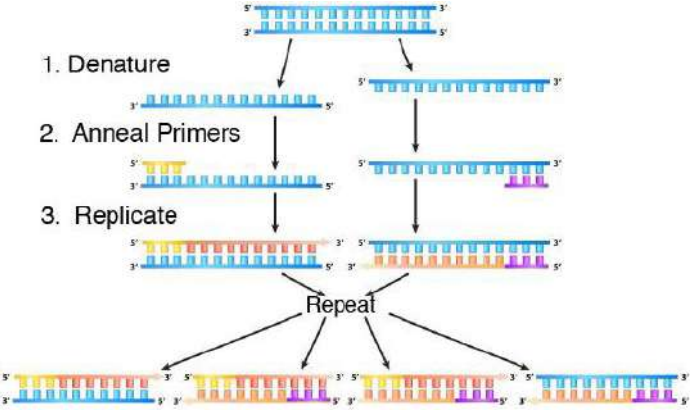


Figure 6: Amplification steps in a PCR reaction

Transcription mediated amplification (TMA): TMA technique is based on two enzymes, RNA Polymerase and Reverse Transcriptase. The Viral RNA target is reverse transcribed into cDNA, and DNA formation is then followed by synthesis of RNA amplicons from DNA by RNA polymerase (Figure 7). It is an isothermal technique that can occur in a single tube in multiplex format and capable of detecting many pathogens in one reaction. TMA based assays are considered more sensitive compared to conventional PCR assays. The final product of a TMA reaction is RNA amplicons, and as RNA can be easily destroyed in a lab setup, the risk of cross-contamination is very low in a TMA reaction compared to PCR. TMA can produce 100-1000 copies of RNA amplicons in one cycle whereas, PCR only doubles with every cycle. This ends up in billions of amplified products in a shorter time. Transcription-mediated amplification offers two advantages in the blood centre setting: first is extraction and amplification takes place in the same tube under isothermal conditions. Second, the test lends itself to a triplex configuration, whereby HIV, HCV and HBV can be measured simultaneously.

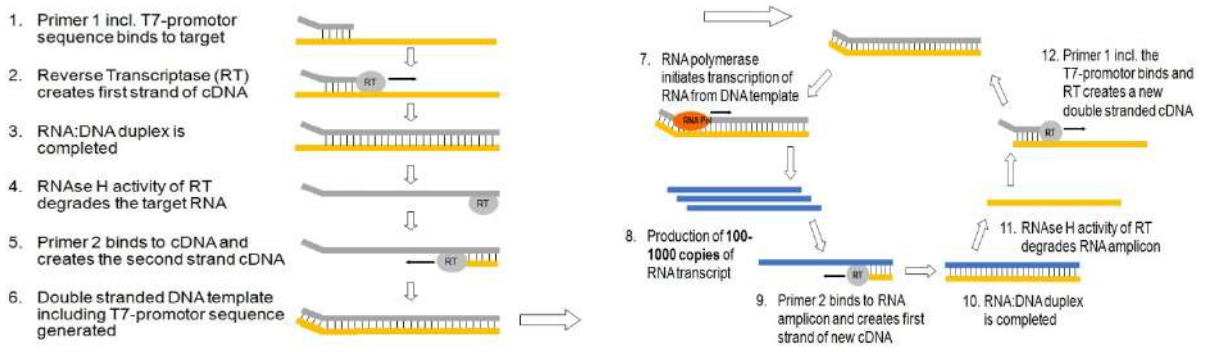


Figure 7: Principals of TMA reaction

Table 13: Major differences between RT-PCR and TMA technology

Parameters	RT-PCR	TMA
Enzymes used	DNA polymerase	Reverse transcriptase and RNA polymerase
Amplified product	cDNA copies	RNA amplicons
Stability of the final product	Very stable	Labile
Cross-contamination	Higher chances	Very low
Amplification condition	Thermal cycling	Isothermal
Instrument	Thermal cycler	Heat block/ water bath
Amplification rate	Double per cycle	Exponential (100-1000 copies per cycle)
Sample handling	Multiple tubes	One tube reaction
Reaction inhibition	Presence of heparin	No heparin inhibition

Testing strategies: Two kinds of testing strategies used worldwide are ID-NAT (Individual donor nucleic acid testing) and MP-NAT (mini-pool testing). As the names suggest, a blood sample from a single donor is tested individually in ID-NAT format. In contrast, in MP-NAT format, samples from different donors are pooled together in a range of 4, 6, 8, 16, 18, 20 to 96 and tested as one sample. However, it has been observed that ID-NAT screening will only slightly increase the detection of infected donors but significantly increase the testing cost as compared to MP-NAT. There are chances of false-positive results with ID-NAT due to higher sensitivity, while there are chances of missed positive donors in MP-NAT due to lower sensitivity.

NAT for HCV: HCV NAT reduces the window period from 60-80 days to a minimum of 1.3 days. During the window phase, the high viral titres of HCV of 10^6–10^9 particles/ml plasma and a rapid viral burst due to viral doubling time of <1 day, as well as the long time to seroconversion, made HCV the best candidate for the application of NAT to identify the highest yield of contaminated donations, even when samples were pooled.

NAT for HIV: In contrast to HCV, the window period for HIV is much shorter and the viral doubling rate, as well as viral load, is much lower during the window phase. HIV NAT reduces the window period from 14-28 days to a minimum of 2.9 days.

NAT for HBV: For HBV, the situation is slightly difficult. The gap between NAT and HBsAg detection of viral infection is short due to the higher sensitivity of serological HBsAg testing (as few as 1000 viral copies/ml (100–1000 IU). The replication rate of HBV is very low, with a doubling time of 2.6 days; therefore, HBV NAT reduces the window period from 42-55 days to a minimum of 10.3 days.

NAT in India: In India, both RT-PCR and TMA NAT testing methods are available. In the Indian multi-centric study[25], blood samples with their serology report were collected from eight blood centres from seven major cities. Twelve thousand two hundred twenty-four samples, when tested by ID-NAT, revealed eight ID-NAT yields (NAT positive, serology negative). The observed ID-NAT yield was 1 in 1528, and keeping that yield

rate in mind, it was estimated that among 5 million annual donations, 3272 TTIs could be interdicted by implementing ID-NAT. Chatterjee et al.[26] have compared the sensitivity of ID- and MP-NAT testing as assessed by dilution of NAT yield samples. The authors observed that all dilutions detected samples with a high viral load, but 67% of samples with a low viral load are missed by MP-NAT and concluded that ID-NAT is an ideal methodology for TTI screening. The selection of ID-NAT and MP NAT testing in India depends on nationwide and individual blood centre-level factors.[27]

The nation-wide factors include (i) Prevalence of TTI viruses in the donor population (ii) voluntary or replacement donors (iii) Lookback programme for donor monitoring (iv) Implementation of the haemovigilance programme in the country (v) Cost v/s benefit comparisons between NAT testing and disease treatment cost. The blood centre-level factors are (i) Different algorithms and testing assays for serological screening of blood donors, and (ii) Regional variations in TTI prevalence.

Interpretations of screening test results: If the screening test is nonreactive, the test result is considered negative, and blood components can be used for transfusion. If a test is reactive during the initial screening, the donor sample is characterized as reactive, and the blood unit should be discarded.

8. Pathogen inactivation of pathogen reduction technologies

Introduction: Two interchangeable terms, pathogen inactivation (PI) and pathogen reduction (PR) have been used for a proactive approach in reducing contaminating pathogens in blood and blood components. But by definition, PI refers to the complete prevention of infectivity by a pathogen, whereas PR refers to decreasing the amount of an infectious pathogen.

Properties of ideal PI agents

a. Should inactivate or remove all types of infectious agents.
b. Should not add neoantigens in the cellular blood components.
c. Should have no effect on the function or life span of a blood component.
d. Should not have any residual toxic effects.
e. Should not involve risk greater than any TTI risk to the patient.

There are two basic mechanisms of PI methods: chemical inactivation and photo-inactivation. The chemical inactivation is usually based on the addition of heterocyclic chemical compounds, which interact with the base groups of the linked nucleotides of nucleic acid (both DNA and RNA). Subsequently, photometric excitation with UV or visible light leads to an excited electronic state which reacts directly with the nucleic acid (photochemical reaction) or secondary activation to form free oxygen radicals (type I) or reactive oxygen (type II) in a photodynamic reaction (e.g., methylene blue). Finally, inhibition of pathogen replication results by cross-linking within or between strands of the nucleic acid. The solvent/detergent (SD) treatment of FFP is different from the general mechanism. In this, the SD damages the lipid envelope of pathogens and also lyse any blood cells present in FFP.

Types of PI methods: There are many FDA approved PI methods available. The selection of methods depends on blood components and the property of pathogens (Table 14 & Table 15).

Table 14: PR/PI agents, susceptible pathogens, and mechanism of action

S. No.	PR/PI agents	Pathogens	Mechanism of action	Log reduction
1.	Solvent/detergent (1% tri-(N-butyl)-phosphate (TNBP) and 1% Triton X-100 or Triton X-45)	Enveloped virus, Intracellular virus	Liquid envelop solubilisation	>6 log
2.	Methylene blue plus light	Enveloped and some non-enveloped virus	Free radical guanidine	>5.45 log

S. No.	PR/PI agents	Pathogens	Mechanism of action	Log reduction
3.	Psoralen (S59 or Amotosalen hydrochloric acid) Ultraviolet Light treatment	Enveloped virus, some non-enveloped virus, intracellular viruses, bacteria (no spores), protozoa, leucocytes	Pyrimidine adducts and cross-links	>6.2 log
4.	Riboflavin plus light		Guanidine oxidation and possible adducts to thymine and adenine	>5.0 log
5.	Frangible anchor linker effector (FRALE) compound) S-303		Nucleic acid adducts	>5.0 log
6	PEN110 (Aziridine compounds)		Guanine adducts	>5.0 log

Table 15: Available PI/PR technologies for different blood components

S. No.	Product	Manufacturer	PR agents	Blood components	Removal step
1.	INTERCEPT	Cerus	Amotosalen + UVA light	Plasma & Platelets	Adsorption device (6–16 hours)
2.	INTERCEPT	Cerus	S-303	Red blood cells	Adsorption device (6–16 hours)
3.	Mirasol	Terumo BCT	Riboflavin + UVB light	Plasma, Platelet, Whole blood & Red blood cells	None
4.	THERAFLEX	Macopharma	UV-C light + agitation	Platelets	Filtration (5–10 min)
5.	THERAFLEX	Macopharma	Methylene blue + visible light exposure + filtration	Plasma	Filtration (5–10 min)
6.	Octaplax	Octapharma	Solvent/Detergent	Plasma	None
7.	Inactine	Vitex	PEN110	Red blood cells	Washing with 6L saline

Toxic effects of PI agents

- THERAFLEX and INTERCEPT treatment of plasma decreases fibrinogen, Factor V and Factor VIII activity by approximately 17% to 30% and thrombin generation capacity is impaired in plasma.
- In vitro testing of INTERCEPT treated platelets showed increased CD61 microparticle formation, higher metabolic rate, accelerated metabolic changes and reduced agonist-induced aggregation responses, but no neo-antigenicity or toxic effects were observed.
- Riboflavin treated platelets also leads to some platelet activation as expressed by increased metabolic activity and P-selectin expression.
- INTERCEPT treated red cells may develop neoantigens caused by the alkylation of proteins by acridine nitrogen mustards.

Key points

- The supply of safe blood to the patient is the primary responsibility of blood centres.
- In India, TTI screening of HIV1&2, HBV, HCV, malaria, and syphilis are mandatory as per DCA & Rule 1945.
- As per the NACO 2017 report, prevalence of HIV, HBV, and HCV in blood donors in India was around 0.12%, 0.89%, and 0.30% respectively.
- The HIV virus is well preserved in refrigerated as well as in frozen blood and it can transmit infection by 90% rate for the infected individual through blood transfusion.

- On infection of HBV, the first marker to appear is HBV-DNA, followed by HBsAg, HBeAg and IgM anti-HBc antibody in the first few weeks of exposure.
- HCV can be transmitted by incompletely inactivated plasma fractionation products like IvIG and Factor VIII, but not by albumin.
- Other hepatitis viruses like Hepatitis A, D, E, G & TTV can also be transmitted through transfusion.
- CMV transmission can be prevented by 10^5 to 10^6 log leucoreduction.
- Bacterial contamination of blood components is a rising concern for transfusion-associated morbidity and mortality.
- Stringent donor selection criteria, diversion pouch in a blood bag, optimum storage of blood components, leucoreduction and bacterial screening testing, can help to reduce septic transfusion reactions.
- The absence of natural pathogenesis history and blood donor selection criteria for emerging infections increase the risk of their transmission through blood transfusion.
- The advancement of serological testing from the first generation to the fourth generation increases sensitivity and specificity and reduces the window period of testing.
- Nowadays, chemiluminescence testing is preferred over ELISA or Rapid testing because of automation, less sample requirement, shorter turnaround time, and STAT facility testing.
- TTI screening testing by NAT testing from RT-PCR or TMA techniques is increasing day by day in India. It reduces the transmission risk of HIV1/2, HBV, and HCV by decreasing their window period.
- Pathogen inactivation of blood components adds an extra layer of safety to prevent TTI.

References

1. Serious Hazards of Transfusion (SHOT), Annual Report 2018. Accessed 20 September 2020, https://www.shotuk.org/wp-content/uploads/myimages/SHOT-Report-2018_Web_Version-1.pdf
2. National AIDS Control Society (NACO) Annual Report 2018-2019. Accessed 25 August 2020, 2020. https://main.mohfw.gov.in/sites/default/files/24%20Chapter%20496AN2018-19.pdf
3. Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N Engl J Med*. Dec 14 1989;321(24):1621-5. doi:10.1056/nejm198912143212401
4. Busch MP, Donegan E, Stuart M, Mosley JW. Donor HIV-1 p24 antigenaemia and course of infection in recipients Transfusion Safety Study Group. *Lancet*. Jun 2 1990;335(8701):1342. doi:10.1016/0140-6736(90)91221-u
5. Desmyter J. AIDS and blood transfusion. *Vox Sang*. 1986;51 Suppl 1:21.
6. Sudha T, Teja VD, Gopal M, Rajesh M, Lakshmi V. Comparative evaluation of TRI-DOT Rapid HIV test with fourth-generation ELISA for the detection of human immunodeficiency virus. *Clin Microbiol Infect*. Oct 2005;11(10):850-2. doi:10.1111/j.1469-0691.2005.01231.x
7. Cesaire R, Kerob-Bauchet B, Bourdonne O, et al. Evaluation of HTLV-I removal by filtration of blood cell components in a routine setting. *Transfusion*. Jan 2004;44(1):42-8.
8. Kumar H, Gupta PK, Jaiprakash M. The Role of anti-HBc IgM in Screening of Blood Donors. *Med J Armed Forces India*. Oct 2007;63(4):350-2. doi:10.1016/s0377-1237(07)80013-x
9. Yap PL, McOmish F, Webster AD, et al. Hepatitis C virus transmission by intravenous immunoglobulin. *J Hepatol*. Sep 1994;21(3):455-60.
10. Bjoro K, Froland SS, Yun Z, Samdal HH, Haaland T. Hepatitis C infection in patients with primary hypogammaglobulinemia after treatment with contaminated immune globulin. *N Engl J Med*. Dec 15 1994;331(24):1607-11. doi:10.1056/nejm199412153312402
11. Castillo I, Rodriguez-Inigo E, Bartolome J, et al. Hepatitis C virus replicates in peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. *Gut*. May 2005;54(5):682-5. doi:10.1136/gut.2004.057281

12. Carreno V. Occult hepatitis C virus infection: a new form of hepatitis C. *World J Gastroenterol*. Nov 21 2006;12(43):6922-5. doi:10.3748/wjg.v12.i43.2000
13. Paloheimo JA, von Essen R, Klemola E, Kaariainen L, Siltanen P. Subclinical cytomegalovirus infections and cytomegalovirus mononculeosis after open heart surgery. *Am J Cardiol*. Nov 1968;22(5):624-30. doi:10.1016/0002-9149(68)90198-7
14. Preiksaitis JK. The cytomegalovirus-"safe" blood product: is leucoreduction equivalent to antibody screening? *Transfus Med Rev*. Apr 2000;14(2):112-36.
15. Drew WL, Tegtmeier G, Alter HJ, Laycock ME, Miner RC, Busch MP. Frequency and duration of plasma CMV viremia in seroconverting blood donors and recipients. *Transfusion*. Mar 2003;43(3):309-13.
16. Bowden RA, Slichter SJ, Sayers M, et al. A comparison of filtered leucocyte-reduced and cytomegalovirus (CMV) seronegative blood products for the prevention of transfusion-associated CMV infection after marrow transplant. *Blood*. Nov 1 1995;86(9):3598-603.
17. Bruce-Chwatt LJ. Transfusion malaria revisited. *Trop Dis Bull*. Oct 1982;79(10):827-40.
18. Parra ME, Evans CB, Taylor DW. Identification of Plasmodium falciparum histidine-rich protein 2 in the plasma of humans with malaria. *J Clin Microbiol*. Aug 1991;29(8):1629-34.
19. Hoppe PA. Interim measures for detection of bacterially contaminated red cell components. *Transfusion*. Mar-Apr 1992;32(3):199-201. doi:10.1046/j.1537-2995.1992.32392213799.x
20. Blajchman MA, Goldman M. Bacterial contamination of platelet concentrates: incidence, significance, and prevention. *Semin Hematol*. Oct 2001;38(4 Suppl 11):20-6. doi:10.1016/s0037-1963(01)90120-9
21. Klein MA, Kadidlo D, McCullough J, McKenna DH, Burns LJ. Microbial contamination of haematopoietic stem cell products: incidence and clinical sequelae. *Biol Blood Marrow Transplant*. Nov 2006;12(11):1142-9. doi:10.1016/j.bbmt.2006.06.011
22. M S, J H, J E. *Handbook of Fluorescence Spectroscopy and Imaging*. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim; 2011.
23. N T, J BA. Electrogenerated chemiluminescence. IX. Electrochemistry and emission from systems containing tris (2, 2'-bipyridine) ruthenium (II) dichloride. *J Am Chem Soc*. 1972;94:1.
24. Rout D, Chaurasia R, Coshic P, Chatterjee K. Chemiluminescence brings renaissance in TTI screening: Primi experientia. *Asian J Transfus Sci*. Jan-Jun 2017;11(1):72-73. doi:10.4103/0973-6247.200777
25. Makroo RN, Choudhury N, Jagannathan L, et al. Multicenter evaluation of individual donor nucleic acid testing (NAT) for simultaneous detection of human immunodeficiency virus -1 & hepatitis B & C viruses in Indian blood donors. *Indian J Med Res*. Feb 2008;127(2):140-7.
26. Chatterjee K, Coshic P, Borgohain M, et al. Individual donor nucleic acid testing for blood safety against HIV-1 and hepatitis B and C viruses in a tertiary care hospital. *Natl Med J India*. Jul-Aug 2012;25(4):207-9.
27. Shyamala V. Nucleic Acid Technology (NAT) testing for blood screening: impact of individual donation and Mini Pool – NAT testing on analytical sensitivity, screening sensitivity and clinical sensitivity. *ISBT Science Series*. 2014;9:315-324.

Section 6

Principles of Immunohaematology

1. Basic principles of immunohaematology

Immunohaematology is a branch of medicine which deals with the study of blood group antigen and antibodies. An immunohaematologist should have a basic understanding of immunology as it involves dealing with immune responses elicited by transfusion of red cells, platelets, and other cellular elements of blood. The following text describes the immune system focusing on its relation to transfusion medicine.

1.1 Immune system in brief

The human body can resist foreign pathogens and toxins, which can harm the body, and this ability is represented as immunity. Before the body could respond to specific threats, the threats are responded to by the certain nonspecific natural defence of the body called together as innate immunity. These include the intact skin and mucous membrane, protective factors like lactic acid and fatty acids in various secretions of the body, lysozymes in tears, nasal secretions etc., as well as the leucocytes and various phagocytic cells in the body.

A more specific immune response towards a pathogen is provided by adaptive immunity, wherein the body responds by forming antibodies as/or activated lymphocytes that attack and destroy specific pathogens. The acquired immunity has memory; the ability to remember the antigen and to improve the immune defines process upon successive exposure. The adaptive or acquired immunity may have either a humoral immune response (formation of antibodies) or a cellular immune response. The main elements of cell-mediated immunity are T-cells and macrophages, which act by direct cytotoxicity or by producing lymphokines. B-cells are responsible for humoral immunity wherein specific antibodies are produced in response to exposure to antigens followed by the clearance of the organism by antibody interaction.

Table 1: Differences between innate and adaptive immunity

Innate immunity	Adaptive immunity
Provides an initial line of defence to pathogens and toxins	It supplements the response of innate immunity
It is present at birth and is nonspecific	It is acquired by contact with the pathogen and is specific to that particular pathogen
The response remains the same with a subsequent encounter with a pathogen	The response gets better with each encounter, and the body develops a memory for a subsequent encounter with the same pathogen
Example: skin, mucous membrane, secretions such as saliva, sweat etc. cytokines and phagocytic cells such as macrophages, dendritic cells, NK cells etc	Example: lymphocytes such as T-cells and B cells and antibodies etc

1.2 Antigen

Any molecule which can bind to an antibody or to a receptor on a T-cell and is capable of generating an immune response may be called an antigen. These may be proteins or polysaccharides.

Types-

1. Complete antigen (MW > 10000D)
2. Haptens (MW <10000D) - Haptens + carrier protein= complete Ag.

Epitope - a specific chemical structure with 3-D configuration present on the antigen. It is the smallest unit of antigenicity, which reacts with the complementary site on the specific antibody.

Paratope- it is a combining area on the antibody corresponding to the epitope of an antigen.

Properties of antigens

The ability of the immune system to recognize and respond to an antigen is sometimes called immunogenicity, and it differs for different antigens. It is important to know the properties of antigens to understand the different responses elicited by the immune system for different antigens.

These properties include chemical composition, degree of foreignness, dosage and antigen density, size of the antigen and the route of administering the antigen. Protein molecules are known to be the most immunogenic, followed by complex carbohydrates. Similarly, molecules having molecular weight >10,000D are known to be better immunogens and the intramuscular or intravenous route is known to elicit more immune responses.

1.3 Antibody

It may be defined as a glycoprotein (immunoglobulin) molecule formed by B-lymphocytes in response to a foreign antigen and specifically recognize and bind to that particular antigen resulting in its clearance from the body.

Properties of antibodies

Antibody molecules are glycoproteins that belong to a family of proteins called immunoglobulins. They are structurally made up of four polypeptide chains (two identical heavy chains and two identical light chains) joined together by disulphide chains (Figure 1, 2). There are five different classes of antibodies, namely IgG, IgM, IgA, IgD and IgE, of which IgG and IgM classes are mostly involved in transfusion medicine.

IgM antibodies (Figure 1)

IgM antibodies are produced by B cells as a primary response to foreign antigens. They are pentameric molecules with five Ig units held together by a Joining (J) chain. It contains 10 potential antigen binding sites and thus capable of causing visible agglutination of antigen-positive red cells in the saline phase. IgM antibodies can activate the classical pathway of complement and can cause intravascular red cell haemolysis. IgM antibodies are incapable of transfer across the placenta.

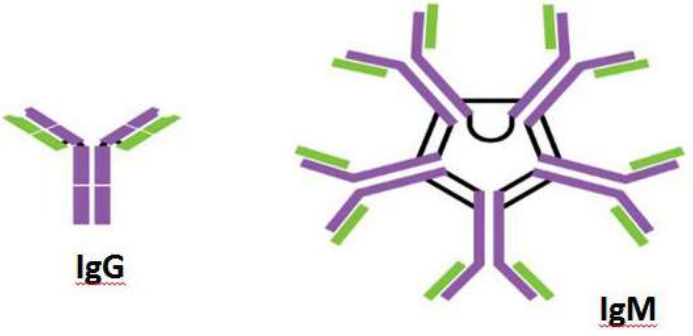


Figure 1: Common immunoglobulin types in immunohaematology

IgG antibodies (Figure 2)

IgG antibody is a monomeric immunoglobulin form that possesses two antigen combining sites. IgG forms approximately 70-75% of immunoglobulin concentration in plasma. It is the principal antibody produced by the body after a second exposure to an antigen. There are four different subclasses of IgG antibodies, namely IgG1, IgG2, IgG3 and IgG4, and minor differences in heavy chains. Due to their small size and bivalent nature, IgG antibodies are not capable of producing visible agglutination of the sensitized red cells suspended in saline and require the use of anti-human globulin (AHG) to produce a visible reaction.

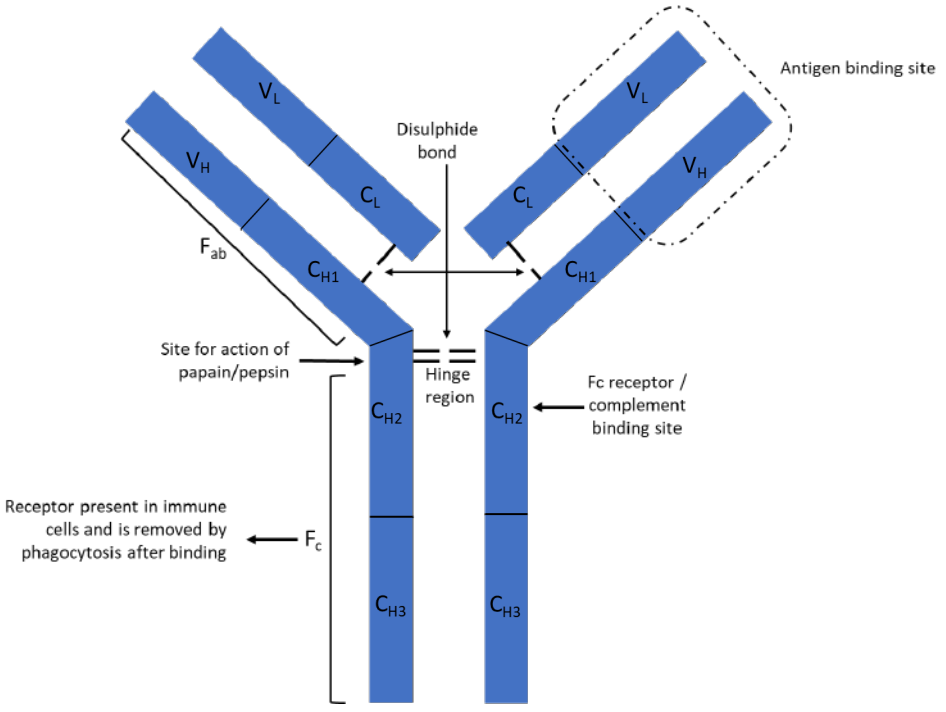


Figure 2: Immunoglobulin structure

IgG antibodies rarely initiate the classical complement pathway and hence rarely cause intravascular haemolysis. These antibodies are responsible for delayed extravascular haemolysis of sensitized red cells. These antibodies can also be transferred across the placenta from mother to fetus and can cause haemolytic disease of the fetus and newborn (HDFN). A comparison between IgG and IgM antibodies is shown in Table 2.

Table 2: Comparison of IgG and IgM antibodies

SN	Characteristic	IgM	IgG
1.	Structure	Heavy chain – Mu Light chain – kappa or lambda Pentameric, joined by J-chain	Heavy chain – gamma Light chain - kappa or lambda Monomeric
2.	Valency	10	2
3.	Serum half-life	5 days	~3weeks
4.	Serum concentration	10%	70-75%
5.	Transfer across placenta	No	Yes
6.	Activation of complement path	Yes	No, sometimes yes, but not efficiently
7.	Haemolysis	Intravascular	Extravascular

Alloantibodies vs autoantibodies

In the context of transfusion, the cellular elements of blood contain a number of blood group antigens which being foreign, can elicit an immune response in the recipient. As these antigens originate from the transfused component of the same species, they are referred to as allogenic antigens or alloantigen. The antigens which are self to the recipient are called as autologous antigens or autoantigens.

The immune response to the transfused antigens is mainly humoral, mediated by B cells and resulting in the formation of antibodies towards foreign antigens called alloantibodies. The immune system recognizes and tolerates self-antigens. The failure to tolerate self-antigens may result in the formation of antibodies to self-antigens, and such antibodies are called autoantibodies.

Immune antibodies vs Naturally occurring antibodies

Antibodies to blood group antigens are produced in response to exposure of the foreign antigen either from transfusion or pregnancy. Red cell antibodies formed due to exposure to foreign red cells are thus called immune antibodies. These antibodies are generally IgG in nature and react best at 37^{o}C. Example of antigens where immune antibodies are formed include antigens belonging to Rh, Kell, Kidd, Duffy, Ss blood group systems etc.

However, antibodies in the case of some blood group systems may be detected even without exposure to foreign antigens in the form of transfusion and pregnancy. A common example is antibodies of the ABO blood group system (anti-A and anti-B). These antibodies are also called naturally occurring antibodies. The antigens in the case of these blood groups have similarity to substances present in the environment, such as pollen grains and part of bacterial membranes, and the antibodies are probably a response to exposure to them. These antibodies are mostly IgM in nature, activate complement, reactive best at room temperature or lower, and are haemolytic if active at 37^{o}C. Other examples include antibodies to antigens belonging to Hh, Ii, Lewis, MN, P blood group systems etc.

Blood group antibodies other than ABO system are sometimes also called as unexpected antibodies as normally one does not expect them to be present in the sera of an individual. They should always be identified before transfusion.

1.4 Antigen-antibody reactions

The antigen and antibodies combine with each other specifically and in an observable manner. The importance of these reactions in the human body is to provide immunity. However, the in-vitro performance of these antigen-antibody interactions is used for the diagnosis of infections and other disorders.

Few important points:

- If an antigen is present over the red cells of an individual, the individual will not form the corresponding antibody.
- If an antibody is detected in the plasma of an individual, he/she should lack that corresponding antigen over red cells.

1.4.1 Types of antigen-antibody reaction

1. **Agglutination:** Antibody-mediated clumping of particles that express antigen on their surface occurs optimally when Antigen and Antibody react in equivalent proportion. e.g.- Red cell agglutination during ABO grouping.
2. **Precipitation:** Development of perceptible, insoluble Ag- Ab complex resulting from a mixture of an equivalent amount of soluble antigen and antibody. Ex- RPR test to screen syphilis.
3. **Agglutination inhibition:** Opposite to what is normally observed in agglutination. This is usually done to detect soluble antigens, which, if present, will combine with the known agglutinin and will result in inhibition of agglutination of the cells with known antigens—e.g.- secretory study to determine the presence of soluble ABO substances in bodily fluids.

4. **Haemolysis:** Antibody-mediated rupture of red cells with a release of intracellular haemoglobin due to activation of complement component by antigen-antibody interaction.

1.4.2 Stages of antigen-antibody reaction

Antigen-antibody reactions generally occur in two stages referred to as the stage of sensitization and the stage of lattice formation.

Primary stage or the stage of sensitization

The binding of the antibody characterizes this stage to an antigen on the red cell membrane. The antigenic determinants on red cell combine with the antigen-binding site (Fab region) of the antibody molecule. Ag- Ab are held together by noncovalent bonds, and no visible agglutination is seen at this stage.

Factors affecting the stage of sensitization (Table 3)

- **Temperature:** IgG antibodies combine optimally with antigens at a temperature of 37ºC. As most of the clinically significant antibodies in transfusion are IgG type, a temperature of 37ºC is used to enhance the antigen-antibody interaction e.g., Anti-D, anti-K etc. In contrast, a few of the antibodies in transfusion medicine are of IgM type, and these interact better at ambient temperature or colder temperatures (4-24ºC), e.g., ABO antibodies.
- **Incubation time:** In order to attain optimal antigen-antibody reaction, a suitable time is required to be given to reach equilibrium. The optimal incubation time will depend on the type of antibody being detected as well as the reaction medium. An incubation period of 30-45 min is considered optimal for most clinically significant IgG antibodies. However, for IgM antibodies, an immediate spin may be sufficient, and no additional incubation period is required.
- **pH:** Antigen-antibody reactions are optimal at a physiologic pH of 7.0. Some antibodies, such as anti-M, may require the pH to be reduced for detection—non-optimal pH results in changes in antigen and antibody structure, affecting the interaction of antigen and antibody.
- **Ionic strength:** In reaction mediums such as isotonic saline, the Na+ and Cl- ions get attracted to the oppositely charged antigen and antibody molecules and provide an anionic barrier to sensitization, slowing the rate of reaction. This barrier can be reduced by lowering the ionic strength of the reacting medium (e.g., using LISS) and increasing the uptake of antibodies by antigens.

Table 3: Factors affecting different stages of antigen-antibody reactions

Factors affecting stage of sensitization	Factors affecting stage of lattice formation
Temperature	Antigen and antibody concentration
Incubation time	Centrifugation
pH of reaction medium	Number of antigenic determinants (zygosity)
The ionic strength of the reaction medium	Distance between red cells (zeta potential)
	Potentiators used

Secondary stage or stage of lattice formation

The secondary stage of antigen-antibody reactions is characterised by cross-linking between the sensitized red cells resulting in a lattice formation, and visible agglutinates.

Factors affecting stage of lattice formation (Table 3)

- **The concentration of antigen and antibody:** The concentration of antigen and antibody have to be in the zone of equivalence for maximum agglutination to be observed. Both antibody excess (prozone) and antigen excess (post zone) result in suboptimal red cell agglutinates. To correct the problem of excessive antibody, the serum may be diluted, whereas for the presence of excess antigens, the serum to cell ratio may be increased.

Immunohaematological testing aims to achieve a zone of equivalence for optimal agglutination. Generally, a ratio of 40:1 is used, which is achieved by using 2 drops of serum and 1 drop of a 5 per cent suspension of cells.

- **Centrifugation:** It forces the sensitized red cells together and helps in a lattice formation. Both the speed and time of centrifugation play an important role in forming the lattice.
- **A number of antigenic determinants:** More the number of antigenic determinants on the red cell more the number of antibodies bound to it. A red cell with a homozygous expression of an antigen (e.g., Jk^a/Jk^a) will result in a stronger agglutination as compared to a red cell with a heterozygous expression of the antigen (Jk^a/Jk^b). This is known as dosage, and it is an important aspect while interpreting the antigrams in antibody screen and identification.
- **Distance between red cells:** Red cells possess a negative charge on their surface, and this attracts the positively charged ions (cations) in the saline solution to their surface. This positive charge around each red cell creates a force of repulsion between them and is called zeta potential. This keeps the red cells at a distance from each other.

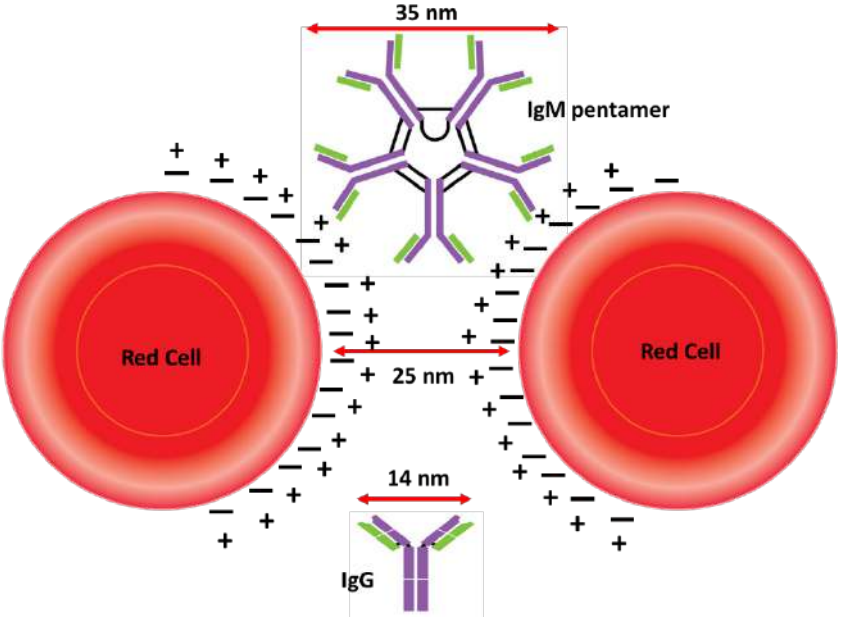


Figure 3: Concept of zeta potential and effect of type of antibody for agglutination

IgM antibodies being pentameric structure and large size can bridge this gap resulting in agglutination of red cells, whereas IgG antibodies cannot do so and require AHG to bridge the gap caused by zeta potential (Figure 3). Other factors that can overcome this distance caused by zeta potential can enhance the lattice formation and be discussed under potentiators.

- **Use of potentiators/enhancement reagents:** A few of the reagents used in immunohaematology tend to reduce the zeta potential and enhance the agglutination or reduce the time of sensitization resulting in a rapid reaction. These reagents are known as potentiators. Examples include anti-human globulin (AHG), albumin, polybrene, polyethylene glycol, low ionic strength solution (LISS), enzymes (e.g., papain) etc. These will be discussed in the reagent section.

1.5 Reagents used in immunohaematology

Antigen and antibody interactions are central to the field of immunohaematology. Most of the investigations involve using either a known antigen to find out an unknown antibody or vice versa. In addition to these, additional reagents either detect agglutination (e.g., anti-human globulin reagent) or enhance the agglutination (e.g., Various potentiators) are also used.

Commercial Antibodies

A good quality antibody reagent contains antibodies specifically directed towards a known antigen present in a high titre. They may be polyclonal or monoclonal.

Polyclonal antibodies

The earliest antibody reagents used were derived from polyclonal sources. In response to a foreign antigen, the body responds immunologically by secreting antibodies from multiple B-cell clones and the corresponding antibody obtained is called the polyclonal antibody. Each clone of B-cell produces antibodies to an epitope of the antigen in question.

These antibodies were earlier obtained either from immunized volunteer donors or by immunizing animals by injecting a source of antigen for which antibodies need to be prepared. The animal responds by producing the antibody by different B-cells, directed against a specific epitope of the antigen. The mixture of these antibodies used to be purified and used as reagent polyclonal antibodies.

Monoclonal antibodies

Monoclonal antibodies are derived from hybridoma cell lines, produced by fusing mouse antibody produced by B lymphocytes with mouse myeloma cells or are derived from a human B cell line through Epstein Barr Virus (EBV) transformation. Each hybridoma cell line produces homogenous antibodies of only one immunoglobulin class, which are identical in their chemical structure and immunological activity. These replaced the need for obtaining antisera from human or animal source. However, these may not detect all antigenic determinants of an antigen and may give a negative reaction if the antibody is derived from a clone lacking a particular antigenic determinant. For this reason, sometimes, more than one monoclonal antibody is blended before use.

Red Cells with known antigens

These include commercial/in-house reagent red cell panels or donor or patient red cells of known phenotype. Examples include A-cells and B-cells to perform serum grouping and red cell reagent panels used for antibody screen and identification.

For blood grouping purpose, donors' cells are typed using known anti-A and anti-B sera. These may be obtained from a single donor or may be used as pooled cells. These may be prepared in-house or may be obtained commercially.

Reagent red cells for antibody screen and identification are generally prepared from O blood group donors. For screening purpose, either a two-cell panel or a three-cell panel is used (Figure 4). Each cell in the panel is derived from an individual donor of O blood group whose cells have been typed for Rh (D, C, c, E, e), Kell (K, k), Kidd (Jk^a, Jk^b), Duffy (Fy^a, Fy^b), MNS (M, N, S, s) and Lewis (Le^a, Le^b) blood group antigens in such a way that they detect all clinically significant antibodies. The manufacturer in a paper provides the antigenic profile called an antigram. Similarly, for antibody identification, a panel of 11-14 red cells is used.

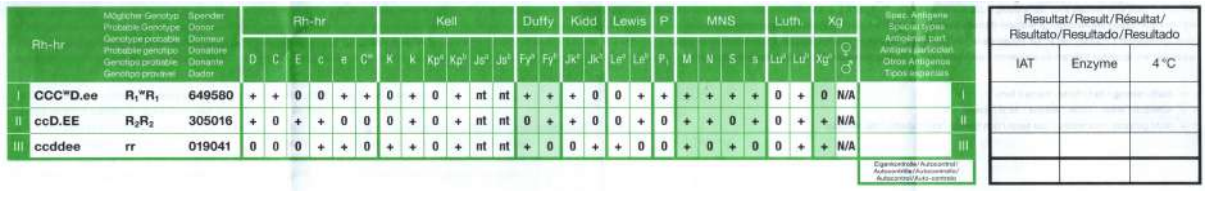


Figure 4: Example of an antigram for interpreting 3-cell panel used for antibody screening

Anti-Human Globulin (AHG)

As is evident from the name, anti-human globulin represents antibodies against Fc portion of human immunoglobulins and complement components. Anti-human globulins are prepared either by classic method or by hybridoma technology. In the classic method of AHG preparation, laboratory animals such as rabbit are injected with human serum or purified globulins and antibodies formed in response to them are identified, isolated, and purified for use. In hybridoma technology, after immunizing laboratory animals with purified human globulin, spleen cells containing antibody-secreting lymphocytes are fused with myeloma cells, and after identifying their specificity and affinity, they are propagated in tissue cultures to produce antibodies. Polyclonal and monoclonal antibodies are obtained using classic and hybridoma techniques, respectively.

The most commonly used AHG is directed towards IgG and C3d. It is called polyspecific if both anti-IgG and anti-C3d are present and monospecific if only one component, i.e., either anti-IgG or anti-C3d, is present. As both IgM and IgA antibodies have been known to fix complement, having antibodies directed to them was not necessary as anticomplement activity will also detect them.

AHG forms the cross-links between red cells sensitized with IgG and/or C3d, bridging the gaps between red cells and helps in a lattice formation. The usefulness of this property will be discussed in the section dealing with the coombs test.

Bovine Serum Albumin (BSA)

BSA has been used as a potentiator in IH tests to enhance the stage of lattice formation by allowing the antibody-coated red cells to come closer together than they would in a saline medium without additives. It is most commonly used in the concentration of around 22-30% for this purpose. It is thought to reduce the zeta potential by increasing the dielectric effect of water and in turn, decrease the repulsion between the red cells to produce the desired effect.

Low ionic strength solution (LISS)

LISS contains sodium chloride, glycine, and salt poor albumin with an ionic strength of 0.03M as compared to that of saline with ionic strength of 0.17M. As zeta potential is due to the ionic cloud across the red cell, lowering the ionic concentration by using LISS increases the rate of antigen-antibody interaction. This enhancement of the stage of sensitization allows for a decreased incubation period. However, it also enhances the cold autoantibodies causing false positivity in the presence of them.

Polyethylene glycol (PEG)

PEG is a water-soluble linear polymer that accelerates red cell antigen-antibody binding by causing steric exclusion of water molecules in the test medium and bringing them closer. PEG promotes the detection of potentially clinically significant antibodies& decrease detection of clinically insignificant antibodies. PEG may enhance warm reactive autoantibodies, and, in such cases, LISS or saline IAT may be preferred to decrease the interference. Centrifugation of PEG and test serum should be avoided because centrifugation can lead to aggregates that are difficult to disperse.

Enzymes

Proteolytic enzymes such as papain, ficin and bromelain are used as potentiators. These act by reducing the negative charge from red cell surface by cleaving sialic acid molecules on red cell membrane and thereby reducing the zeta potential. Enzymes enhance reactivity to some blood group antigens such as Rh, Kidd and Lewis antigens, and they also denature some blood group antigens such as M, N, S, Fy antigens and thus suppress reactivity towards these antigens. For this reason, enzymes are more commonly used to separate a mixture of antibodies showing specificity towards enzyme enhanced/ resistant and enzyme sensitive antigens.

IgG sensitized red cells

These are also known as check cells or coombs control cells. These represent cells that have been sensitized with known IgG antibodies in vitro and are used as a control for negative AHG reactions. On adding check

cells, a negative AHG result would show positivity due to the reaction between unutilized AHG reagent and the sensitized red cells. Thus, these are used to check the possibility of false-negative coombs results.

2. Blood group genetics

Genetics is the study of the mechanism of how different traits or characteristics are passed on from one generation to the next generation. The following text will describe the basic principles of genetics and terminologies as they apply to blood group antigens.

2.1 Chromosome

A chromosome is a thready structure and is an organized package of deoxyribonucleic acid (DNA) present in the nucleus of a cell. Humans have 23 pair of chromosomes, with one chromosome of each pair contributed by one parent. Of the 23 pairs, 22 pairs are called autosomes. The 23rd pair of chromosomes are called as sex chromosomes and are denoted as X and Y, which define the gender of an individual.

2.2 Gene

A gene is considered a basic heritable unit responsible for the transmission of traits or characteristics from parents to offspring. It is a segment of a specific sequence of DNA occupying a specific location on a chromosome and encodes a particular protein.

2.3 Gene locus/ loci

Specific physical location of a gene or DNA sequence on a chromosome.

2.4 Alleles

These are alternative forms of a gene that can occur at the same place or locus on a homologous chromosome. A person inherits two alleles for each gene, one from each parent. If the alleles are the same, the person will be called homozygous for that gene, and if the alleles are different, the individual will be called heterozygous for that gene. For e.g. A, B, and O genes are alleles. If a person inherits A alleles from both the parents (AA), he will be called homozygous for the A gene, but if he inherits one A allele and one O allele, he will be called heterozygous for A gene (AO).

Knowing the zygosity of a blood group allele is of importance in some blood groups. An individual inheriting a heterozygous allele of a blood group (e.g., Jk^a/Jk^b) will have a single dose of antigen being expressed over red cells (one Jk^a antigen and one Jk^b antigen), resulting in a weaker antigen density or weaker antigenic expression. Whereas if the allele is in a homozygous state (Jk^a/Jk^a), a double dose of the antigen will be expressed over the red cells (both Jk^a antigens), resulting in strong antigenic expression. This effect is also known as the dosage effect.

2.5 Genotype

Genotype represents the total genetic makeup of an individual. It however, is used in reference to a particular locus/locus or to a trait or set of traits. Concerning the blood groups, all the alleles of a blood group system present at its locus will represent the genotype of that blood group system. E.g., *BO* or *BB* genotype for B blood group, *CDe*/*cde* genotype for Rh blood group system.

2.6 Phenotype

An individual's observable traits or the physical expression of the inherited traits constitute its phenotype. The antigens resulting from the genes that are expressed will form the blood group phenotype. e.g. Blood group B, D+C+c+E-e+ phenotype resulting from the expression of genotype described above, respectively.

In blood centres, phenotypes can be directly identified by typing the blood group antigens using known antisera whereas genotype may possibly be inferred/predicted from the phenotype. Family studies or molecular tests may be required to determine the genotype of an individual or a trait.

2.7 Polymorphism

A gene is said to be polymorphic if more than one allele occupies that genes locus, and each of these alleles occur in the population at a rate of at least 1%. Events that lead to these polymorphisms can occur at the level of the chromosome, gene, or nucleotide. The most common event is change in the single nucleotide in the DNA, called single nucleotide polymorphism (SNP), and is responsible for polymorphism in most blood groups.

2.8 Pedigree

A pedigree is a graphical representation of familial traits and provides a simple graphical record for communicating genetic data. Each generation is recorded on a new line with the oldest member entered on the left, and the symbol for the male in mating is entered on the left. The other common symbols used to prepare the pedigree chart are shown in Figure 5. It is useful to show the mode of inheritance of a trait and is also useful to identify individuals at risk.

2.9 Inheritance patterns

Inheritance of a trait will depend on whether the gene for the trait is located on autosome or sex chromosome as well as whether the gene is dominant or recessive.

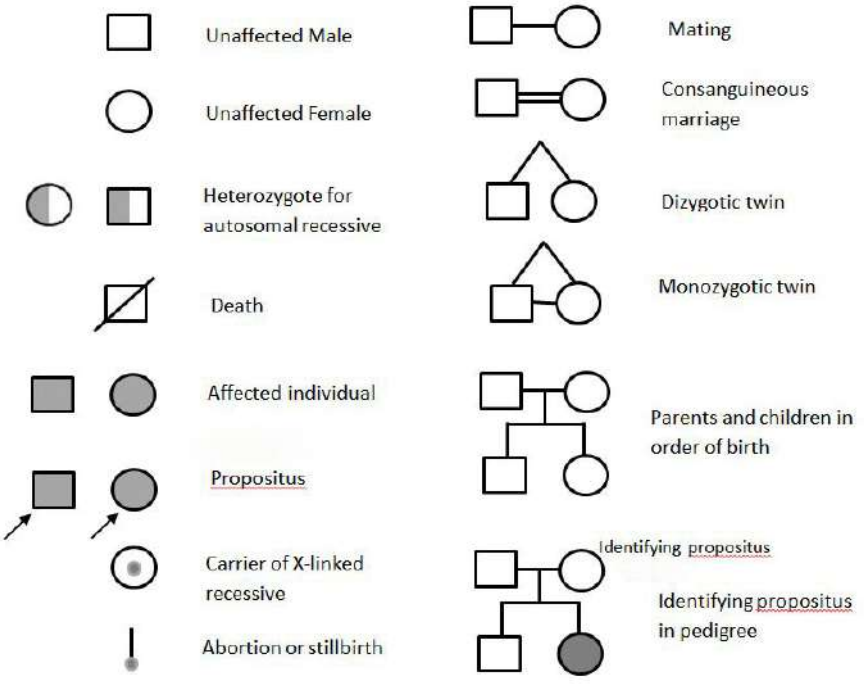


Figure 5 Symbols used to prepare pedigree charts

2.9.1 Autosomal dominant inheritance

In this type of inheritance, the trait will be expressed regardless of the zygosity of the allele. Both male and female express the allele and pass it equally to sons and daughters. These show a vertical pedigree pattern i.e., each person with an autosomal dominant trait has a parent with that trait. The ratio of persons with and without trait in the family is almost always 1:1.

ABO blood group system is an example of this type of inheritance where A and B are dominant over the O allele, and the presence of either of them along with O allele will result in either A or B blood group irrespective of whether a single allele is present, or both the alleles are present. AO and BO will type as A and B blood groups as A and B are dominant over the O allele.

2.9.2 Autosomal recessive inheritance

The trait will be expressed only when present in homozygous condition and a recessive allele has been inherited from both parents. Both the genders will inherit the trait, and the pattern of expression is horizontal, with pedigree showing breaks. When both parents are homozygous for the trait, all the children will express the trait. When both the parents are heterozygous, then only one of the children will inherit the trait in a homozygous condition and express the trait.

The simplest example from blood groups could be the expression of D antigen. A child born to D positive parents who are heterozygous for D (Dd) could be D negative if he inherits both the parents' recessive alleles (dd).

2.9.3 X-linked recessive inheritance

The trait will be expressed very much higher in males than in females. The father will pass the allele for the trait to all the daughters, but they will not express it. A woman with heterozygosity for the trait will pass the allele to half of the sons who express it and half of the daughter who does not express it. The father will never pass the allele to the son. Haemophilia inheritance is a classic example of an X-linked recessive trait.

2.9.4 X-linked dominant trait

The trait is expressed and passed by both genders. Father with the trait will pass the trait to every daughter but never to the sons. A mother with the trait in homozygous condition will pass the trait to all the sons and daughters, whereas a mother with heterozygous alleles for the trait will pass the trait to 50% of sons and daughters. Xg^a blood group system is an example of this type of inheritance.

2.9.5 Codominant inheritance

If neither of the two alleles is dominant and if present, both the alleles will be expressed, it is called co-dominance. A and B alleles in the ABO blood group system are an example of co-dominance. If both A and B alleles are present in an individual, both A and B antigens will be expressed, and the person will phenotype as AB blood group. A number of blood group alleles are codominant, where the presence of alleles of both types of result in the phenotypic expression of both the antigens.

2.10 Mendel's laws

Mendel postulated few principles applicable to the genes of most diploid organisms and in turn to the inheritance of various blood group systems.

Mendel postulated that hereditary characteristics are determined by indivisible units of information (now known as genes). These units occur in pairs in individuals (alleles) of which one is a dominant allele which may mask the traits of the recessive allele. This was called the **principle of dominance**. This pair of alleles separate at the time of gamete formation such that a gamete carries only one pair of the allele, and the pair is restored after fertilization. This was referred to as the **principle of segregation**. A gene/set of genes represents each phenotype and during segregation and reassortment, the alleles of different genes reassort independently of each other. This is called as **law of independent reassortment.**

Blood groups also follow the Mendelian principle. This can be explained using the example of the ABO blood group system. An individual with A blood group can have *AA* or *AO* genotype. He may have both A genes or an A gene and an O gene. 'A' being the dominant gene will always express and mask the traits of the O gene so that an individual with AO genotype also gets typed as A blood group. The gametes from an individual with an AO genotype will carry either A or O allele. These alleles will be inherited discreetly and independently from other blood group genes located on other chromosomes.

2.11 Hardy Weinberg principle

This principle states that the frequency of alleles and genotype in a population will remain constant from one generation to the other if any evolutionary influences are absent. To apply this principle to a population, the following criteria must be fulfilled.

- The population studied must be large.
- Organisms reproduce by the random union of gametes.
- No migration, gene flow, mutation or selection occurs in the population.
- Allele frequencies are equal in both genders.
- Generations are non-overlapping.

For a trait (e.g., Kell blood group), if there are two alleles at a locus with frequencies, namely K=p and k=q Hardy Weinberg principle states that p+q =1 if the criteria's described above are fulfilled.

This principle can be used to calculate the genotype frequencies if the phenotype frequency of one of the alleles is known. For example, if the phenotype frequency of K is known, then the genotype frequency of both K and k alleles can be calculated.

Frequency of K allele = p

Frequency of k allele = q

Then the frequency of KK genotype = p^2

Frequency of Kk genotype = 2pq

Frequency of kk genotype = q^2

Combining the entries i.e., $p^2+2pq+q^2 = 1$ as the frequency of genes must remain 1 through generations. As we know the phenotype frequency of K say x% or 0.0x so,

$p^2+2pq = 1-q^2 = 0.0x$ or $q^2 = 1-0.0x$. which will give the genotype frequency of q or k allele.

As the sum of p+q=1, by knowing the value of q, we can calculate the value of p as well.

However, there are certain limitations of this principle. Gene flow may happen due to the mixing of the population by migration, and non-random mating may also occur in a population. In the example above, K null phenotype also exists and thus will affect the calculation of gene frequency. Despite this, the principle remains the best way to calculate the gene frequency of the blood groups.

2.12 ISBT blood group terminology

Initially, the naming of identified blood group antigens was very inconsistent, and the blood groups were either named after the patient who made the first antibody or donor whose cells carried the antigen. For example, the Kell blood group was named for Mrs Kelleher, in whom the anti-Kell antibodies were identified to be the cause of Haemolytic disease of Fetus and Newborn in her infant. The remaining blood groups were either assigned an alphabetical or numerical notation. As the blood groups increased substantially, it was felt that a uniform and consistent nomenclature was required.

International society of blood transfusion (ISBT) established a working party on 'Red Cell Immunogenetics and Blood Group Terminology' in 1980 to address this problem and to devise and maintain a genetically based numerical terminology for red cell surface antigens. The summarized way of representing blood group antigens, phenotypes, and alleles in ISBT format is presented below.

Blood group antigens–Each blood group antigen within a system is identified by a six-digit number. The initial three digits represent the numerical designation given to the blood group system, and the last three digits represent the antigen specificity. For example, Fy^a antigen within the Duffy blood group system will be identified as 008001 in ISBT nomenclature, where 008 represents Duffy blood group system and 001 represents Fy^a specificity. Alternatively, it can also be written as FY001 where FY is the ISBT system symbol and 001 is Fy^a specificity. It may also be represented as FY1 after removing the zeros.

Phenotype - Phenotypes are represented by a system symbol, followed by a colon, followed by a list of antigens separated by commas. For example, the traditional phenotype Fy(a+b-) will be represented as FY:1, -2 in ISBT nomenclature. Here FY represent Duffy blood group system, 1 represent FY1 antigen, and -2 represents absent FY2 antigen.

Alleles are represented by the system symbol, followed by an asterisk and antigen number, all italicized. For example, the allele for Fy^a will be represented as *FY*1*.

3. Antiglobulin test

Coombs, Mourant and Race first described the antiglobulin test (also called Coombs' test) in 1945. Antiglobulin test is used to detect either in-vivo sensitization of red cells by antibodies or to detect red cell antibodies present in plasma after sensitizing them in-vitro onto red cells. The test helps in differentiating immune causes of haemolysis from non-immune causes and in identifying clinically significant red cell alloantibodies capable of causing red cell haemolysis.

Antiglobulin test is based on the following principle:

- Antibody molecules (IgG) and complement components are globulins.
- Antihuman globulin (AHG) is an antibody against human globulin molecules that reacts with them either bound to the RBCs or present free in the serum.
- Washed red cells coated with human globulins (IgG and complement) are thus agglutinated by AHG, resulting in visible agglutination.

3.1 Need for antiglobulin testing

Human red cells coated with IgG or complement either with autoantibodies or with alloantibodies can undergo haemolysis. In-vitro detection of these antibodies either on the

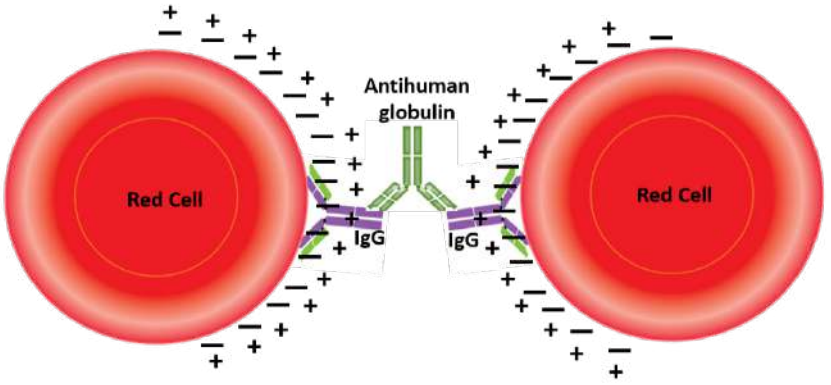


Figure 6: Use of AHG to agglutinate IgG sensitized red cells

sensitized red cells or in the serum is difficult as the red cells sensitized by IgG or complement will not result in visible agglutination. This is due to the inability of these molecules to bridge the gap between two sensitized cells caused by zeta potential due to their smaller size (Figure 6). These antibodies, however, result in significant haemolysis and decreased survival of either own cells or transfused red cells. Antihuman globulin tends to bridge this gap resulting in visible agglutination and thus help in their detection.

3.2 Types of AHG tests

There are two types of AHG tests

a. Direct antiglobulin tests
b. Indirect antiglobulin tests

3.3 Direct antiglobulin test / direct Coombs test (DAT/DCT)

DAT is used to detect in-vivo sensitization of red cells where the addition of AHG directly into a suspension of washed red cells of the patient will result in visible agglutination (Figure 7). It thus helps in differentiating immune causes of red cell destruction from the non-immune causes.

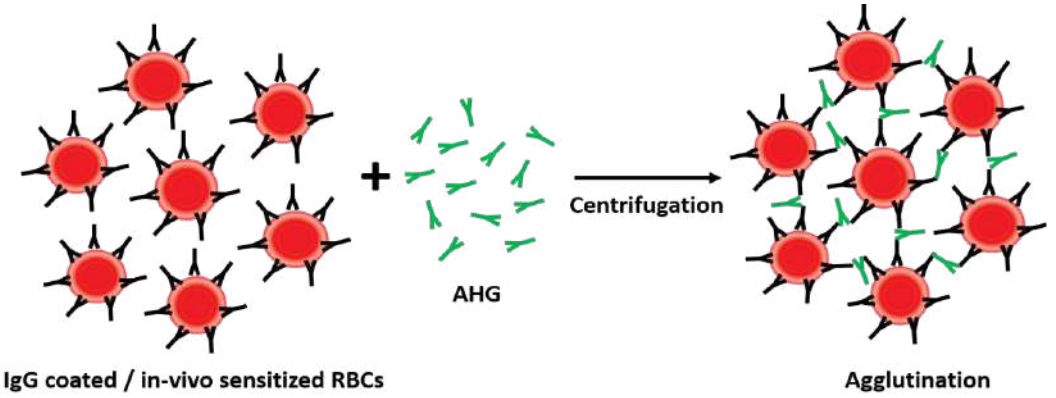


Figure 7: Principle of direct coombs test

DAT is used for the investigation of:

- Autoimmune haemolytic anemia (AIHA)
- Haemolytic disease of fetus or newborn (HDFN)
- Drug-induced immune haemolytic anemia.
- Haemolytic Transfusion Reactions

Other causes where DAT could be positive include:

- Antibodies acquired passively from donor plasma, immunoglobulins etc.
- Nonspecific adsorption of proteins onto red cells in conditions such as hypergammaglobulinemia, administration of intravenous immunoglobulin etc.
- Passenger lymphocyte syndrome e.g.in transplanted organs
- Complement activation in conditions such as bacterial infections.

Performing a direct antiglobulin test

Sample: EDTA-anticoagulated blood samples are preferred as EDTA prevents the in-vitro fixation of complement by chelating the calcium required for complement activation.

Procedure: Washing of the red cells (using normal saline kept at room temperature) removes free plasma globulins and complements (minimum washing for 3 times). Thereafter, DAT is performed by adding Polyspecific antiglobulin reagents (anti-IgG and anti-complement) to freshly washed red cells directly followed by centrifugation.

Test interpretation: The test is positive when agglutination is observed after centrifugation of the test tube. The test is negative when there is no agglutination after centrifugation, provided the 'check cells' gave a positive reaction. The test is invalid when check cells give a negative reaction.

Result inference: A positive DAT result in a patient with haemolytic anaemia indicates that the most likely diagnosis is one of the immune haemolytic anaemias. However, positive DAT does not always indicate the presence of haemolytic anaemia. Therefore, an interpretation of the positive DAT requires knowledge about the patient's diagnosis; drug history, pregnancy, history of the previous transfusion along with the other laboratory parameters indicative of haemolytic anaemia (falling Haemoglobin/haematocrit, increased indirect bilirubin, increased reticulocytes, increased serum Lactate Dehydrogenase, decreased haptoglobin, peripheral smear showing the presence of agglutinates or schistocytes). Thus, a dialogue with the consulting physician is important, and the clinical correlation and laboratory data should be considered while evaluating a positive DAT result.

DAT reactivity may also differ depending on the technique performed as the sensitivity of different techniques depends upon number of IgG molecules coating the red cells also differs (Table 4).

Table 4: Sensitivity of DAT with different methods

Methods of antiglobulin test	Sensitivity (IgG molecules / red cell)
Conventional tube technique	300 – 500
Gel technique	120 – 180
Flow cytometry	30 - 40

3.4 Indirect antiglobulin tests / Indirect Coombs test (IAT/ICT)

An indirect antiglobulin test is used to detect the presence of red cell antibodies in patient's plasma by sensitizing the red cells in-vitro and then detecting the in-vitro sensitized red cells using antiglobulin reagent (Figure 8). It helps to identify red cell alloantibodies in response to previous exposure of foreign antigens by transfusion or pregnancy.

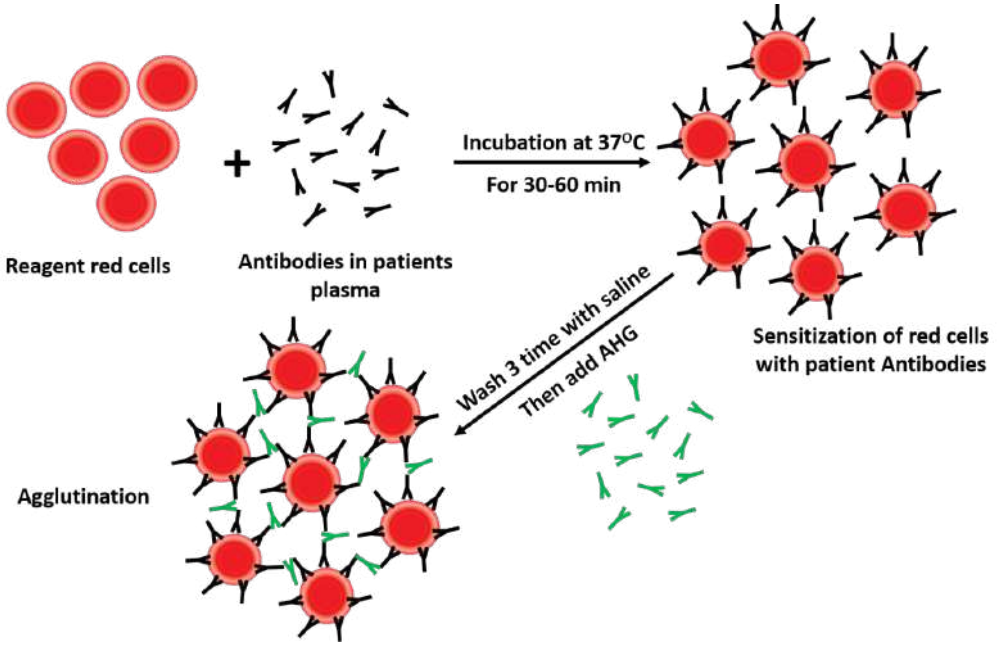


Figure 8: Principle of indirect antiglobulin test

Applications of IAT

- Red cell antibody screening
- Identification of red cell alloantibodies
- Titration studies of incomplete antibodies, e.g., Anti-D titration
- Compatibility testing
- Phenotyping of red cell antigens using IgG antisera.

Performing an indirect antiglobulin test

Sample: EDTA anticoagulated sample/serum sample

Procedure: Patients' serum/plasma is incubated with washed allogeneic red cell suspension at 37°C for 30-60 min to sensitize the cells. The tubes are centrifuged, and agglutination or haemolysis, if any, is noted, followed by washing the mixture to remove unwanted free plasma globulins and complement components. The washed sensitized red cells are then mixed with the AHG reagent and centrifuged before looking for agglutination.

Red cells used for IAT may be prepared in-house, or commercial red cell panels may be used. For antibody screening in patients, a 2-cell panel or a 3-cell panel may be used. For antibody screening in a blood donor, a pool of 2-3 red cells may be used.

Test interpretation: The test is positive when agglutination is observed either after initial centrifugation following incubation at 37ºC or after centrifugation following the addition of AHG. The test is negative when there is no agglutination after both the centrifugation, provided the 'check cells' gave a positive reaction. The test is invalid when check cells give a negative reaction.

Result inference: A positive IAT result will indicate the presence of red cell allo / autoantibodies in the test serum/plasma and should prompt further investigation in the form of antibody identification. Positive agglutination / IAT during compatibility testing would indicate that the donor cells are incompatible with antibodies in the patient's plasma.

3.5 Probable sources of error in antiglobulin testing

False-positive results

1. Auto-agglutinable cells
2. Bacterial contamination of cells or saline used in washing
3. Cells with a positive DAT used for IAT
4. Dirty glassware
5. Over centrifugation or over-reading
6. Polyagglutinable cells
7. Preservative dependent antibodies in LISS reagents
8. Contaminating antibodies in the antihuman globulin reagent

False-negative results

1. Improper procedure
   a. Failure to add test serum, enhancement medium or Anti-human globulin reagent may lead to the negative test result.
   b. Too heavy red cell concentration may mask weak agglutination.
   c. Too light suspension may be difficult to read.
   d. Improper/insufficient serum: cell ratio.
   e. Inadequate washing of cells: result in neutralization of the antiglobulin serum by trace amounts of residual globulin.
   f. Test cells, test serum, and antiglobulin serum lose reactivity if improperly stored.
   g. Over-centrifugation may pack cells so tightly that agitation required to resuspend cells breaks up agglutinates.
   h. Under-centrifugation may not be optimal for agglutination.
   i. The optimum temperature for reactivity of the antibody must be maintained during incubation to achieve maximal coating of the cells.
2. Contamination with human serum: Neutralization of the AHG reagent may occur if the reagent vial gets contaminated with human serum.
3. Elution of antibody from the red blood cells may take place if the test procedure is interrupted or delayed, particularly during the washing phase.
4. Complement: Anticoagulants such as ACD, CPD, or EDTA chelate calcium, preventing activation of complement in the plasma sample.
   a. As some antibodies may be detected only in presence of active complement. E.g., Anti-Jk^a and Anti-Jk^b, use of plasma rather than serum may lead to a false-negative reaction.
   b. Similarly, the use of old or improperly stored serum will also have impaired complement activity.
   c. Rare antibodies notably may be only detected when Polyspecific AHG is used (containing anti-complement component).

5. pH of Saline: An optimum pH of 7.0-7.2 is necessary as saline with low pH can decrease the sensitivity of the Anti-human globulin test. Some antibodies may require saline to be at a specific temperature to retain antibody on red blood cell: Use 37ºC or 4ºC saline.

3.6. Factors affecting the sensitivity of coombs tests:

These factors are similar to the factors which affect different stages of antigen-antibody interactions and have already been discussed in detail in section 1.4.2.

4. ABO blood group system

In 1900, Karl Landsteiner discovered the ABO blood groups and classified human blood into A, B and 0 groups. A fourth blood group AB was discovered by Landsteiner's associates, Von Decastllo and Sturli in 1902.This marked the beginning of the whole subject of blood group serology and made blood transfusion practicable.

The four groups are determined by the presence or absence of blood group antigens (agglutinogens) on the red blood cells, and accordingly, an individual's group is A, B, AB, or O (O denotes the absence of A and/ or B antigens). ABO antigens are also present on lymphocytes, thrombocytes, organs, endothelial cells, and epithelial cells. In addition, it has been shown 'that corresponding to antigens-A and B; there are naturally occurring antibodies anti-A and anti-B (agglutinins) in the plasma/ serum of individuals whose red cells lack the corresponding antigen. (Table 5).

Table 5: The ABO antigens and corresponding antibodies

Antigens on RBC	Antibody in plasma/ Serum	Blood Group
A	Anti-B	A
B	Anti-A	B
A and B	None	AB
None	Anti-A, Anti-B	O

4.1 Genetics and biochemistry

Antigens of the ABO system are detectable at 5 to 6 weeks of gestation. Newborns demonstrate weaker antigens, but ABO antigens are fully developed by two to four years of age.

4.1.1 Genetics

In 1924 Bernstein discovered that ABO antigens follow simple Mendelian genetics. ABO system gene is located on chromosome 9 occupied by one of the three allelic genes A, B or O. The coding region of ABO is organized into seven exons; exons 6 and 7 constitute 77% of it (Figure 9). The expression of the A and B genes is codominant.

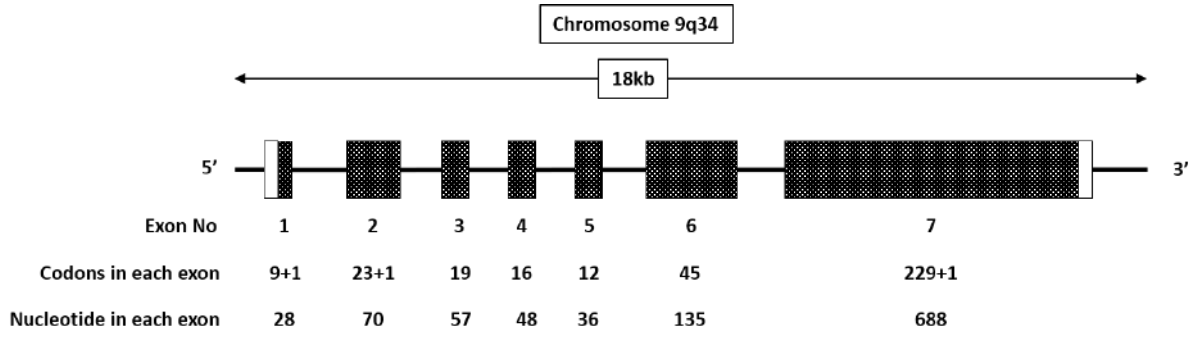


Figure 9: Genomic organization of the ABO gene, showing the seven coding exons with codons and nucleotides in each exon.

In the laboratory, the presence or absence of A or B antigens and their corresponding antibodies is used to determine the phenotypic expression of the inherited genes. Since the O gene does not produce an antigen, it cannot be detected directly, so the lack of A and B antigens on the cells indicates O blood group.

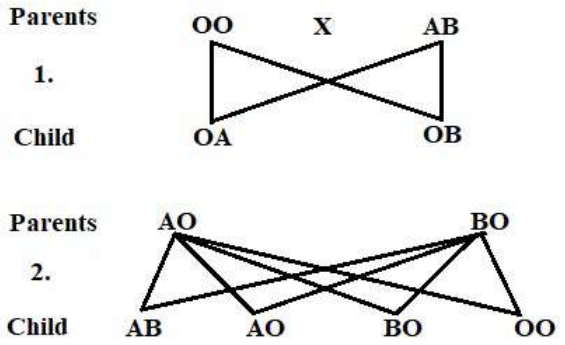


Figure 10: Inheritance of ABO blood groups

The child of the parents in example 1 would test either A or B, and in example 2; the child would be AB, A, B or O. (Figure 10). The presence of O genes could not be determined in the laboratory. But the fact that the child inherited the O gene could be determined only by family studies. Based on this, the possible phenotypes and genotypes in the ABO blood group system are given below (Table 6)

Table 6: Phenotypes and genotypes in ABO blood group system

Phenotypes	Genotypes
A_1	A_1A_1, A_1A_2, A_1O
A_2	A_2A_2, A_2O
B	BB, BO
A_1B	A_1B
A_2B	A_2B
O	OO

4.1.2 Biochemistry

Expression of A, B, and H genes does not result in the direct production of antigens. Rather, each gene codes for producing an enzyme known as a transferase (Table 7). Each transferase catalyzes the transfer of a carbohydrate molecule to an oligosaccharide chain. The attached carbohydrate provides antigenic specificity.

Table 7: Summary of ABH gene and corresponding transferase enzymes.

Gene	Transferase
H	α-L-fucosyltransferase
A	α-3-N-acetyl-D-galactosaminyl Transferase
B	α-3-D-acetyl-D-galactosyl Transferase
O	No Transferase Produced

ABH antigens can be expressed on different oligosaccharide chains attached to either a protein or a lipid molecule. The oligosaccharide is a carbohydrate molecule linked either in simple linear forms or in a complex structure with a high degree of branching. Type 1 and type 2 chains differ in the manner in which terminal galactose joins the subterminal N-acetyl glucosamine. The basic precursor substance (Figure 11) has short

chains of sugars (oligosaccharides). In the type 1 chain, the carbon-1 of galactose is linked to carbon-3 of N-acetyl glucosamine (1-3) linkage (Figure 11 A). In type 2 chain, the carbon-1 of galactose is linked to carbon-4 of N-acetylglucosamine (1-4) linkage (Figure 11B). In the red cell membrane, both glycolipids and glycoproteins with ABH activity are present. In the plasma, only glycoproteins in the soluble form are found. Body serous and mucous secretions contain only glycoproteins insoluble form.

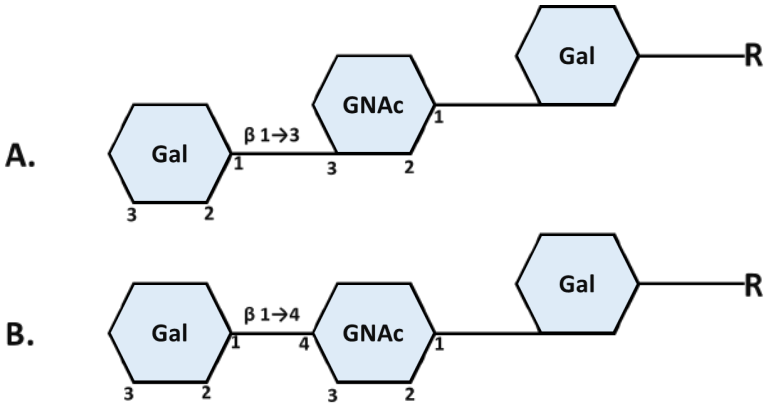


Figure 11: Basic precursor chains. A. type-1 and B. type-2 oligosaccharide chains (Gal: D-Galactose, GNAc: N-Acetylglucosamine, R-Rest of the molecule attached to the protein/lipid)

The basis precursor substance (oligosaccharide) is converted by an enzyme L-fucosyl transferase (a product of H gene) to H substance by adding the sugar L-fucose to the terminal D-galactose of the precursor substance. The H substance is partially converted by the specific transferases, namely N-acetylgalactosaminyl transferase and D-galactosyl transferase (the products of A and B genes) to A and B antigens by the attachment of N-acetyl-galactosamine and D-galactose respectively, to H substance (Figure 12). The O gene is an amorph (no gene product), and group O cells contain only H substance. Some H substance remains unconverted. Thus, all A and/or B cells normally contain some H substance along with A and/ or B antigens. The amount of H substance on red cells in order of diminishing quantity is, $O > A_2 > A_2B > B > A_1 > A_1B$

The expression of A and B genes is dependent on H gene expression. Most individuals are homozygous for the H Gene (i.e., HH). Since its allele h is anamorphic gene, it has no observable effects on precursor substance. The blood group resulting from homozygous hh condition is called the Bombay (O_h) blood group.

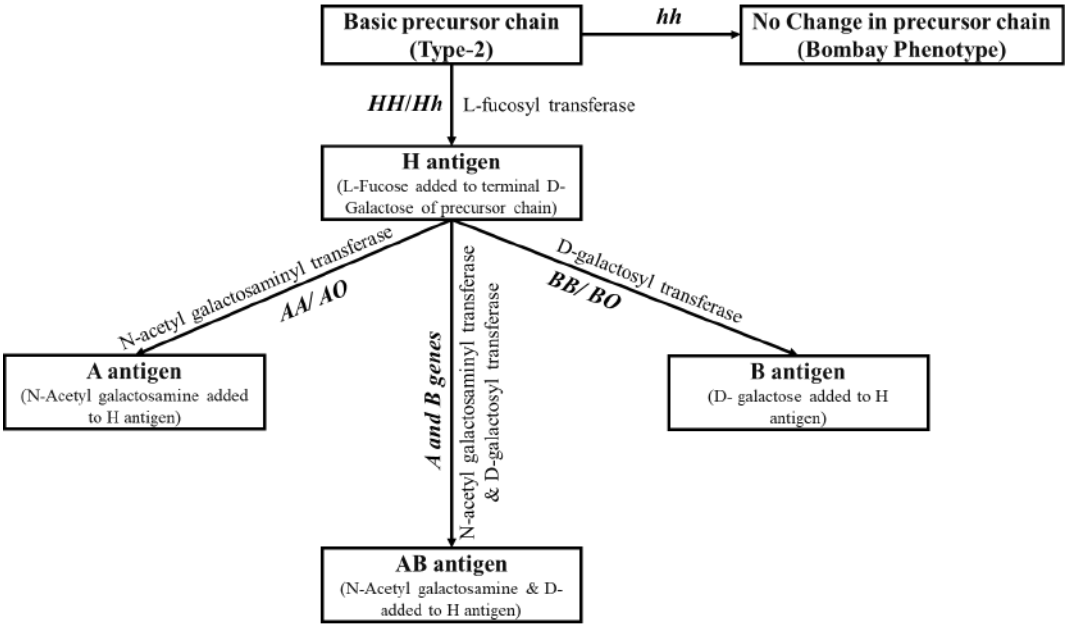


Figure 12: Simple diagram of the formation of A, B and H specific structures

4.2 Secretor status

A, B and H antigens are present on red cells and widely distributed throughout the body tissues except in the central nervous systems. A B and H substances are also found in the secretions of 80% of the population. The ability to secrete A, B and H substance is determined by the presence of the secretor gene (Se) in either the homozygous SeSe or heterozygous Sese state, which is inherited independently of the ABO and Hh genes. Normally all secretors secrete H, in addition to A and/or B substance. (Table 8)

Table 8: Secretor status

Blood group	Substance secreted
O	H
A	A & H
B	B & H
AB	A, B & H
Oh	Nil

4.3 ABO subgroups

ABO subgroups represent phenotypes showing weaker and variable serologic reactivity with human polyclonal anti-A, anti-B, and anti-AB reagents. Some unusual ABO genes affect the activity of the gene products and may result in subgroups of A and B. The weaker serologic reactivity of ABO subgroups is attributed to the decreased number of A and B antigen sites on their red blood cells.

4.3.1 Subgroups of A

Subgroups of A are phenotypes that differ quantitatively or qualitatively from the A antigen carried on the RBCs and found in the saliva of secretors. A_1 and A_2, the two major subgroups of A, constitute 99% or more of group A people tested. The cells of approximately 80% of all group A (or AB) individuals are A_1 (or A_1B), and the remaining 20% are A_2 (or A_2B) and weaker subgroups.

It is not necessary to classify group A patients or donors as A_1 or A_2 except when the individual's serum contains anti-A1. Anti-A1, reacting at 22°C or lower, has no clinical significance, but it is clinically significant if it reacts at 37°C; Anti-A1 causes discrepancies between ABO cell and serum grouping and also cause crossmatch incompatibility. Any patient of group A_2, or A_2B having anti-A1 reactive at 37°C should be given A_2 or A_2B group blood. Quantitative and Qualitative differences in A_1 and A_2 red blood cells are as shown in Table 9.

Table 9: Quantitative and qualitative differences in A_1 and A_2

Differences	Group A_1	Group A_2
Quantitative		
Reaction with Anti-A in Forward Grouping	4+	4+
Number of Antigen Sites-Adults	1,000,000	250,000
Number of Antigen Sites-Newborn	300,000	140,000
Qualitative		
Reaction with Anti-A1	Positive	Negative
Anti-A1 in Serum	Absent	May Be Present
α-3-N-acetyl-D-galactosaminyl Transferase Activity	Normal Activity	Diminished Activity
Branching	Normal	Decreased

Weak subgroups of A

Subgroups weaker than A_2 occur infrequently. They are characterised by the declining number of A antigen sites on red cells and a reciprocal increase in H reactivity. Adsorption and elution techniques may be necessary for the detection of antigens on the surface of red cells. Weaker variants of A are mainly A_3, A_x, A_m and A-intermediate. (Table 10)

Classification of weak A subgroups is based on:

1. Degree of agglutination with Anti-A, Anti-A1 and Anti-AB.
2. Degree of H reactivity on the red Cells
3. Presence or absence of Anti-A1 in the serum
4. Presence of A and H substance in the saliva of secretors
5. Adsorption and elution studies
6. Family (pedigree) studies

4.3.2 Subgroups of B

Subgroups of B exist e.g., B_3, B_x and B_m, but they are rare and even less common than subgroups of A. They can be classified based on reactions shown in Table 11. Widespread use of monoclonal Anti-A and Anti-B reagents has lessened the problems of weak A or B subgroups because monoclonal antibodies can agglutinate cells with weak or aberrant antigen expression.

Table 10: Subgroups of A

Subgroup	Cell typing				Unexpected antibodies in serum	Substance in saliva (If secretor)
	Anti-A	Anti-B	Anti-AB	Anti-H		
A_3	++mf	0	++mf	3+	Sometimes anti-A1	A, H
A_x	wk/0	0	2+	4+	Almost always anti-A1	H
A_{end}	wk mf	0	wk mf	4+	Sometimes anti-A1	H
A_m*	0/wk	0	0/+	4+	–	A, H
A_y*	0	0	0	4+	–	A, H
A_{el}*	0	0	0	4+	Usually, anti A1	H

* A specificity demonstrated only by adsorption and elution
0=negative, mf=mixed field, wk=weak

Table 11: Subgroups of B

Subgroup	Cell typing				Unexpected antibodies in serum	Substance in saliva (If secretor)
	Anti-A	Anti-B	Anti-AB	Anti-H		
B_3	0	00mf	++mf	3+	–	B, H
B_x	0	wk	wk	3+	Weak anti-B	H
B_m*	0	0/wk	0/wk	3+	–	B, H
A_{el}*	0	0	0	3+	Sometimes weak anti-B	H

* B specificity demonstrated only by adsorption and elution
0=negative, mf=mixed field, wk=weak

4.4 Bombay phenotype (O_h)

The Bombay blood group was first reported in 1952 by Bhende in Bombay, India. The frequency in India is around 1:7600. More than 130 Bombay phenotypes have been reported in various parts of the world. It is inherited as an autosomal recessive trait. It lacks the H gene and is homozygous for its allele h (hh). This group is characterized by the absence of A, B and H antigens on the red cells. The serum of these persons contains anti-A, anti-B and anti-H, which reacts with all O blood groups. They are non-secretor of A, B and H substances in saliva. Bombay blood group patients can only be transfused with blood from another Bombay group.

4.5 Para-Bombay phenotype

The para-Bombay phenotype RBCs either completely lack H antigens or have small amounts of H antigen present. The genetic basis for the para-Bombay phenotype is either a mutated H gene (FUT1) with or without an active Se gene (FUT2) or a silenced H gene with an active Se gene. In laboratory testing, red cells from para-Bombay individuals may (or may not) have weak reactions with anti-A and anti-B reagents. The notations A_h, B_h and AB_h describe Para-Bombay A, Para-Bombay B, and Para-Bombay AB blood group, respectively.

4.6 B(A) and A(B) phenotype

The B(A) phenotype is an autosomal dominant phenotype characterized by weak A expression on group B red cells. Serologically, red cells are strongly reactive with anti-B and weakly reactive with monoclonal anti-A (<2+), and they possess a strong anti-A in their sera. In general, the agglutination is weak with fragile, easily dispersed agglutinates. Testing the sample with polyclonal anti-A or a different monoclonal anti-A should resolve the discrepancy.

An A(B) phenotype has also been described with monoclonal anti-B. The A(B) phenotype was associated with elevated H antigen and plasma H-transferase activity. It is hypothesized that the increased H precursor on these cells may permit the synthesis of some B antigen by the A-glycosyltransferase.

4.7 Antibodies of ABO system

The ABH system is unique for the presence of naturally occurring antibodies against missing ABH antigens. These antibodies begin to appear during the first few (3-4) months of life, probably from exposure to ABH antigen like substances in the environment. These antibodies reach a peak by 10 years of age and then gradually decreases. Additional situations that exhibit reduced ABO antibody levels are summarized in Table 12.

Table 12: Conditions with decreased levels of ABO antibodies

- Age related
  - o Newborns and young infants
  - o Elderly individuals
- Immunodeficient individuals
  - o Congenital conditions
- Congenital hypogammaglobulinemia
- Congenital agammaglobulinemia
- Immunosuppressed patients
  - o Immunosuppressive therapy
  - o Chronic lymphocytic leukemia (CLL)
  - o Bone marrow transplant
  - o Multiple myeloma
  - o Acquired hypogammaglobulinemia
  - o Acquired agammaglobulinemia

Anti-A or anti-B antibodies are usually naturally occurring and are mostly IgM. However, some IgG and IgA antibodies are also present. IgG anti-A and anti-B are found more commonly in group O individuals than in A or B individuals. O group individuals may have a high titre of anti-A and anti-B as they are both IgM and IgG and are referred to as high titre O group individuals. High titre antibodies in O groups are important in two situations:

1. O group donors as Apheresis platelets donor.
2. In pregnancy, if the mother is of group O and the baby is of group A or B, the chances of ABO HDN is more as IgG type of anti-A or anti-B can easily cross the placenta.

The IgM antibodies activate complement and often strongly react in vivo and in vitro. Soluble blood group substances neutralize the IgM antibodies.

Anti-A (Anti-A + Anti-A1)

The antibody anti-A is found in group B and group O individuals. Anti-A of group B serum appears from simple studies to contain separable anti-A and anti-A1 and reacts well with A_1 and A_2 cells but not as well with weaker subgroups of A.

Anti-B

The antibody anti-B is found in group A and O individuals and react almost with all B group cells but less effectively with weaker variants of the B group.

Anti-AB

The antibody anti-AB is found in group O individuals, and it reacts with both A and B cells. Anti-A and anti-B specificity cannot be separated by differential adsorption.

Anti-A1

This is found in 1-8% of A_2 and 22- 30% of A2B individuals. Anti-A1 lectin is manufactured from seeds of *Dolichos biflorus* and is available commercially.

Anti-H

Anti-H very rarely occurs as cold reactive agglutinin in individuals with very low levels of H antigen on their cells and have little clinical significance. However, anti-H found in the Bombay group (O_h) can often be potent and reacts strongly at 37°C. It is an IgM antibody capable of binding complement and causing RBC lysis. Anti-H is manufactured from the lectin of *Ulex europaeaus.*

4.7.1 Clinical significance of ABO antibodies

ABO antibodies are capable of causing both Haemolytic Disease of the Fetus and Newborn (HDFN) and Haemolytic Transfusion Reactions (HTR). ABO compatibility is also significant in solid organ transplantation. In ABO-incompatible organ transplant, pre-and post-transplant ABO antibody titre and plasmapheresis to reduce the titre of the incompatible antibody will assist in achieving a positive outcome.

4.8 ABO system and disease association

ABH antigens, besides being found on red cells, are widely distributed throughout the body. Disease association with a particular blood group may light their biological role. Table 13

Table 13: ABO system and disease association

Disease	Risk factor	Blood group
Vascular disorders, venous and arterial thromboembolism, coronary heart disease, ischemic stroke, myocardial infarction	Reduced clearance of von Willebrand factor and FVIII	Groups A > AB > B

Disease	Risk factor	Blood group
Dementia, cognitive impairment	Coagulation factors	Groups AB > B > A
Colon/Rectum Cancer	Type 1 and 2 chains; Lewis antigens	Secretors: "A-like" antigens expressed
Plague, cholera, tuberculosis, mumps	Antigen profile	Group O
Smallpox, Pseudomonas aeruginosa	Antigen profile	Group A
P. falciparum malaria	Receptor/antigen profile	Groups A, B
H. pylori	Strain-dependent	Group A; 95% non-O
Gonorrhoea, tuberculosis, S. pneumoniae, E. coli, Salmonella	Antigen profile	Group B
Smallpox, E. coli, Salmonella	Antigen profile	Group AB
Von Hippel-Lindau and Neuroendocrine	Multiple tumours	Group O
Pancreatic Cancer	H. pylori strain	Group B > AB > A

4.9 ABO grouping discrepancy

Discrepancies are detected when forward and reverse grouping fails to tally each other and hence require further investigations. Common sources of clerical and technical errors resulting in ABO discrepancies are related to one or more of the following:

Clerical issues

- Mislabelled specimen or testing tubes.
- Improper recording of test results

Technical issues

- Not following manufacturer's instructions
- Deleted procedural step.
- Missed or under interpreted weak reactions
- Incorrect interpretation of serological reactions
- Missing or incorrect reagents in test samples
- Equipment malfunction, centrifuge time or incorrect speed
- Contaminated antisera or cells
- Incorrect cell suspension

Clinical issues

These discrepancies occur due to problem inherent to the patient/donor. To solve this, essential information such as age, the patient's/donor's medical condition, medication, recent transfusion history and pregnancy must be considered.

4.9.1 General approach for solving discrepancy

- When investigating ABO group, always remember that RBC and serum grouping reactions are strong (3+ to 4+), and the weaker reactions (<+2) typically represent the discrepancy.
- When a blood group discrepancy is encountered, all results must be recorded, but the interpretation of the ABO type must be delayed until the discrepancy is resolved.
- Obtain fresh blood sample from the donor unit or patient to rule out discrepancy due to contamination or unidentified samples.
- Repeat the test before additional investigations are carried out.
- Repeat cell grouping with fresh antisera (a different lot if possible) as appropriate.

- Perform a direct anti-globulin test on the cells to detect if cells are coated with antibodies in case of previous transfusion or AIHA.
- Quality assurance of reagents, correct technique, careful observations, and interpretation of results resolve many problems.

ABO discrepancies may be arbitrarily divided into four major categories, and examples of ABO discrepancies are given in Table 6.14.

4.9.2 Group I discrepancies

These discrepancies are associated with unexpected reactions in the reverse grouping due to weakly reacting or missing antibodies. These group of discrepancies are more common than other groups.

Common causes are:

a. Newborns
b. Elderly patients
c. Patients with leukemia or lymphoma
d. Patients on immunosuppressive drugs
e. Patients with congenital or acquired agammaglobulinaemia or immunodeficiency diseases.
f. Patients with bone marrow or haematopoietic progenitor stem cell (HPC) transplants
g. Patients whose existing ABO antibodies may have been diluted by plasma transfusion or exchange transfusion.
h. ABO subgroups
i. Substances in plasma or serum

Resolution

The discrepancy can be resolved by enhancing the reaction with prolonged incubation (30-45 min) at room temperature or at 4^0C for 15-30 min. It can also be resolved by increasing the cell: serum ratio.

Substances in plasma or serum

Blood group substances (A and/or B substance) may be in excess amount in any individual's blood. Red cell suspension in the serum or plasma in such individual may neutralize the antibodies in the testing reagents, and they fail to react with the corresponding antigen(s) on the red cells. This type of discrepancy is resolved by washing the red cells three times with normal saline.

4.9.3 Group II discrepancies

This is due to weak or missing antigens resulting in unexpected reactions in the forward grouping.

Common causes are:

a. Subgroups of A or B
b. Leukemia and Hodgkin's disease
c. The "acquired B"
d. Antibody coated red cells (DAT Positive)

Resolution

This can be resolved by enhancing the reaction with prolonging incubation (30-45 min) at room temperature or at 4^0C for 15-30 min. Use Anti-AB and Anti-A1 antisera for subgroups detection. Adsorption- elution and saliva testing may be required for weak subgroups.

Acquired B antigen

Acquired B antigen is usually found in A_1 individual with diseases of the lower intestinal tract:

- Carcinoma of the colon or rectum

- Intestinal obstruction
- Gram-negative septicaemia

In A_1 individual with acquired B antigen, cells agglutinate with anti-A and anti-B, but the reaction with anti-B is a weak or mixed field. Anti-B present in patients' serum does not react with autologous cells. Acquired B antigen should be suspected when the red-cell group appears as AB, and the serum has Anti-B.

To identify an acquired B antigen:

1. Observe the strength of agglutination with anti-A and anti-B. The reaction with anti-A is usually much stronger than with anti-B.
2. Test the patient's serum with his cells: Anti-B in the patient's serum does not agglutinate red cells with acquired B antigen.
3. Test the saliva for the presence of A and B substance. If the patient is a secretor, A substance is present but not B.
4. Use acidified anti-B antisera. It will agglutinate only true B antigens and not acquired B antigens (acidify anti-B typing reagent to pH 6.0 by adding 1 or 2 drops of 1 N HCl to 1 mL of anti-B antisera, and measure with a pH meter.

Antibody coated red cells (DAT positive)

Red cells may be coated with antibodies, and a direct antiglobulin test may be positive (see section 3.3 for details).

When red cells are coated with antibodies, they mask the antigens on the surface of the red cells and give false-negative results.

Resolution:

- When the cells are coated with IgM, non-coated red cells can be obtained by
  1. Wash the cells with warm (37°C) saline.
  2. 45°C elution technique, followed by warm washing.
  3. Treatment of IgM coated red cells with dithiothreitol or 2-mercaptoethanol also provide non-agglutinated samples.
  4. Gentle elution to remove the adsorbed antibody. Repeat the direct antiglobulin test and ABO grouping.
- When the cells are coated with IgG, non-coated red cells can be obtained by
  1. IgG immunoglobulin may be partially removed by 45°C elution.
  2. Treatment of IgG-coated red cells with chloroquine diphosphate may be used to remove IgG immunoglobulin from red cells.

4.9.4 Group III discrepancies

These discrepancies between forward and reverse groupings are caused by protein or plasma abnormalities and result in the rouleaux formation or pseudo agglutination. This can be due to:

a. Elevated levels of globulin
b. Elevated levels of fibrinogen
c. Plasma expanders
d. Wharton's jelly in cord blood samples

Resolution:

Rouleaux is a stacking of erythrocytes that adhere in a coin like fashion, giving the appearance of agglutination. It can be resolved by washing the cells with normal saline 3-4 times. If the serum/reverse grouping is affected, perform saline replacement technique:

- Reagent cells and patient serum centrifuged to allow antigen and antibody to react.

- Serum is removed and replaced by an equal volume of saline (saline disperses cells).
- Tube is mixed, centrifuged, and re-examined for agglutination, rouleaux disperses but not true agglutination.
  - Wharton's jelly: Wharton's jelly in the cord blood may interfere with serologic testing and may cause a discrepancy in ABO & Rh typing. This discrepancy is resolved by properly washing cord blood red cells three times with normal saline.

4.9.5 Group IV discrepancies

Discrepancies between forward and reverse groupings are due to miscellaneous problems such as:

a. Autoantibodies
b. Alloantibodies
c. Mixed field agglutination as in Chimera
d. Cis-AB

Autoantibodies

- Cold autoantibodies e.g., anti-I or anti-HI etc, may cause problems in a reverse grouping. It can be resolved by doing serum grouping after absorbing autoantibodies with auto cells.
- Auto anti-I may react with all adult cells at cold temperature. Cord cells, which exhibit less I reactivity than adult cells, are not generally agglutinated.

Unexpected alloantibodies:

- Anti-A1 in A_2 & A_2B group individuals:

  Resolution is made by testing patient red cells with anti-A_1 lectin and serum with A_1, A_2, and O cell along with auto-control.
- Unexpected antibodies that react at room temperatures such as anti-P1, anti-Le^a, anti-Le^b, anti-M and anti-N can agglutinate cells used for reverse grouping with incubation but may not be demonstrable on the immediate spin if reverse grouping cells happen to have corresponding antigens. Such discrepancy can be resolved by screening and identifying antibodies with panels of cells and selecting A, B and O cells lacking corresponding antigens.

Mixed-field agglutination as in chimera

A chimera is an individual with two separate cell populations, e.g. A cells and O cells. It is very rare. It can be artificial (transient) or permanent.

Artificial (transient) chimera may occur due to

- Transfusion of group O red cells to group A or group B.
- Fetomaternal haemorrhage
- Bone marrow transplantation when the ABO group of the donor is different than that of the recipient.
- Intrauterine transfusion
- Exchange transfusion

One of these cell populations will live only for a short time, making this type of chimera a transient phenomenon.

Permanent chimera may occur when two cell populations exist throughout life, e.g.

- In twins, when an exchange of blood occurs in utero due to vascular anastomosis, both cell populations grow, and both are recognized as self.
- Dispermy (when two sperm fertilize one egg) and result in two cell populations.

Resolution:

In such cases, cell typing can give a mixed field pattern of agglutination. Patient history and perhaps cell separation studies are the best ways to readdress these types of discrepancies.

Cis-AB

It refers to the inheritance of both AB genes from one parent carried on one chromosome and an O gene inherited from the other parent. This results in the offspring inheriting three ABO genes instead of two. RBCs with the cis-AB phenotype (a rare occurrence) expresses a weakly reactive A and B antigen. The serum of most cis-AB individuals contains a weak anti-B, which reacts with all ordinary B RBCs, yet not with cis-AB RBCs. Molecular study and serum transferase levels will help to resolve this discrepancy.

Table 14: Examples of ABO discrepancy

No.	Forward		Reverse			Auto	Cause	Resolution
	Anti-A	Anti-B	A1c	Bc	Oc			
1	4+	0	1+	4+	0	0	Subgroup of A, probable A_2 with anti-A_1	Test patient cells with Anti-A_1 lectin, test patient serum with A_1, A_2 and O cells along with auto control
2	0	0	0	0	0	0	Group O newborn/ elderly patient on immunosuppressive / patient with hypogammaglobulinemia	Check medical records of patient, incubate at RT for 30 min or at 4°C for 15 min
3	4+	4+	2+	2+	2+	2+	Rouleaux	Wash RBCs; saline replacement technique
4	0	0	4+	4+	4+	0	O_h Bombay	Test with anti-H lectin
5	4+	2+	0	4+	0	0	Group A with acquired B antigen	Check medical records of patient; Use acidified anti-B; Saliva testing. Test serum against autologous cell
6	4+	4+	2+	0	2+	0	Group AB with cold alloantibody	Do antibody screen and panel, identify room temperature antibody, repeat serum type with antigen negative reagent cells or perform serum type at 37°C
7	4+	0	1+	4+	1+	1+	Group A with cold autoantibody	Enzyme-treat RBCs and perform auto adsorption at 4°C or perform prewarmed testing

4.10 Universal RBC / Stealth RBC

Two approaches have been used to produce universal group O donor red blood cells (RBCs) from groups A, B, and AB RBCs. The first approach was enzyme-converted group O (ECO) RBCs, and the second was PEGRBC.

The first approach involves cleavage of the terminal immunodominant sugars from carbohydrate chains on the RBC membrane. ECO RBCs have been produced from whole units of B RBCs and transfused successfully to humans. Group A RBCs (especially A_1RBCs) have been more difficult. New sources of enzymes have produced ECO RBCs from A_1 and A_2.

The second approach interferes with an antibody reaching its specific antigen on the RBC membrane by bonding polyethene glycol (PEG) to the RBC. PEG will attract water molecules, yielding a combination that may block most RBC antigens, including A and B antigens. But it has reduced in vivo RBC survival, and PEG is immunogenic in animals and humans.

5. Rh blood group system

The RH blood group system is the most important protein blood group system. It comprises 54 antigens numbered from Rh1 to Rh61 with 7 numbers obsolete. The Rh system is highly immunogenic and complex, with numerous polymorphisms and clinically significant alleles. The five major antigens of the Rh system are D, C, E, c and e. They are coded by two closely linked genes RHD and RHCE. However, the expression "Rh-positive" and "Rh-negative" has come to indicate the presence and absence of D antigen, respectively.

5.1 Discovery of Rh system

In 1939, Levine and Stetson described an antibody in the serum of a group O mother who delivered a stillborn fetus and subsequently developed symptoms of haemolytic transfusion reaction when transfused with her husband's group O blood. They noted that the responsible antibody causing haemolytic transfusion reaction developed in the mother through an antigenic factor from the fetus. The antibody was not named at that time.

A year later, in 1940, Landsteiner and Wiener immunized rabbits and guinea pigs with red cells of rhesus monkeys. The serum of immunized rabbits contained an antibody named anti-Rh, which agglutinated the red cells in approximately 85% of white people tested. Its antigenic determinant was called the Rh factor. The antibody discovered by Levine and Stetson in the mother was subsequently re-examined and found identical inactivity as the anti-RH antibody found by Landsteiner and Wiener. This work led to the discovery of the Rh system.

In late 1940, Wiener and Peters demonstrated that an antibody similar to anti-Rh, responsible for haemolytic transfusion reactions in patients transfused with ABO compatible blood. Later, the evidence established that the animal anti-rhesus of Landsteiner and Wiener was not identical to the human antibody called an anti-Rh antibody, but by that time, the Rh blood group system had already received its name. The anti-Rhesus formed by animals demonstrated by Landsteiner and Wiener was renamed anti-LW in their honour for first reporting it.

In 1943 and 1944, Fisher and Race discovered four additional antigens in the Rh system and thus established five Rh antigens, viz. D, C, E, c and e and their corresponding antibodies anti-D, anti-C anti-E, anti-c and anti-e. A subsequent discovery has brought the number of Rh related antigens over 50, many of which exhibit quantitative variations. But in most Transfusion Medicine settings, five principal antigens D, C, E, c and e and their corresponding antibodies account for more than 99% of clinical issues involving the Rh system.

5.2 Nomenclature of Rh system

Currently there are four systems of nomenclature for Rh blood group namely Fisher-Race, Weiner, Rosenfield and ISBT nomenclature.

5.2.1 Fisher Race: dce Terminology

It is still the most commonly used terminology for Rh. In the 1940s Fisher and Race postulated the theory of three closely linked genes D, C, and E and their alleles d, c, and e. The allele d has not been identified; the notation d indicates the absence of D gene. In Fisher's terminology, the same letter designation is used for both gene and gene products (antigens) except that by convention, the symbols for the gene are always printed in Italics. (Table 15)

According to the Fisher Race theory, a person inherits a set of Rh genes from each parent (haplotype) that constitutes the person's genotype (e.g., *DCe*/*DCe*). The phenotype is determined by the antigens expressed on the red cells (e.g., DCe). Placing parenthesis around in phenotype e.g. (D) indicates weakened antigen expression.

Table 15: Comparison of Weiner and Fisher- Race nomenclature

Rh-Hr of Wiener			CDE of Fisher Race		Shorthand
Gene	Agglutinogen	Factors	Gene complex	Antigen	Notation
R_0	Rh_0	Rh_0hr'hr"	Dce	Dce	R_0
R_1	Rh_1	Rh_0rh'hr"	DCe	DCe	R_1
R_2	Rh_2	Rh_0hr'rh"	DcE	DcE	R_2
R^z	Rh_z	Rh_0rh'rh"	DCE	DCE	R_z
r	rh	hr'hr"	dce	ce	r
r'	rh'	rh'hr"	dcE	cE	r'
r"	rh"	hr'rh"	dCE	CE	r"
r^y	rh_y	rh'rh"	dCe	Ce	r^y

5.2.2 Wiener: The Rh-hr terminology

Wiener favoured the concept of multiple allelic gene at one locus resulting in a complex gene, and he gave the Rh-Hr terminology. He believed that the immediate gene product is a single entity agglutinogen (haplotypes) on the surface of red cells and that each agglutinogen has a number of factors (antigens) each of which is recognized by its own specific antibody. For example, the gene complex R_1 gives rise to the agglutinogen (haplotypes) Rh_1 which possesses the three blood factors (antigens) Rh_0, rh' and hr''. (see table 6.16). In Wiener's terminology, the gene complexes were designated by a single italic letter *R* and *r* with superscript. The gene product agglutinogen (haplotypes) were designated by Roman type Rh and rh with subscripts. The symbol for individual factors were Roman characters in boldface type with **Rh_0** representing **D** and **rh', rh'', hr'** and **hr''** representing C, E, c and e factors (antigens) respectively.

The shorthand phenotype notation mostly used employs single letters R and r in Roman type with subscripts. Thus, R_1 indicates C, D and e antigens; R_2 indicate c, D and E antigens and so on. (Table15).

5.2.3 Rosenfield: numeric terminology

As the blood group system expanded, it became difficult to assign names to new antigens using existed terminologies. Rosenfield and his associates proposed a system that assigns a number to each antigen of the Rh system in the order of discovery or recognized relationship to the Rh system. (Table 16).

This system has no genetic basis but simply demonstrates the presence or absence of the antigen on the red cell. A minus sign preceding a number designates the absence of the antigen on the red cell. If an antigen has not been typed for, its number will not appear in the sequence. An advantage of this nomenclature is that the red cell phenotype is thus succinctly described.

For the five major antigens, D is assigned Rh1, C is Rh2, E is Rh3, c is Rh4 and e is Rh5. For red cells that type D+ C+ E+ c negative, e negative, the Rosenfield designation is Rh: 1, 2, 3, -4, -5. If the sample was not tested for e, the designation would be Rh: 1, 2, 3, -4. All Rh system antigens have been assigned a number (Table 16)

5.2.4 International Society of Blood Transfusion committee: updated numeric terminology

With the discovery of newer antigens and a need for uniform terminology, the International Society of Blood Transfusion (ISBT) came up with the universal numeric terminology. This nomenclature is both machines- and eye- readable and is based on the genetic basis of the blood group. Each authenticated antigen is assigned a six-digit number where the first three digits indicate the blood group system and the last three digits the antigen specificity.

004 is the number assigned for the Rh blood group system by ISBT. It is followed by three digits depicting each antigen under this system. E.g., 004001 represents D antigen. In 2008 the ISBT committee recognized RH-associated glycoprotein (RHAG) as a new blood group system and assigned the number 030.

5.3 Genetics

Now it is well known that Rh proteins are coded by two closely linked genes *RHD* and *RHCE,* present on the short arm of chromosome 1p36.11. Like ABO, Rh genes exhibit codominant expression. *RHD* codes for the presence or absence of D antigen and *RHCE* encodes the CE antigen in four combinations (CE, Ce, cE, ce), respectively. Both *RHD* and *RHCE* genes have 10 exons and are 97% identical.

Table 16: Wiener, Fisher-Race and Rosenfield nomenclature

Wiener	Fisher Race	Rosenfield
R_1r	DCe/dce	Rh: 1, 2, -3, 4, 5
R_1R_1	DCe/DCe	Rh: 1, 2, -3, -4, 5
rr	dce/dce	Rh: -1, -2, -3, 4, 5
R_1R_2	DCe/DcE	Rh: 1, 2, 3, 4, 5
R_2r	DcE/dce	Rh:1, -2, 3, 4, 5
R_2R_2	DcE/DcE	Rh: 1, -2, 3, 4, -5
r' r	dCe/dce	Rh: -1, 2, -3, 4, 5
r' r'	dCe/dCe	Rh: -1, 2, -3, -4, 5
r" r	dcE/dce	Rh: -1, -2, 3, 4, 5
r" r"	dcE/dcE	Rh: -1, -2, 3, 4, -5
R^0r (R^0R^0)	Dce/dce (Dce/Dce)	Rh: 1, -2, -3, 4, 5
r' r" (r^yr)	dCe/dcE (dCE/dce)	Rh: -1, 2, 3, 4, 5

Another gene important for the expression of Rh antigens is *RHAG*. It is located on chromosome 6p21. It determines the successful expression of Rh antigens by coding for the Rh-associated glycoprotein (RhAG). It forms complexes with the Rh protein within the RBC membrane. Mutations in this gene can cause absent/ decreased expression of RhD and RhCE proteins.

5.4 Rh antigens - phenotype and genotype

The phenotype denotes the expression of antigens on red blood cells, which can be determined with anti-sera. The genotype denotes the gene complex that codes for the antigen in a particular individual.

The phenotype of an individual can be reported by detecting common Rh antigens on red cells by five antisera: anti-C, anti-c anti-D anti-E and anti-e. However, an individual's genotype with respect to Rh cannot be predicted precisely by serological methods and is largely based on the frequencies of antigens, gene complexes, and individual gene.

An individual inherits one combination from each parent, giving rise to genotype like CDe/cde (R_1r) etc. Such thirty-six possible genotype combinations could occur. The number of phenotypes would, however, depend upon the type of anti-sera used for testing. Rh system antibodies show dosage phenomenon.

5.5 Biochemistry of Rh antigens

Rh antigens are found only on red cells and are an integral part of the red cell membrane. They are highly hydrophobic, non-glycosylated proteins that span the red cell membrane 12 times with few extracellular loops. The proteins encoded by both genes (RHD and RHCE) differ by 32-35 amino acids. The protein coded by RHCE gene carries both C/c and E/e antigens. The amino acid position 103 determines C or c expression. Similarly, amino acid position 226 determines the E or e expression. (Figure 13)

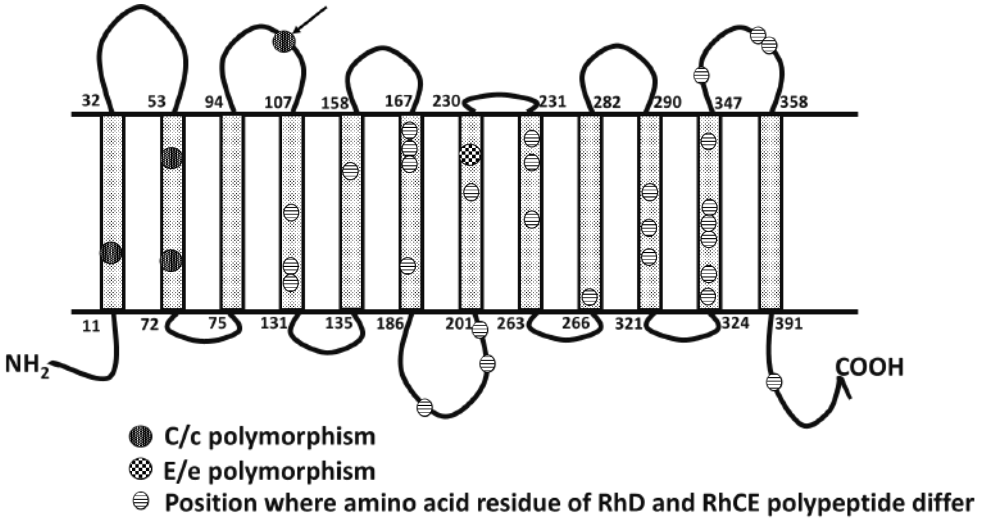


Figure 13: The predicted 12-transmembrane domain model of RhD and RhCE polypeptide. C/c and E/e polymorphisms are depicted with the most important C/c site arrowed. Differences in RhD and RhCE polypeptides marked in the diagram. (Representational presentation only)

Currently, more than 275 alleles of *RHD* and 50 *RHCE* alleles have been discovered. Newer antigens are formed due to single nucleotide polymorphisms in the gene arrangement. The RHAG protein is 38% identical to RhD/RhCE protein. Rh proteins have an important structural role in the erythrocyte membrane. The Rh complex is linked to the membrane skeleton through CD47-protein 4.2 interactions and Rh/RhAG-ankryin interactions.

5.6 D antigen

Unlike ABO antigens, Rh antigens are present only on red blood cells. Rh(D) antigen is clinically the most important in the Rh system because it is highly antigenic. An individual is designated as Rh-positive or Rh-negative depending on the presence or absence of D antigen on the red cells. In India, 94.61% of the population is Rh(D)-positive, and 5.39% is Rh(D) negative, but this percentage varies slightly in different parts of the country. In Caucasians, 85% of the population is Rh(D)-positive, and 15% is Rh(D)–negative.

The D antigen is made of various epitopes that were originally defined by anti-D antibodies made by sensitized individuals. D epitopes are highly conformational, and the amino acid changes have resulted in altered D antigens. Altered D antigens are classified into Weak D, Partial D, Del, and non-functional RhD.

5.6.1 Weak D

Red cells with a reduced amount of D antigen requiring an Indirect Antiglobulin Test (IAT) for detection are termed as Weak D (D^u). D^u red cells are agglutinated by some anti-D sera and not by others, but mostly these react by the AHG technique. Weak D is caused by SNP that alter the aminoacid sequences in the intracellular or transmembrane portion of Rh protein, affecting the insertion of protein in the membrane.

There are two grades of weak D. Red cells of the higher grade of weak D are agglutinated by certain anti-D sera, while red cells of the lower grade are detectable only by AHG test (IAT). The incidence of weak D in India is reported to be between 0.3-0.5% (Bhatia, 1985). A 2017 review estimates 0.2%-1% of routine RhD blood typing result in a “serological” weak D phenotype. Among the various types of weak D, type 1, 2 and 3 are the most common, accounting for almost 90%. Most of the weak D individuals do not make anti-D when transfused with D positive blood.

Clinical significance of weak D (D^u)

- **Donor Typing** – Weak D is much less antigenic in comparison to D; however, D^u red cells may be destroyed if transfused to a person already having anti-D. Also, D^u red cells, if transfused to D negative patients, can

sensitize them, which can cause HDN later (D negative females of reproductive age group). Hence, weak D testing has to be done on all D negative donor units before transfusion. Those that turn out positive should be labelled Rh(D)-positive.

- **Recipient typing** - There is controversy in handling weak D recipients. Weak D testing is currently not required for a recipient. The weak D recipients are classified as Rh(D) negative and safely transfused with Rh(D) negative blood. Some centres choose to test recipient for weak D also and transfuse weak D recipient with weak D positive blood.
- **Weak D testing in Infants**- Weak D positive infant can suffer from HDN if the mother possesses anti-D antibodies. Weak D testing in newborns plays an important role in determining who should receive anti-D immunoprophylaxis with Rh immunoglobulin (RhIG). Newborn infants of Rh(D) negative mothers are tested for D, and weak D and RhIG is recommended for the mother of D positive or weak D positive infants to prevent potential immunization.

5.6.2 Partial D

Partial D individuals type as D positive but can make anti-D when they are exposed to conventional D antigen. Earlier known as "category D", these variant forms are caused due to changes in the extracellular part (epitopes) of Rh proteins. The majority of partial D phenotypes are caused due to exchange of genetic material from *RHCE* to *RHD*. This can result in the loss of D epitopes on Rh protein forming new antigens. DVI is the most common form of partial D found in Europeans.

5.6.3 D_{el} (D-Elution)

These red cells have an extremely low level of D antigens that can be detected only by adsorption and elution studies and not by any of the routine serological methods (not even IAT). These are caused due to mutations in the *RHD* gene that causes decreased expression.

5.6.4 D-Negative phenotype

The frequency of "Rh-negative" individuals varies among different ethnic groups. In India, the overall D negative prevalence is 5%, but it varies from region to regions, such as 2.55% in Maharashtra, 6.49% in Uttarakhand and 0.53% in Sikkim. In Whites, the prevalence of Rh-negative phenotype is 15%, whereas it is lesser in Africans (6%) and Asians (1%)

In Caucasians, D negative phenotype is because of *RHD* gene deletion, while in Africans and Asians, it is because of gene rearrangements and mutations. The pseudo-*RhD* gene is mainly responsible for the Rh-negative phenotype in African individuals.

5.7 Other Rh antigens - C/c and E/e

Other common Rh antigens are C, c, E and e. These are less immunogenic than the D antigen. D is considered to be the most potent immunogen, followed by c and E. The order of immunogenicity is as follows

$$D > c > E > C > e$$

More than 50 *RHCE* alleles have been identified, which give rise to compound and variant C/c and E/e antigens. Altered C and altered e are most commonly encountered resulting from nucleotide changes in the *RHCE* gene. Individuals with altered C/c/E/e antigens might type positive for the antigen and still make antibodies to them.

5.8 Variants of Rh antigen

About 100 Rh antigens have been discovered so far. A variant of C/c viz. C^w, C^u, C^x, C^v and of E/e viz. E^w, E^u, e^s and compound antigens viz. G (present on cells with C and D), f (combination of c and e) etc., have been described. The commonest among C/c variants is $C^{w.}$

5.9 G antigen

G antigen is found on red cells with either D or C antigen. Antibody to G antigen can appear as anti-D plus anti-C and cannot be separated. Its significance lies in the fact that anti-G antibody in a D-Negative woman

who delivered D-(C+) infant can mimic anti-D. Such mothers can still make anti-D when exposed to D positive cells and hence are candidates for RhIG prophylaxis. Adsorption elution studies can distinguish Anti-D, -C and -G.

5.10 RH null syndrome

The absence of all Rh antigens characterizes Rh null. "Regulator type" Rh null is caused by mutations in the *RHAG* gene. In this type, normal Rh genes are present. However, they are unable to express themselves. "Amorph type" Rh null is caused by nucleotide changes in the *RHCE* gene and deletion of the *RHD* gene.

Rh null red cells exhibit membrane abnormality (stomatocytes), resulting in their reduced survival. In addition, these individuals are more prone to develop antibodies to the Rh system.

5.11 Rh antibodies

Rh antibodies are clinically the most significant after anti-A and anti-B antibodies. Almost all Rh antibodies result from immunization by pregnancy or blood transfusion. They are immune (IgG) antibodies except, very rarely, for a few examples of anti-E and anti-C^w that occur without any stimulus, and which may be saline (IgM) antibodies. Rh(D) is considered to be the most antigenic, followed by c and E.

Although few Rh antibodies react in saline, most react best in high protein, antiglobulin or enzyme tests. Even saline-reactive anti-D usually react to higher dilution by AHG test. The reactivity of Rh antibodies can be enhanced by enzyme treatment of red cells. Rh antibodies are reactive at 37^{o}C and do not bind complement (though it can occur in rare cases) when they combine with corresponding antigens. Hence Rh antibodies cause extravascular haemolysis.

Rh antibodies are the major cause of haemolytic disease of fetus and new-born (HDFN) and lead to the destruction of transfused Rh-positive red cells (HTR). Anti-c causes the most severe HDN following anti-D, among the Rh antibodies. Rh immunization persists for many years. Even if the level of circulating antibodies falls below the detectable threshold, subsequent exposure to antigen results in rapid secondary response and antibody formation. Rh antibodies show dosage effect.

5.12 Reagents for Rh typing

The different types of anti-Rh (D) sera available are:

1) Polyclonal (human) anti-Rh(D) serum.

   Potentiating substances such as albumin, enzyme, AHG serum are used in test to bring about agglutination with polyclonal (human) IgG anti-D.

2) High protein Anti-D reagents for slide test or rapid tube test

   These reagents contain IgG anti-D obtained from human plasma. Macromolecular additives are added to optimize their reactivity in slide/tube test. However, chances of false positivity are high because of the protein content. Hence manufacturer's direction is to be strictly followed, and results should be validated with controls.

3) Anti-D sera for saline tube test (low protein antisera)

   Two kinds of saline active anti-Rh(D) sera available are:

   - The traditional saline reagent is made from raw material containing predominantly IgM antibodies which agglutinate Rh(D) antigens positive cells suspended in saline. These are low protein-based and can be used to type IgG coated cells. These are relatively scarce due to the non-availability of raw material. Another drawback is their high cost of production and lengthy incubation time. Since they are composed of IgM mainly, they cannot be used for Weak D testing.
   - Saline-active reagents prepared from IgG antibodies have been chemically modified to convert them to agglutinate in the saline medium. These can be used in both the slide and tube method. Manufactured Rh controls are not required if simultaneous ABO typing is done. However, separate control needs to be run if Rh typing alone is done or the sample tests as AB Rh-positive.

4) Monoclonal Rh(D) antibodies.
   - Monoclonal anti-D reagents are made predominantly from human IgM antibodies, requiring no potentiators and agglutinate most D positive red cells from adult and infants in the saline system.
   - Monoclonal anti D reagents usually promote reactions stronger than those with polyclonal IgG reagents, but they may fail to agglutinate red cells of some partial/weak-D antigen categories. Adding a small amount of polyclonal anti-D to the monoclonal antibodies provide a reagent that will react with partial/weak-D antigen red cells in the AHG test.

     The types of monoclonal anti-D reagents are:

     1. IgM anti-D Monoclonal reagent
     2. Blend of IgM and IgG anti-D Monoclonal antibodies reagent
     3. Blend of Monoclonal IgM and Polyclonal (human) IgG anti-D

The monoclonal IgM anti-D is highly specific, and saline reacting equally well at room temperature and 37°C. They are good for slide test or immediate spin tube tests and routine Rh(D) typing in tubes.

Blend of IgM and IgG (monoclonal) anti-D or blend of IgM (monoclonal) and polyclonal (human) IgG anti-D reagents can be used for testing weak D (D^u) by IAT/AHG. Mostly blended IgM and IgG (monoclonal) anti-Rh(D) or blended monoclonal IgM and polyclonal (human) IgG anti-Rh(D) antibodies are used now in routine.

Controls of Rh(D) typing

Known O Rh(D) positive (O, R_1R_2) and O Rh(D) negative (O, rr) cells are used as positive and negative control cells. AB serum/6% to 10% bovine albumin/diluent control supplied with anti-D reagent can be used as negative control according to manufacturer's direction with polyclonal anti-D reagents and high protein sera.

It has been recommended that the test for Rh typing must be done with two anti-Rh(D) sera from two different manufacturers or with two different batches from the same manufacturer.

5.13 Rh phenotyping

Rh typing generally refers to determining the D antigen status, whereas the term "Rh phenotyping" refers to the testing of the red cells for the presence or absence of all five principal Rh antigens in order to determine the phenotype. Commercial antisera specific for the five major Rh antigens are available that can be used for testing on slide/tube/ gel card method.

5.14 Rh genotyping

Genotyping employs molecular techniques in order to determine the gene sequence of the antigen. Since the *RHD* and *RHCE* genes are closely linked, the exchange of genetic material between them is possible, giving rise to a variety of novel alleles and antigens. These novel antigens can be picked up and assigned to a blood group system with the aid of genotyping. For more on the molecular testing, check the section on molecular blood grouping.

5.15 Clinical significance of Rh

Transfusion

Rh(D) typing is a critical component of pretransfusion testing. When Rh antibodies are detected, it is necessary to provide an antigen-negative crossmatch compatible unit for transfusion. Extended phenotyping and matching for Rh and Kell would be desirable if resources permit. Rh antibodies are IgG class and can cause clinically significant haemolysis when transfused with antigen-positive blood. They are reactive at 37°C. Policies regarding D typing and transfusion of blood components should be based on patient population, risk of immunization to D, and limited supply of D-negative blood components.

Haemolytic disease of the fetus and newborn

HDFN caused due to Rh antibodies is very severe because these antigens are well developed on fetal cells, and the antibodies are IgG type that can readily cross the placenta. RhIg immunoglobulins have been successful

in preventing HDFN in susceptible D negative mothers. RhIg needs to be given to unsensitized Rh(D) negative mothers who deliver Rh(D) positive fetus. This has to be given within 72hrs of the delivery. Hence, that makes typing the infants for D antigen necessary.

The red cells from an infant suffering from HDN are coated with immunoglobulin. The infant's red cells may be so heavily coated with antibody that all antigen sites are occupied, leaving no antigenic site to react with anti-D. The "blocking" phenomenon should be suspected if the infant's cells have a strongly positive DAT, and the cells do not react with anti-D. The antibodies coated on the infant's red cells are removed by the heat elution method, and the red cells free from antibody are used for Rh typing and cross-matching. For elution technique, see chapter on special methods.

5.16 LW antigen system

Since the LW and Rh antigens are strongly related phenotypically, a discussion of the LW blood group system is often integrated with that of the Rh system. It was found that anti-Rh obtained by injecting rhesus monkey cells into rabbits and guinea pig (Landsteiner and Wiener) was not identical to the human antibody called an anti-Rh antibody, but by that time, the Rh blood group system had already received its name, and these antibodies were termed as anti-LW.

LW antigen is a high-frequency antigen system and genetically independent from the Rh system. Phenotypically, there is a similarity between Rh and LW system. Anti-LW reacts strongly with most D positive cells, weakly (some not at all) with D- negative red cells, and never with Rh null cells. Sometimes anti-LW is found as a warm auto-immune antibody in warm type acquired immune haemolytic anaemia (AIHA).

6. Other blood groups

There are >300 red blood cell antigens identified so far classified into >35 blood group systems or into other three classes (Table 17).

Routinely for blood transfusion, ABO and Rh D compatibility is considered most important; however, many of the other blood groups are clinically significant as antibodies formed due to previous exposure by transfusion or pregnancies are capable of causing haemolytic transfusion reaction and haemolytic disease of the fetus and newborn. For blood transfusion purposes, these are taken care of by recipient red cell antibody screening, identification and by providing antigen-negative crossmatch compatible blood. Patients who receive multiple transfusions are more likely to form these antibodies. A sound understanding of other blood groups is required to identify the antibodies and provide RBC antigen-negative blood or extended phenotype matched blood.

Some of the other blood groups may have naturally occurring antibodies that are mostly IgM and do not react at body temperature and are not clinically significant but are nuisance antibodies and interfere with in vitro immune-haematological tests.

Many blood group phenotypes have disease associations; therefore, transfusion medicine specialists should have a sound understanding of blood groups beyond ABO and Rh.

6.1 Duffy (FY) blood group system (008)

History

In 1950 a red cell antibody was identified in a multi transfused haemophiliac patient (Mr Duffy) by Cutbush, Mollison and Parkin (1950), and a blood group system was named after the patient. The antibody thus identified was named as anti-Fy^a and the corresponding antigen as Fy^a. The antithetical antigen was named as Fy^b.

Genetics, antigens, and phenotypes:

Duffy gene (DARC) is located on chromosome 1, and expression is identified by 3 antithetical genes, Fy^a and Fy^b, which are codominant and a silent gene *Fy* which results in four phenotypes Fy (a+b-), Fy (a-b+), Fy (a+b+) and Fy(a-b-). The frequency of Duffy blood group system antigens varies greatly between different

populations. The silent recessive gene Fy, which is very rare in the white population, has a high frequency in homozygous form (FyFy), resulting in Fy(a-b-) phenotype in black African populations particularly in malaria-endemic regions. Duffy antigens are present in many cells of the body.

- Duffy glycoproteins serve as a receptor for *Plasmodium vivax* and *Plasmodium knowlesi;* hence Fy(a-b-) red blood cells are resistant to invasion by these parasites.
- These antigens are sensitive to most proteolytic enzymes, i.e., papain, ficin and bromelain but not to trypsin.
- The antigens also progressively deteriorate in RBC suspensions made in saline or LISS solutions; they are, however, well preserved in anticoagulated blood and RBC preservative solutions.

Antibody characteristics:

- Anti–Fy^a and anti-Fy^b are IgG antibodies which form in respective antigen-negative person following exposure by transfusion or pregnancy.
- Like any other IgG antibody against RBC antigens, they can be detected and identified by IAT technique using antigen-positive cells.
- However, unlike many other IgG antibodies against RBC antigens, they cannot be identified by enzyme techniques as enzymes destroy Duffy antigens. This fact, however, is useful to differentiate and identify antibodies where antibodies to multiple antigens are suspected.

Clinical significance:

- Duffy system antibodies are capable of causing severe extravascular haemolytic transfusion reactions and HDFN.
- Antigen negative and IAT crossmatch compatible blood should be transfused to patients with antibodies.

6.2 Kidd (Jk) blood group system (009)

History:

The Kidd blood group system was discovered by Allen et al (1951) through an antibody identified in sera of Mrs Kidd whose child suffered from immune HDFN. This 'new' antibody was named as anti Jk^a. In 1953, Plaut et al identified antithetical antibody anti Jk^b.

Genetics, antigens, and phenotypes:

The two main antigens of Kidd blood group system Jk^a and Jk^b are expressed due to pair of co-dominant alleles on chromosome 18. In some individuals, specially Polynesians, South American and South African Indian populations, the gene is a silent recessive gene *Jk* resulting in Jk(a-b-) RBC phenotype. These Jk(a-b-) RBC are resistant to lysis by 2 M Urea (Urea lysis test).

Antibody characteristics:

- Anti Jk^a and anti Jk^b are IgG antibodies (incomplete) which form in respective antigen-negative person following exposure by transfusion or pregnancy (Immune antibodies). These antibodies however activate complement in a much more efficient way than other IgG antibodies resulting in a greater likelihood of intravascular haemolytic transfusion reaction.
- The antibodies are detected by the IAT technique, although some weak examples react by enzyme technique. Some of these antibodies may require complement for detection therefore it is recommended to use a fresh serum (and not plasma) for their detection.
- The avidity and titre of anti-Kidd antibody often decrease rapidly in an immunized person; therefore, it should be recorded and available in-patient records once identified. It may not be detected in subsequent samples for transfusion demand; even then, the antigen-negative IAT crossmatch compatible blood should be transfused.
- Anti-Jk3 can be a potent IgG antibody detected by IAT in Jk(a-b-) individuals.

Clinical significance:

Transfusion:

- Both anti-Jk^a and anti-Jk^b are known to cause severe acute and delayed haemolytic transfusion reactions.
- Anti Jk3 should be considered clinically significant and Jk(a-b-) IAT compatible blood should be sought from rare donor registry.
- Haemolytic disease of newborns
- The antibodies are known to rarely cause HDFN.

6.3 Kell blood group system (006)

History:

The Kell antigen system was discovered in 1946 by Coombs, Mourant and Race.

Genetics, antigen characteristics and phenotypes:

The Kell blood group system is extremely complex. The locus for Kell antigens *(KEL)* is present on chromosome 7. Twenty-four Kell system alleles have been identified. The Kell system is also associated with the Kx (019, locus on X chromosome) and Gerbich blood group system (020, locus on chromosome 2).

The two main antithetical antigens of this system are K (KEL 1) and k (KEL 2); the possible phenotypes being K+k-, K+k+, and K-k+. After the ABO and Rh antigens, the K antigen is the most immunogenic RBC antigen. The system, however, has more than 30 antigens ascribed to it; the other well-known antithetical antigen pairs are Js^a/Js^b and Kp^a/ Kp^b, k, Kp^b and Js^b are high-frequency antigens.

- Kell antigens are well expressed on fetal red cells and erythroid precursors.
- Kell antigens are type II membrane glycoproteins sensitive to disulphide bond reducing agents, i.e., DTT and AET but is resistant to papain, ficin, trypsin, α-chymotrypsin, and glycine.
- Kell antigen glycoproteins are linked to Xk protein that expresses Kx blood group antigen (019) by a single disulphide bond. The absence of Xk protein results in decreased expression of both Kell glycoproteins and Kell antigens (McLeod phenotype); this happens despite the gene for Kell (K/k) being normal.
- In contrast, Ko or Knull phenotype results from the inheritance of homozygous recessive silent gene Ko and none of the Kell antigens are expressed.
- Kell antigens are enzymatically active and can cleave biologically inactive peptide big-endothelin-3 to create biologically active vasoconstrictor endothelin-3.
- Kell antigens are also present on myeloid progenitor cells, and Kell glycoprotein has been in seen testis, lymphoid tissues and with Xk protein in skeletal muscles.
- All the Kell antigens are destroyed by DTT.

Antibody characteristics:

- Anti-K and anti-k are usually IgG antibodies, detected in vitro by IAT and sometimes by enzyme techniques.
- Anti-K reacting at temperatures below 37^{o}C have also been reported.
- Although anti-K is usually an 'immune' antibody produced in response to exposure by transfusion or pregnancy, cases with naturally occurring anti-K have also been reported, which probably result due to bacterial infection such as Escherichia coli.
- As k-antigen is a high-frequency antigen in most populations, if a patient develops anti-k, it is very difficult to provide k antigen-negative blood, and rare donors registries should be contacted to arrange blood.

Clinical significance:

Transfusion:

Anti–K and anti–k are clinically significant and cause severe haemolytic transfusion reaction, including delayed haemolytic transfusion reaction. A patient with anti-K or anti-k should be provided respective antigen-negative,

IAT crossmatch compatible blood. Multi-transfused patients should be preferably transfused K-antigen, and the various Rh antigen matched blood to prevent the formation of immune antibodies.

Haemolytic disease of the fetus and newborn:

Antibodies of the Kell blood group cause severe haemolytic disease of fetus and newborn. The severity of anaemia in HDFN caused by anti-Kell antibodies is much more pronounced than caused by other blood group antibodies, often causing fetal death and is not related to the titre of anti-Kell antibodies. This is because, in addition to immune haemolysis, anaemia is also caused by suppression of erythropoiesis by anti-Kell antibodies as Kell antigens are well expressed on fetal and neonatal red cells and also on erythroid precursors. As a result, the rise in bilirubin is also not proportionate to the degree of anaemia. Amniocentesis, therefore, does not indicate the severity of the disease.

Kp^a and Kp^b antigens

Kp^a and Kp^b antigens result due to other codominant allelic pair of the Kell blood group system. Kp^a is a low incidence antigen (<2%), and Kp^b is a high incidence antigen (>98%). Antigen and antibody characteristics are similar to K and k-antigens and likewise the clinical importance also. If antibodies are detected, antigen-negative IAT crossmatch compatible blood needs to be transfused.

Js^a and Js^b:

Js^a antigen is found mostly in blacks (~ 20%), whereas in whites, the incidence is < 0.1%. Js^b is a high incidence antigen (>98%). Anti-Js^a is rarely seen. Anti-Js^b is mostly detected in blacks and is IgG type and known to cause HDFN and HTR.

Kx Blood group system (019)

This is not part of Kell system and is only mentioned here to understand its relationship to Kell blood group system and to differentiate them.

The only antigen of this system is Kx which is located on Xk protein encoded by gene *Xk* on X chromosome. The absence of this gene makes a person Kx negative as in very rare X linked disorder. As males have only one X chromosome, Kx negative males have McLeod's syndrome characterized by the presence of acanthocytosis, late-onset muscular, neurologic, and psychiatric symptoms. They also have a very weak expression of Kell antigens on RBCs despite inheriting normal Kell genes, termed as McLeod's phenotype. These persons can make anti-Kx and anti-Km on exposure which are clinically significant and can cause severe HTRs (Not HDFN as only males have the disease and form these antibodies).

Kx negative individuals may also have a deletion of *CYBB,* which results in chronic granulomatous disease.

6.4 Indian blood group system (023)

History:

The first antigen of the system (In^a) was described in 1973 by Badakere et al and named for its finding in an Indian population. Giles established an antithetical relationship of salis antibody with anti-In^a and therefore named it anti-In^b. In 1995, it was formally recognized as an independent blood group system. In^a and In^b were given the antigen numbers IN1 and IN2. In 1988, Spring and colleagues demonstrated IN location on the leukocyte homing & adhesion molecule, CD44. In 2007, two new high-incidence antigens were identified, namely IN3 (INFI), IN4 (INJA). In 2017, another high-frequency antigen, IN5 (INRA) was discovered by SR Joshi in India.

Genetics, antigen characteristics and phenotypes:

Indian blood group system antigens are carried on the CD44 glycoprotein. CD44 molecule is a glycoprotein and major adhesion molecule present on the surface of numerous different cell types (ability to bind hyaluronan, a component of the extracellular matrix). It is also a major red cell membrane component of apparent MW 80 kDa encoded by *CD44 gene* located on the short arm of chromosome 11 (11p13) and spans 50 to 60 kb of

DNA & consists of 20 exons. In^a and In^b are antithetical antigens, of which In^a (IN1) is a low-frequency antigen and In^b (IN2) is a high-frequency antigen. IN3, IN4 and IN5 all are high-frequency antigens.

- Estimated 6000 to 10 000 copies of CD44 per red cell.
- Destroyed by the proteases papain, pronase, trypsin, and chymotrypsin, but resistant to sialidase. Also destroyed by disulphide bond reducing agents like AET and DTT though higher concentrations of the AET/DTT may be required.
- Reduced expression (25-38%) of Ina in cord samples and pregnant women.
- Normal adult number of Ina in 3–6 months after birth.
- No such weakness of In^b on cord red cell samples.
- CD44 is present in serum and In^b can be detected in serum by haemagglutination inhibition.

Antibody characteristics:

- Indian antigens appear to be good immunogens. In one report 30 of 39 In(a–) donors immunized for anti-D production with D+ In(a+) RBCs made anti-In^a induced by both transfusion and pregnancy.
- Though anti-Inb produced in an un-transfused woman during her first pregnancy has also been reported.
- Antibodies to Indian blood group antigens can be detected by direct agglutination of red blood cells (RBCs) in saline medium but react stronger by the indirect antiglobulin test (IAT).
- Antibodies are known to cause HTRs and reduced survival of transfused RBCs. Some observations on the In^b antigen and evidence that anti-In^b causes accelerated destruction of radiolabelled red cells.

Clinical significance:

Transfusion:

Indian blood group antibodies can cause acute haemolytic transfusion reaction and reduced survival of radiolabelled RBCs, as mentioned earlier; hence if detected, antigen-negative blood should be transfused.

Haemolytic disease of the fetus and newborn:

Neither anti-In^a nor anti-In^b has been reported to cause HDFN. In(b+) cord cells from babies born to mothers with immunoglobulin, IgG1 anti-In^b does not usually give a positive direct antiglobulin test (DAT), and anti-In^b cannot usually be detected in the infants' sera. In one case with maternal anti-In^b of high titer-RBCs obtained from the baby yielded a positive DAT, and anti-In^b could be eluted from baby RBC; however, no sign of HDN was there. It is postulated that binding of anti-In^b to CD44 on fetal monocytes and macrophages could have a blocking effect on FcγR1.

6.5 Lewis blood group system (007)

History:

In 1946, Mourant discovered the antibody anti-Le^a. The antithetical antibody was discovered by Anderson in 1948. In 1951, Grubb demonstrated the presence of soluble Lewis antigens in plasma and saliva. Sneath and Sneath demonstrated that Lewis antigens on RBCs are adsorbed from plasma.

Genetics, antigens, and phenotypes:

Le^a and Le^b antigens are not synthesized on RBC and are adsorbed from plasma. Besides being present on secretions and RBCs, Lewis antigens are also found on platelets, endothelium, kidney, genitourinary and gastrointestinal epithelium.

The alleles of the Lewis system are dominant Le, and amorphic gene le and the possible genotypes are LeLe, Lele and lele. If the genotype is lele there would be no Lewis substances in saliva or RBC regardless of the secretor status, and the RBC phenotype would be Le(a-b-).

If a person inherits the Lewis gene, i.e., genotype LeLe or Lele, then Lewis substances in saliva and RBC are determined by the secretor status. Secretion of Le^a in body fluids is independent of secretor gene so if a

person is non-secretor then only Le^a substance would be secreted in plasma and saliva and adsorbed on RBC, resulting in RBC phenotype as Le(a+b-). Secretion of Le^b substance is dependent on inheritance of Secretor gene (Sese or SeSe) so a secretor would have both Le^a and Le^b substances in saliva but as Le^b is preferentially adsorbed onto RBC resulting in phenotype Le(a-b+). Table 18. shows the interaction of Lewis and secretor genes and resulting PRBC phenotypes and substances in secretions.

Lewis antibodies:

- Lewis antibodies are not clinically significant (Table 19).
- These are mostly IgM and naturally occurring antibodies in Le(a-b-) individuals; anti-Le^a is more common than anti-Leb.
- Occasionally Le(a+b-) individuals can produce anti-Le^b.
- Le(a-b+) individuals do not make anti-Le^a because they have Le^a substance in their secretions.
- The presence of Lewis antibodies does not require transfusion of antigen-negative blood. The units required to be only crossmatched compatible at 37ºC AHG crossmatch.
- Lewis antibodies are more reactive with enzyme-treated cells than untreated cells.

Other interesting facts:

- After transfusion to Le(a-b-) individuals, Le(a+) and Le(b+) RBCs lose their Lewis antigens to become Le(a-b-) in few days.
- Lewis antigens weaken during pregnancy so much so that woman may become Le(a-b-) though she is not so genetically.
- Lewis antigens are weakly expressed during the neonatal period, and correct adult phenotype often does not express until 6 years. The phenotype Le(a+b+) may be observed in children whose adult phenotype would be Le(a-b+) during the transient developing phase.

6.6 MNS blood group system (002):

History:

Landsteiner and Levine discovered the M and N antigens in 1927. S and s antigens were discovered by Walsh and Montgomery (1947) and Sanger and Race (1947).

Genetics, antigens, and phenotypes:

The genes for MN antigens and Ss antigens are closely associated. Family studies show the tight linkage between *M/N* and *S/s*. M & N alleles are codominant, and so are genes for S and s antigens. A small proportion of blacks are S-s- and in most cases, they are also negative for a high-frequency antigen called U; such persons can make anti-U in addition to anti-S and anti-s upon immunization. Some S-s- cells are U+. However, the U antigen is very weak. The alleles for M and N antigens are located on glycoprotein Glycophorin A (GPA, CD235A) and are trypsin sensitive, whereas S, s and 'N' are located on Glycophorin B (GPB, CD235B) and are trypsin resistant. However, M, N, S, s, 'N' all are destroyed by treatment of RBCs by papain, bromelain or ficin.

Antibodies:

Clinical relevance of antibodies is shown in Table 19.

Antibodies against M & N antigens are mostly naturally occurring and not clinically significant. However, if the antibody is clinically significant in laboratory testing, antigen-negative and crossmatch compatible blood should be transfused. In contrast, anti-S and anti-s and anti-U antibodies form upon exposure to respective antigens and are generally IgG antibodies reactive at 37ºC and are capable of causing HTRs and HDFN.

6.7 P blood group system and GLOBOSIDE collection (003 and 028)

These blood group systems are considered together as some of the antigens which were initially considered part of P blood group system are now assigned to Globoside collection (028).

History:

The P blood group system was discovered by Landsteiner and Levine (1927) by immunizing rabbits with human red cells, and the antibody thus obtained was called anti-P, which classified humans red cells into P+ and P-. Later P antigen was designated as P1, and P+ designated as phenotype P1 and P- as P1- were designated as phenotype P2. Both P1 and P2 individuals, however, have P antigen on their red cells. Hence antigens on P1 RBCs are P and P1, and on P2 RBC are P+P1-. With this designation, another group of individuals were identified who were P-P1- and were termed as p phenotype. In 1957, Matson and Co-workers described Pk phenotype, which had a high-frequency P^k antigen but lack P antigen. The p phenotype individuals were also found to lack P^k antigen in addition to P antigen.

Genetics, antigens, and phenotypes:

The genes for P1 and P^k are located on chromosome no. 22 and the gene for P antigen is located on chromosome no.3. Thus, the antigens designated to this system were P1 and high-frequency P and P^k antigens, and the phenotypes identified so far were P_1, P_2, P_1^k, P_2^K and p. Subsequently, however, high-frequency P, P_k and LKE antigens assigned to a separate blood group system Globoside collection (028) as shown in Table 20.

So, as of now P blood group system (003) has only one antigen that is P_1 and two phenotypes P_1 and P_2. Whereas Globoside collection (028) has P, P^k antigens and 3 phenotypes P_1^k, P_2^k and p.

Antibodies:

- Anti-P1 is usually an IgM type antibody. It is commonly encountered as a cold agglutinin but occasionally reacts at 37°C. However, some rare examples may bind complement and react by IAT.
- Anti-P1 that binds complement and reacts by IAT may cause transfusion reactions.
- Other antibodies that are not part of the P blood group system are noted here:
- Anti-PP1P^k (previously called anti-Tj^a) is a rare, potent antibody found in the very rare type p individuals. The antibody reacts at all temperatures by all methods and is frequently present as a haemolysin. It causes transfusion reactions and is a potential cause of recurrent abortions. It rarely causes HDFN.
- Anti-P is found in the serum of all P^k individuals and will haemolyze P1 and P2 cells in the presence of complement. Anti-P is also found in cases of paroxysmal cold haemoglobinuria (PCH). PCH is a haemolytic disease which occurs mainly in children following a viral infection. The sera from such patients give a positive Donath-Landsteiner test.

Clinical significance

Transfusion:

Anti-P1 is not generally considered to be clinically significant even when reactive at 37ºC, and it is not usually necessary to select antigen-negative blood. The crossmatched blood is considered compatible if negative by IAT.

Haemolytic disease of the fetus and newborn:

Anti-P1 has not been reported to cause HDFN.

Other Features:

- Anti-P1 is usually not of clinical significance.
- P1 antigen is weakly expressed at birth.

- P1 substance can be found in various flatworms and hydatid (tapeworm) cysts in sheep livers. P1 substance from avian sources, e.g., pigeon egg white, can be used in inhibition tests.
- The frequency and avidity of anti-P1 is increased in P1- individuals suffering from helminth infestations (parasitic worm, e.g., hookworm).

6.8 Ii blood group system (027)

History:

Ii blood group system was discovered by Weiner et al in 1956.

Genetics, antigens, and phenotypes:

The *I* gene **(***IGnT, CGNT2***)** is located on chromosome 6 and codes for β-1-6 N-Acetyl-glucosamine transferase, which converts linear i into branched I antigen.

I and i antigens are present on all cell membranes. The red cells of almost all healthy adults have I antigen. Fetal and neonatal red cells are i phenotype and do not have I antigen. During the first 18 months of life, red cells gradually come to react strongly with anti-I and weakly with anti-i.

An increase in i-antigen on adult red cells can also be observed in chronic haemolytic disorders and is an indication of stressed erythropoiesis.

Very rarely, adults may have little or no I-antigen, and this phenotype is denoted as i_{adult}, it is an abnormal recessive phenotype caused by mutations in the *I* gene (*IGnT* or *CGNT2*). Two genetic disorders are associated with an increase in i-antigen on adult red cells; In the Asian population, the i_{adult} phenotype can be associated with congenital cataracts and a marked increase in i-antigen is also observed in HEMPAS (Hereditary erythroblastic multinuclearity with positive acidified serum lysis test).

Antibody characteristics

- Anti-I antibodies are usually typed IgM antibodies that frequently occur as cold reacting autoantibodies or cold agglutinins. They are seldom seen as an alloantibody.
- However, potent auto anti-I cold reacting antibodies can cause cold autoimmune haemolytic anaemia.
- The antibodies may be detected at 37°C by enzyme or IAT methods, but they are not normally clinically significant.
- Anti-i is occasionally found in patients recovering from diseases such as infectious mononucleosis (glandular fever).
- Anti-I may be associated with anti-H, forming antibodies with anti-HI specificity.

Clinical significance

Transfusion

- Although most anti-I antibodies are not clinically significant, some examples of auto anti-I with a wide thermal range can be seen in cold haemagglutinin disease. Should these patients require blood transfusions, they may be given the least incompatible blood, warmed in validated blood warmer before infusion.
- The patient's samples may be difficult to type if the patient's cells are auto agglutinated. The red cells may need to be washed with warm saline before testing.
- Crossmatching must be very carefully performed to ensure that the auto anti-I is not masking clinically significant antibodies.

Haemolytic disease of the fetus and newborn

Ii system antibodies have not been implicated in HDFN.

6.9 Lutheran blood group system (005):

History:

The Lutheran blood group system was first discovered by Callender et al. in 1945 and fully described by Callender and Race in 1946. The antithetical antibody was discovered by Cutbush and Chanrain in 1956 thereafter; the original antigen was named Lu^a and the antithetical antigen as Lu^b.

Genetics, antigens, and phenotypes:

The Lutheran locus is a part of a linkage group located on chromosome 19, including Secretor, Lewis, H and LW loci. The main antithetical antigens are Lu^a (low frequency) and Lu^b (high frequency) though 19 antigens (Lu6/Lu9, Lu8/Lu14, Au^a/Au^b and other 10 other high-frequency antigens) have been so far identified. The expression of Lutheran antigens is variable, and dosage effects may be seen.

Taking into consideration Lu^a and Lu^b four phenotypes have been described as Lu(a-b+), Lu(a+b+), Lu (a+b-) and Lu(a-b-) or null type.

Antibody characteristics:

The antibodies may be stimulated by pregnancy or transfusion.

- Anti-Lu^a is usually an IgM type antibody reacting by saline techniques. Reactions with anti-Lu^a often show a typical 'mixed field' result.
- Anti-Lu^b is usually an IgG type antibody reacting best by IAT.
- Anti-Lu^a is seldom seen and rarely causes a problem in the crossmatching laboratory as compatible blood can be easily found.

Clinical importance:

Transfusion

- Lutheran antibodies have been reported to cause mild or delayed transfusion reactions.
- IAT crossmatch compatible blood should be transfused.
- The provision of Lu(b−) blood may be difficult, but the antibody is seldom seen.

Haemolytic disease of the fetus and newborn

Lutheran antibodies have not been reported to cause severe HDFN as the antigens are only weakly expressed on cord cells.

Table 20 summarizes the other blood group systems which one may infrequently come across in clinical practice.

Table 17: Blood group antigen classification

Systems	Collections (200 series)	Low incidence antigens (700 series)	High incidence antigens (901 series)
Systems consist of one or more antigens controlled at a single gene locus or by two or more very closely linked homologous genes with little or no observable recombination between them	*Collections (200 series)* consist of serologically, biochemically, or genetically related antigens, which do not fit the criteria required for system status.	*700 Series* or low incidence antigens with an incidence of less than 1% and cannot be included in a system or collection.	*901 Series* or high incidence antigens with an incidence of greater than 90% and cannot be included in a system or collection.
Blood group system 001 to 030	Ii and Glob collections, Cost, Er, Vel	By, Chr^a, Bi, Bx^a, Pt^a, Re^a, Je^a. Li^a, Milne, RASM, JFV, Kg, JONES, HJK, HOFM, SARA and REIT	Lan, At, Jr, Emm, AnWj, Sd, PEL, MAM

Table 18: Interaction of Secretor and Lewis genes

Genotype Secretor	Genotype Lewis	Lewis Antigens in saliva	RBC phenotype
SeSe or Sese	LeLe or Lele	Le^a, Le^b	Le (a-b+)
Sese	LeLe or Lele	Le^a	Le(a+b-)
SeSe or Sese or sese	lele	None	Le (a-b-)

Table 19: Important features of other common clinically important blood group systems

ISBT System Name (No.)	Chromosome location	Associated blood group Antigens [Null phenotype]	Clinical Significance	
			HTRs	HDFNs
MNS (002)	4q	M, N, S, s, U, He, Mi^a (MNS7), Mur (MNS10), Vw + may more [En(a-); U-; M^kM^k]	Possibility: Yes with • Rare anti-M and anti-N active at 37°C, • Anti-S, anti-s, anti-U, and others	• Anti-M rarely • Anti-S, anti-s, anti-U cause severe HDFN
P1Pk (003)	22q	P1, P^k	Very rarely if active at 37°C	No
Lutheran (005)	19q	Lu^a, Lu^b and much more Recessive Lu (a-b-)	Possible • Mild DHTR with anti-Lu^a, anti-Lu^b. • AHTRs with anti-Lu8.	No
Kell (006)	7q	K, k, Kp^a, Kp^b, Ku, Js^a, Js^b and many more [K_0 or K_{null}]	Cause severe AHTRs & DHTRs	Cause severe HDN
Lewis (007)	19p	Le^a, Le^b, Le^{ab}, Le^{bh}, ALe^b, Ble^b, [Le(a-b-)]	Not considered clinically significant generally	No
Duffy (008)	1q	Fy^a, Fy^b, Fy3, Fy5, Fy6 [Fy9a-b-)]	Can cause AHTRs and DHTRs	Can cause HDFN
Kidd (009)	18q	Jk^a, Jk^b, Jk3, [Jk(a-b-)]	The common cause of DHTRs, anti-Jk^a and anti-Jk3, also cause AHTRs	Not usually
Kx (019)	Xp	Kx [McLeod phenotype]	Anti-Kx and anti-Km can cause severe HTRs	Antibodies found only in males
Indian (023)	11p	In^a (IN1), In^b (IN2), IN3, IN4, IN5	Possible	Not reported
Globoside (028)	3q	P [P-]	AHTRs	No, but high rate of spontaneous abortions

Table 20: Salient features of other blood systems

ISBT Number	Blood group system name	Main antigens	Chromosome location	HTR	HDFN
010	Diego	Di^a, Di^b, Wr^a, Wr^b	17	Reported, Very rare	Reported, very rare
011	Yt	Yt Yt^a, Yt^b	7	Reported, very rare	No

ISBT Number	Blood group system name	Main antigens	Chromosome location	HTR	HDFN
012	Xg	Xg Xg^{a}	X	No	No
013	Scianna	Sc1, Sc2	1	No	No
014	Dombrock	Do^{a}, Do^{b}, Gy^{a}, Hy, Jo^{a}	12	AHTRs, DHTRs reported	No
015	Colton	Co^{a}, Co^{b}, Co3	7	Acute & Delated HTRs reported	Reported mild to severe
016	Landsteiner-Weiner	LW	19	No	No
017	Chido/ Rodgers	CH/RG	6	No	No
018	H	H	19	Anti-H in Bombay phenotype can cause severe intravascular HTRs	Anti-H in Bombay phenotype can cause severe HDFN
019	Kx	Kx	X	Anti-Kx & anti-Km reported to cause severe HTRs in McLeod's Syndrome	Antibodies found only in males
020	Gerbich	Ge2, Ge3, Ge4	2	No	Only few cases reported
021	Cromer	Cr^{a}	1	No	No
022	Knops	Kn^{a}, Kn^{b}	1	No	No
024	Ok	Ok^{a}	19	No	No
025	Ralph	MER2	11	No	No
026	John Milton Hagan	JMH	15	1 case reported	No
027	I	I	6	Reported in i_{adult} phenotype with anti I	No
028	Globoside	P	3	Intravascular HTRs	No, but high rate of spontaneous abortion
029	Gill	GIL	9	No	No
030	RHAG	RHAG1, RHAG2, RHAG32	6	No	No

7. Human platelet antigen system

Platelets play an important role in maintaining the integrity of endothelium of blood vessels and contributes to haemostasis. The interaction between platelet glycoprotein with the extracellular matrix is critical for this function. Platelets express a variety of antigenic markers on their surface. These antigens and the immune responses are important in alloimmune, autoimmune, and drug-induced immune syndromes involving platelets. Lately, the role of platelet has been observed in various pathological conditions ranging from heart disease to autoimmune disease to even cancer. These involvements of platelets are through ligand receptors interactions involving many glycoproteins which are expressed on their cell membrane.

The investigation is done to explore the biochemical nature, function, and molecular biology of platelet membrane glycoproteins (GPs). The various gene encodes this platelet membrane GPs which are expressed in different forms due to single nucleotide polymorphisms (SNPs). The amino acid changes resulting from these SNPs induce changes in the glycoprotein structure to form antigens that can elicit antibodies through exposure from pregnancy or platelet transfusions.

7.1 Antigen nomenclature

Human platelet antigens (HPAs) are expressed on six different platelet membrane glycoproteins: GPIIb, GPIIIa, GPIba, GPIbb, GPIa, and CD109. Around 33 antigens are expressed on these glycoproteins and are referred to as "platelet specific" but can be found on other cells (leukocytes and endothelial cells). Of these, twelve antigens are clustered into six bi-allelic groups (HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, and HPA-15). For the remaining, alloantibodies against the thetical but not the antithetical antigen have been observed.

The nomenclature for HPAs consists of numbering the antigens in their order of discovery, with the higher-frequency antigens designated "a" and the lower-frequency antigens designated "b." HPAs for which antibodies against only one of the two antithetical antigens have been detected are labelled with a "w" for "workshop," such as HPA-6bw.

7.2 Polymorphisms of the glycoprotein complexes

a. IIb /IIIa: The gene encoding GPIIb/IIIa complex, GPIIb (integrin, aIIb, CD41) and GPIIIa (integrin bIIIa, CD61) are encoded by the genes ITGA2B and ITGB3 and is located on the long arm of chromosome 17.

The complex, a heterodimeric integrin, consists of non-covalently associated α and β subunits, which act as a receptor-ligand for fibrinogen, fibronectin, vitronectin and von Willebrand factor (vWf). There are approximately 50-80,000 copies of the heterodimer per platelet, and it requires Ca^{2+} ions for its function. Upon platelet activation, GPIIb/IIIa undergoes a conformational change that permits ligands to bind via the Arg-Gly-Asp (RGD) tripeptide. GPIIb is only expressed on platelets, but GPIIIa also forms a complex with αv to form αv/β3, which is a receptor for vitronectin and other proteins and is expressed on platelets and endothelial cells.

Integrins are essential for platelet adhesion and aggregation because they serve as receptors for ligands, such as fibrinogen, collagen, fibronectin, von Willebrand factor (vWF), and other extracellular matrix proteins. The binding of fibrinogen by GPIIb/IIIa results in platelet aggregation, which leads to the formation of the "platelet plug" to stop bleeding.

The epitopes on GPIIb/IIIa complex are the most frequent targets of antibodies detected in all immune platelet disorders. As demonstrated in the bleeding disorder of Glanzmann's Thrombasthenia, the platelet GPIIb/IIIa is absent or dysfunctional due to inherited mutations in the encoding genes. These patients, when exposed to normal platelets by transfusion or pregnancy, can make is antibodies against GPIIb/IIIa.

GP (IIb) exhibits 6 HPA antigens, and GP (IIIa) carries 14 HPA antigens constituting twenty of the 33 HPA on this complex. HPA-1a is expressed on GP(IIIa). HPA-1a antibodies are the most frequently detected alloantibodies and cause the majority (85%) of serologically confirmed FNAIT cases. Immunization to HPA-1a is highly correlated with carrying the class II human leucocyte antigen (HLA), HLA-DRB3*0101, and more recently, HLA-DRB4*01:01. HPA-1a antibodies are produced by 2% of individuals with the platelet type HPA-1b/1b. Antibodies specific for HPA-1b are commonly detected in patients with post-transfusion purpura (PTP).

HPA-4 antigens are also expressed on GPIIIa and have been implicated in FNAIT, PTP and platelet refractoriness (PR). The HPA-3 antigens are expressed on GPIIb, but antibodies against HPA-3a and HPA-3b are seldom detected because assays like the MACE and MAIPA, can denature and induces conformational changes in the proteins that interfere with the binding of HPA-3 antibodies.

Additional 17 different low-frequency platelet antigens are expressed on GPIIb and GPIIIa. HPA-9bw has been implicated in multiple cases of FNAIT.

b. Ib/V/IX: The GPIb/V/IX (CD42) complex forms the vWF receptor on platelets. It is involved in the initial stages of platelet adhesion at high shear stress to damaged vessel wall via vWf in the subendothelial matrix. GPIbα and GPIbβ are covalently linked by a single disulphide bond and are also non-covalently associated with the other two components, GPIX and GPV. There are approximately 25,000 copies of GPIb/IX and 12,000 copies of GPV per platelet, and the whole complex is functionally associated with the low-affinity Fc receptor FcγRII (CD32).

The primary vWf binding site has been localized within GPIbα. GPIba carries HPA-2a/2b, and GPIbb carries HPA-12bw. Antibodies against HPA-2a, -2b, and -12bw have all been implicated in causing FNAIT.

The chromosomal localisation of the relevant genes is known: the GPIbα gene is on chromosome 17, the GPIbβ gene is on chromosome 22 and the GPIX and GPV genes are both on chromosome 3.

Deficiency of the GPIb/V/IX complex can lead to Bernard Soulier Syndrome (BSS) which occur due to mutations in the encoding genes GPIBA, GPIBB, or GP9. This disorder is characterized by prolonged bleeding time, thrombocytopenia, and the presence of "giant platelets" and can produce antibodies when they are exposed to the protein complex on normal platelets through transfusions or pregnancy. GPIb/IX is also a common target of platelet autoantibodies.

c. Ia/IIa: The GPIa/IIa (CD49/CD29, integrin α2β1) complex or VLA-2 (very late antigen) is another integrin and is found on activated T lymphocytes and several other cell types. The principal ligand of this αβ heterodimer is collagen in exposed sub-endothelium. There are approximately 800–2800 copies of GPIa/IIa per platelet.

The GPIa protein carries the HPA-5a/5b, -13bw, -18bw and -25bw antigens. Antibodies to these antigens can be associated with FNAIT. Antibodies to HPA-5 antigens are second only to anti-HPA-1a, in patients with FNAIT and are also frequently detected in patients with PTP. The HPA-13 mutation is unusual in that it alters the function of the GP, as platelets from HPA-13bw-positive individuals have a reduced response to collagen in aggregation studies and reduced spreading on a collagen surface.

CD109: The HPA-15 allo-antigens are localized on CD109, a 175-kDa glycosylphosphatidylinositol (GPI)-linked GP found on platelets, monocytes, granulocytes, stimulated T-cells and CD34-positive myeloid progenitor cells and is a member of α2-macroglobulin/complement superfamily. CD109 may be involved in cell-substrate and cell–cell interactions and has been reported to bind and negatively regulate the signalling of transforming growth factor b. Presence of HPA-15 antibodies in seen in 0.22% to 4% of maternal sera in patients with suspected FNAIT, and in sera of patients with immune platelet refractoriness.

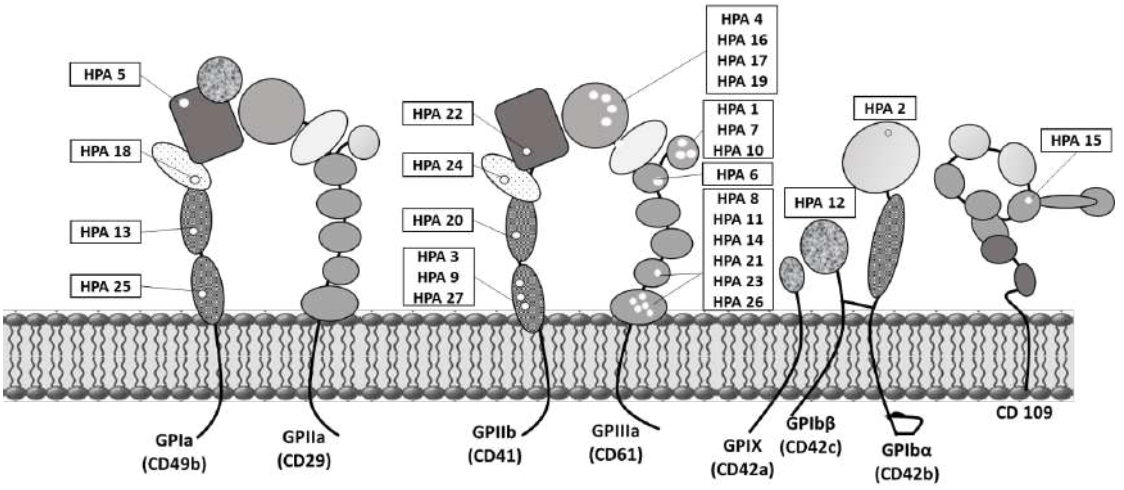


Figure 14: Platelet glycoprotein (GP) structures, GPIIb/IIIa, GPIa/ IIa, GPIb/IX and CD109, and the predicted locations of the 27 different human platelet alloantigen (HPA) biallelic groups they express.

7.3 Other antigens on platelets

a. ABO and other blood groups:

GPIIb and platelet endothelial cell adhesion molecule (PECAM-1/CD31) carry the largest amount of A and B antigens which are attached to these GP through saccharides. The expression of A and B antigens on platelets are variable with 5% to 10% of non-group O individuals having extremely high levels of these antigens. The "high expresser" individuals have highly active glycosyltransferases, attaching A and B antigens more efficiently. Subgroup A2 red cell phenotype expresses lower levels of A on their red cells than A1 individuals; they do not express detectable A antigens on their platelets which makes possible transfusion of A2 platelets to group O patients even with high-titres anti-A.

Often platelets are transfused irrespective of ABO compatibility; however, at times the use of mismatched platelets may result in lower post-transfusion recovery rates. Immunoglobulin G (IgG) A, B antibodies in blood group O recipients are reactive with transfused platelets carrying large amounts of A or B antigens, resulting in platelet transfusion refractoriness. The recovery of ABO mismatch transfused platelets can also be influenced after the transfusion of group O platelets as donor plasma might be reactive with soluble A or B in the recipient plasma to form immune complexes that bind to transfused (and autologous) platelets via FcγRIIa, thereby influencing the survival of the transfused platelets. Although other red cells antigens (e.g., Le^a, Le^b, I, i, P, Pk, and Cromer) are also present on platelets, there is no evidence that these antigens significantly reduce platelet survival in vivo.

b. GPIV/CD36*:*

GPIV binds several different ligands, including low-density lipoprotein cholesterol, thrombospondin, Types I and IV collagen, and malaria-infected red cells. GPIV/CD36 is expressed on platelets, monocytes/macrophages and nucleated erythrocytes and belongs to the Class B scavenger receptor family. Antibodies to CD36 are seen in CD36 deficient individuals when transfused with normal platelets and can cause FNAIT, PTP, and platelet refractoriness.

c. GPVI:

GPVI is a major collagen receptor on platelets and a member of the Ig superfamily. GPVI interactions with collagen exposed on the extra-cellular matrix result in platelet activation and aggregation. To date, no HPAs have been identified on GPVI, but platelet autoantibodies formed against GPVI have been reported to cause a mild form of autoimmune thrombocytopenia. GPVI autoantibodies induce shedding of GPVI from platelets, resulting in reduced collagen binding.

d. HLA:

HLA is present on all nucleated cells of the body. HLA associated with platelets is the main source of class I HLA in whole blood and is expressed as integral membrane proteins, whereas smaller amounts may be adsorbed from surrounding plasma. HLA-A and -B locus antigens are significantly represented, but there appears to be only minimal platelet expression of HLA C. Class II HLA is not present on the platelet membrane. Several factors influence the development of HLA antibodies after transfusions, such as underlying disease, immunosuppressive effects of treatment regimens, and whether the blood components contain a significant number of leukocytes. Transfusion of leukocyte reduced (LR) blood components has reduced HLA alloimmunization.

HLA antibodies can develop following pregnancy and present in more than 32% of women who have had four or more pregnancies. Although, HLA antibodies have also been identified in 1.7% of never pregnant or transfused women and men with no previous transfusions.

7.4 Clinical consequences of HPA alloantibody formation

a. Neonatal alloimmune thrombocytopenia (NAIT)

NAIT, also known as feto- and neonatal alloimmune thrombocytopenia (FNAIT), occurs as a consequence of maternal immunization against fetal platelet alloantigens inherited from the father. Maternal IgG alloantibodies then cross the placenta and cause immune destruction of platelets *in utero*, a situation analogous to haemolytic disease of the newborn, except that approximately 30% of cases occur in the first pregnancy. NAIT, previously thought to be a rare disease, is the most common cause of severe thrombocytopenia in an otherwise healthy term neonate. A study of 25,000 pregnancies showed that anti-HPA-1a antibodies complicate approximately 1/350 pregnancies, and the incidence of severe thrombocytopenia due to anti-HPA-1a is 1/1200 live births.

Clinical severity ranges from asymptomatic thrombocytopenia up to 10% having intracranial haemorrhage, and this can result in severe neurological damage or death. Subsequent pregnancies are usually affected, with similar or increasing severity. Treatment of severely affected cases is usually by transfusion of HPA-compatible platelets, either *in utero* or post-delivery. Approximately 80–90% of cases are due to anti-HPA-1a,

5– 15% are due to anti-HPA-5b, and the remainder is caused by other HPA antibodies. Mothers may also be immunized against HLA antigens, but the involvement of HLA antibodies in NAIT has not been proven. Individuals with certain HLA haplotypes with HLA-DRB3*0101 allele are more likely to develop antibodies against HPA-1a antigen.

A serologic diagnosis of NAIT may be made by 1) testing maternal serum for platelet antibodies using assays that can differentiate platelet-specific from non-platelet-specific reactivity and 2) performing platelet genotyping on parental deoxyribonucleic acid (DNA). Demonstration of platelet specific (HPA) antibody in the maternal serum and the corresponding presence of the antigen in the paternal platelet typing confirms the diagnosis.

Treatment of acutely thrombocytopenic newborn includes the administration of intravenous immune globulin (IVIG) with or without antigen-compatible platelet transfusions that are sometimes supported by the mother's washed platelets.

b. Post-transfusion purpura (PTP)

PTP is a rare but severe disease that occurs approximately a week after transfusion of any blood product containing platelets or platelet membranes. A conservative estimation of its incidence suggests that it occurs in 1/50,000-1,00,000 transfusions. The typical patient is a middle-aged or older woman, although PTP has occasionally been reported in men. All patients have had previous exposure to allogeneic platelet antigens or HLA antigens through either pregnancy or blood transfusion. Severe thrombocytopenia usually occurs within 5-10 days of the precipitating transfusion, and at the same time, high-titre complement-fixing HPA alloantibodies can be detected in the patient's serum. The majority of cases involve anti-HPA- 1a, although other specificities have been reported, which are almost always associated with antigens on GPIIb/IIIa.

The pathogenesis is unknown; the patients are presumably sensitized by previous pregnancy or transfusion and respond to the second challenge of incompatible platelets by making high-titre HPA (and often HLA) antibodies. The resulting immune destruction of transfused platelets may contribute to the transfusion reactions that commonly occur. However, it does not explain the simultaneous destruction of the patient's own platelets, which are negative for the antigen concerned. Two mechanisms have been proposed. The destruction of transfused platelets may release alloantigen that is adsorbed onto the surface of the patient's own platelets, followed by attachment of alloantibody and consequent removal from the circulation. Alternatively, several groups have proposed the simultaneous but short-lived formation of autoantibodies; however, as yet no one mechanism has been shown to be operating in all cases of PTP.

PTP is a life-threatening disease as bleeding is often severe. Platelet transfusion is generally not recommended in PTP as both random, and HPA compatible platelets are usually ineffective in achieving increments. Most patients respond to high- dose intravenous immunoglobulins, which is regarded as the most optimal first-line therapy. In the small number of patients who have been left untreated, spontaneous recovery usually occurs within 1–4 weeks of the onset of thrombocytopenia. As per the Serious Hazards of the Transfusion surveillance program, the frequency of PTP has rather dramatically decreased coincidently with the introduction of leukocyte reduced blood components.

c. Refractoriness to platelet transfusion

This condition is defined as an inadequate increment in the platelet count following transfusion of random ABO-identical donor platelets. It is a common complication in patients receiving multiple platelet transfusions. Most cases of refractoriness have a non-immune cause; however, a significant proportion (26–71%) of patients on long-term platelet transfusion will develop HLA antibodies. Approximately 10% of patients, refractory to random donor platelets, also develop HPA antibodies.

Responses to platelet transfusions are often determined 10 to 60 minutes and 24 hours after transfusion by calculating either a corrected platelet count increment (CCI) or a posttransfusion platelet recovery (PPR), both of which normalize transfusion responses for patient blood volume and platelet dose. 1-hour posttransfusion CCI of less than 5000 and 24-hour CCI of less than 7500 after two consecutive transfusions adequately define the refractory state.

The specificity of HPA antibodies in this clinical setting has only been established in a few relatively small studies, but antibodies against the low-frequency alloantigen HPA-1b, and 5b and 2b appear to be the most common.

Platelet alloimmunisation is one cause of refractoriness, and there are multiple nonimmune-related reasons when transfused platelets may not yield the expected increase in platelet count, such as sepsis, disseminated intravascular coagulation, and the administration of certain drugs.

7.5 Drug-Induced thrombocytopenia

Drugs commonly implicated include quinine, sulpha drugs, vancomycin, GPIIb/IIIa antagonists, and heparin. Both drug-dependent and non-drug-dependent antibodies may be produced. Non-drug-dependent antibodies, although stimulated by drugs, do not require the continued presence of the drug to be reactive with platelets and are serologically indistinguishable from other platelet autoantibodies. These antibodies cause thrombocytopenia of sudden and rapid onset that usually resolves within 3 to 4 days after the drug is discontinued.

a. Heparin-induced thrombocytopenia

It can develop in up to 5% of patients who are treated with unfractionated heparin. There is a baseline reduction in platelet count by approx. 30% to 50% occurring within 5 to 14 days after the primary exposure to heparin. The platelet count is often less than 100,000/μL but usually recovers within 5 to 7 days upon discontinuation of heparin.

The mechanism of HIT includes the formation of a complex between heparin and platelet factor 4 (PF4), a tetrameric protein released from platelet alpha granules. Antibodies (IgG, IgA, and some IgM) are produced to the complex, and IgG in the complex attaches secondarily to platelet receptor FcγRIIa, resulting in platelet activation with subsequent thrombin generation. The antibody may also bind to complexes formed at other sites such as on endothelial cells and monocytes. More than 50% of patients with HIT develop thrombosis; patients may develop stroke, myocardial infarction, limb ischemia, deep venous thrombosis, or ischemia of other organs. An alternative (non-heparin) anticoagulant (e.g., a direct thrombin inhibitor) maybe required to prevent thrombosis.

Testing for drug-dependent platelet antibodies

a. PF4 ELISA
b. ^{14}C-serotonin release assay (SRA)
c. Heparin-induced platelet-aggregation test
d. Heparin-induced platelet-activation test.

The PF4 ELISA and the SRA are both more sensitive and specific than the platelet-aggregation test for the detection of heparin-dependent platelet antibodies.

Autoimmune or immune thrombocytopenic purpura

Immune thrombocytopenic purpura (ITP) is an immune platelet disorder in which autoantibodies are directed against platelet antigens, resulting in platelet destruction. Females are twice as likely to be affected as males. Chronic ITP, which is most common in adults, may be idiopathic or associated with other conditions, such as human immunodeficiency virus infection, malignancy, or other autoimmune diseases. Acute ITP is mainly a childhood disease characterized by the abrupt onset of severe thrombocytopenia and bleeding symptoms, often after a viral infection. Autoantibodies are reactive with several platelet surface-membrane structures, most often including GP complexes IIb/IIIa, Ia/IIa, and Ib/IX, but that can also include GP IV, GPV and GP VI.

Treatment with IVIG or anti-D immunoglobulin infusion is given for raising the platelet counts. Steroids are used less often because of their serious side effects in children. Splenectomy, if used, is reserved for children whose disease is severe and lasts longer than 6 months; this condition is similar to chronic ITP in adults. Rituximab and various thrombopoietin receptor agonists have been used as second-line therapies for acute ITP.

7.6 Laboratory typing of platelet antigens.

Platelet genotyping*:* Genotyping for HPA alleles in combination with detection of platelet antibodies in the patient's serum to aid in the diagnosis of FNAIT, PTP and platelet refractoriness (PR). Genotyping of DNA is the gold standard. Two of the most popular methods are PCR-SSP with a gel endpoint and 5′ exonuclease techniques.

Antigen Capture Assays: Platelet glycoprotein ACAs are used to determine the HPA that is recognized by platelet antibodies in a patient's serum. The assays developed include Modified Antigen Capture ELISA (MACE) and Monoclonal Antibody Immobilization of Platelet Antigens (MAIPA).

7.7 Methods used for the detection of platelet-specific antibodies.

Assays using intact platelets

HPA antibody techniques:

1. Platelet immunofluorescence test (PIFT)
2. Monoclonal antibody immobilisation of platelet antigens (MAIPA)
3. Solid-phase red cell adherence assay (SPRCA)
4. ELISA-based techniques.

SPRCA: An assay that is widely used for the detection of platelet-specific antibodies and for platelet cross-matching. The main limitations are its subjective endpoint and failure to distinguish platelet-specific from non-platelet-specific antibodies.

Flow cytometry:

Alloantibodies that are specific for labile epitopes and unreliably detected by antigen capture assays (ACAs) can be detected with intact platelets using flow cytometry. It does not differentiate between platelet-specific (i.e., platelet-glycoprotein-directed/HPA) and non-platelet-specific anti-bodies (i.e., HLAs or autoantibodies).

8. Human neutrophil antigen system

Antibodies against granulocyte (neutrophil) antigens are implicated in the following clinical syndromes: neonatal alloimmune neutropenia (NAN), transfusion-related acute lung injury (TRALI), immune neutropenia after HPC transplantation, refractoriness to granulocyte transfusion, and chronic benign, autoimmune neutropenia of infancy (AIN). Around nine neutrophil antigens carried on five different glycoproteins have been characterized and given human neutrophil alloantigen (HNA) designations by the Granulocyte Antigen Working Party of the ISBT.

8.1 Human neutrophil antigens

a. Antigens on FcγRIIIb:

The first granulocyte-specific antigen detected was NA1, later named "HNA-1a." Three alleles of HNA-1 have now been identified: HNA-1a, HNA-1b, and HNA-1cand are located on the protein FcγRIIIb (CD16b), which is present only on the surfaces of neutrophils. Individuals (approximately 0.1%) who express no FcγRIIIb (CD16 null) and who can produce antibodies that are reactive with FcγRIIIb. Antibodies to HNA-1a and -1b have been implicated in TRALI and NAIN.

b. Antigens on CD177:

The HNA-2 antigen was initially described as neutrophil-specific antigen NB1, and later the glycoprotein was identified as CD177. The cellular expression of the HNA-2 antigen is restricted to neutrophil granulocytes. HNA-2 is expressed differentially on neutrophil subpopulations with one or two neutrophil subsets expressing the glycoprotein and another one lacking the glycoprotein. A number of different SNPs have been demonstrated

on CD177, but no alloreactivity of antisera developed by HNA-2-deficient individuals has been found so far; thus, it is considered that HNA-2 antibodies are isoantibodies. Interactions between CD177 and the endothelial cell membrane protein PECAM-1(CD31), suggests a role for CD177 in neutrophil transendothelial migration to sites of infection. Antibodies against HNA-2 have been implicated in NAN, TRALI, and neutropenia in marrow transplant recipients.

c. Antigens on CTL2:

HNA-3a and HNA-3b are carried on the choline transporter-like protein 2 (CTL2), and a SNP in the gene (*SLC44A2*) accounts for the polymorphisms. CTL2 is expressed on both T and B lymphocytes, and small amounts are present on platelets. HNA- 3a antibodies are usually agglutinins that develop in women after pregnancy, and HNA-3a antibodies are the most frequent cause of fatal TRALI.

d. Antigens on CD11a and CD11b:

HNA-4a and HNA-5a both are high-prevalence antigens and present on monocytes and lymphocytes as well as granulocytes. HNA-4a is carried on the CD11b/18 (Mac-1, CR3, $\alpha_m\beta_2$) glycoprotein. CD11/18 plays an important role in neutrophil adhesion to endothelial cells and phagocytosis of C3bi opsonized microbes. Alloantibodies against HNA-4a interfere with CD11b/18-dependent neutrophil adhesion and enhance neutrophil respiratory burst. Antibodies against HNA-4a are also implicated in NAN.

HNA-5a is carried on CD11a/18 (LFA-1, αb) glycoprotein, which plays a role in neutrophil adhesion to endothelial cells. Antibodies are found in a chronically transfused patient with aplastic anaemia and have also been associated with NAN.

Neutrophils do not express ABH or other red cell group antigens, but they do express small amounts of Class I and II HLA only upon activation.

8.2 Immune neutrophil disorders

a. Neonatal alloimmune neutropenia:

NAN is caused by maternal antibodies against antigens on fetal neutrophils; the most frequent specificities are against HNA-1a, HNA- 1b, and HNA-2 antigens. NAN may also occur in the children of women who lack the FcγRIIIb protein. Neutropenia in NAN can occasionally be life-threatening because of increased susceptibility to infections. Management with antibiotics, IVIG, granulocyte colony-stimulating growth factor, and/or plasma exchange may be helpful.

b. TRALI:

TRALI is an acute, often life-threatening reaction characterized by respiratory distress, hypo- or hypertension, and noncardiogenic pulmonary oedema that occurs within 6 hours of transfusion. The causative antibodies are most often found in the transfused plasma of the blood donor, which causes activation of primed neutrophils that are sequestered in the lungs. The activated neutrophils undergo oxidative burst releasing toxic substances that damage pulmonary endothelium and resulting in a capillary leak and pulmonary oedema. Class I and II HLA and HNA antibodies have all been implicated in TRALI. Recently HNA and Class II HLA antibodies only, but not Class I HLA antibodies, were significantly associated with TRALI.

c. Autoimmune neutropenia:

Autoimmune neutropenia occurs in both adults and in infants. When present in adults, it may be idiopathic or be secondary to such diseases as rheumatoid arthritis or systemic lupus erythematous or bacterial infections. Autoimmune neutropenia of infancy usually occurs between the ages of 6 months and 2 years. The autoantibody has specificity, usually HNA-1a or -1b, in about 30% of the patients. The condition is generally self-limiting, with recovery usually occurring in 7 to 24 months. The condition is relatively benign and can be managed with antibiotics.

8.3 Testing for granulocyte antibodies and antigens

It is difficult to maintain the integrity of granulocytes for testing that are stored at various temperatures; therefore they are isolated from fresh blood on each day of testing. Class I HLA antibodies that are often present in a patient's sera complicate detection and identification of granulocyte antibodies.

Granulocyte agglutination test:

This was one of the first tests developed for the detection of granulocyte antibodies. It is typically performed by overnight incubation of small volumes of isolated fresh neutrophils with the patient's serum in a microplate. The wells are viewed under an inverted phase microscope for neutrophil agglutination or aggregation.

Granulocyte immunofluorescence tests:

Neutrophil-bound antibodies are detected with fluorescein isothiocyanate-labelled antihuman IgG or IgM with either a fluorescence microscope or a flow cytometer.

Mixed passive agglutination:

Sera to be tested are incubated with neutrophil extract in wells of U-bottom Terasaki plates. Antibody binding is detected using sheep erythrocytes coated with antihuman IgG. The assay has been shown to detect antibodies specific for HNA-1a, -1b, -2a and -3a.

Monoclonal-antibody capture assays:

The monoclonal-antibody capture, or monoclonal-antibody immobilization of granulocyte antigens (MAIGA) assay, allows the detection of antibodies to specific neutrophil membrane glycoproteins. It can be used to detect antibodies to FcγRIIIb (CD16), NB1 gp (CD177), leucocyte function antigen-1 (LFA-1 or CD11a) and complement component C3bi receptor (CR3 or CD11b). In addition, this assay detects antibodies to HNA-1, -2, -4 and -5. The assay allows recognition of antibodies to specific neutrophil glycoproteins even when antibodies to HLA antigens are present.

HNA typing:

Any methods used in HPA typing can be applied to HNA typing with simple modifications to the primer and probe sequences. Because the splicing defect that results in CD177 deficiency is not known, typing for HPA-2/CD177 requires testing for CD177 on freshly isolated neutrophils using specific monoclonal antibodies and the granulocyte immune-fluorescence test.

9. Molecular blood grouping

DNA-based testing for blood group antigens has become commonplace in a number of clinical situations. These include typing for minor antigens in multiple transfused immunized patients to determine risk for production of additional blood group antibodies, patients with positive direct antiglobulin test (DAT) and serum autoantibody, patients facing chronic transfusion therapy, and for locating antigen-negative blood when no serologic reagent is available, as well as in prenatal medicine to assess risk for haemolytic disease of the newborn (HDFN) and to guide Rh immune globulin (RhIg) therapy for pregnant women.

Determination of blood group antigens by DNA methods (genotyping) is an indirect method for predicting an individual's blood group phenotype, in contrast, to direct testing by serologic methods using a specific antibody (phenotyping).

Most blood group antigens result from single-nucleotide gene polymorphisms (SNPs) inherited in a straightforward Mendelian manner, making assay design and interpretation fairly straightforward. However, ABO and Rh blood groups are more complex. There are >200 different alleles encoding glycosyltransferases responsible for ABO type, and single point mutation in A or B allele can result in inactive transferase, i.e., group O phenotype. Next-generation sequencing (NGS) technology holds promise for routine ABO typing by DNA methods. For Rh system, testing for common antigens D, C/c, and E/e are fairly straightforward in most individuals, but antigen expression is more complex in diverse ethnic groups. There are >200 RHD alleles

encoding weak D or partial D phenotypes and >100 RHCE alleles encoding weak, altered, or novel hybrid Rh proteins. RH genotyping, particularly in minorities, requires sampling of multiple regions of the gene(s) and algorithms for interpretation.

The most commonly used methods for the determination of RBC human erythrocyte antigens (HEAs) and human platelet antigens (HPAs) are semiautomated polymerase chain reaction (PCR) using fluorescent probes with automated readout. Automated methods increase the number of target alleles in PCR, allowing the determination of numerous antigens in a single assay. Most platforms currently available are based on fluorescent bead technology.

Knowledge of the molecular basis of most blood group systems has given rise to the development of DNA-based methods for blood group determination. Several multiplex molecular testing platforms have become available to predict phenotypes based on blood group genetics in recent years. High-throughput tools have the potential for application in mass-scale testing of donor blood.

The molecular methods available for red cell genotyping can be divided into the following categories:

- Low to medium throughput:
  - Polymerase chain reaction (PCR)
  - PCR- restriction fragment length polymorphism (RFLP)
  - PCR- sequence-specific primer (SSP) or PCR- allele-specific primer (ASP)
  - Real-time PCR (RT PCR)
  - DNA sequencing and pyrosequencing
- High throughput:
  - Microarrays based systems (e.g., BloodChip, BeadChip and Genome Lab SNP stream).

9.1 Assays based on classical PCR (low throughput)

The identification of SNP by PCR is relatively simple. It is used to amplify a specific sequence of DNA. The reaction consists of three steps:

1. Denaturation at 95^{0}C to separate double-stranded DNA.
2. Annealing for binding of primer to single-strand DNA at 55–65^{0}C.
3. Extension at 72^{0}C for the creation of a complementary DNA copy.

The PCR products can be analyzed using different methods, which can include electrophoresis to separate the fragments, based on size, through an agarose gel with the fragment bands visualized by ethidium bromide staining and UV light.

PCR-RFLP

The first blood group genotyping experiments involved the amplification of relevant sequences of blood group genes followed by restriction fragment length polymorphism. It is based on the introduction or loss of restriction sites by SNP of interest. The alleles are differentiated after PCR by digestion of the products with a restriction enzyme. Alleles are discriminated by the fragment pattern visualized by gel electrophoresis after digestion, and the fragment pattern compared with known controls tested in parallel.

PCR-SSP

In this assay, a primer that only detects the allele of interest is used in the PCR, and a band is observed on the gel only when the gene of interest is present. Because the lack of a PCR product indicates an allele is not present, it is necessary to use internal control in all reaction tubes. Commercially available kits can be used to test for ABO variants, RHD/RHCE variants and other blood group such as K1/K2, FYA/FYB and JKA/JKB.

Multiplex PCR

Multiplex PCR is a molecular technique for amplification of multiple targets in a single PCR experiment. In this assay, more than one target sequence can be amplified by using multiple primer pairs in a reaction

mixture. As an extension to the practical use of PCR, this technique has the potential to produce considerable savings in time and effort within the laboratory without compromising on the utility of the experiment. Initial optimization of multiplex assays can be technically challenging and difficult. Wagner et al. showed the use of a multiplex PCR-SSP in donor screening who were negative for high-incidence antigens like: Kp^b, Co^a, Yt^a and Lu^b.

9.2 Medium-throughput Assays

Real-time PCR

Real-time PCR is an improvement to conventional PCR as both amplification and visualization of the product are achieved within a single tube using fluorescence detection. Its key feature is that the amplified DNA is detected as the reaction progresses in real-time. Three different methods can be used to detect products as nonspecific fluorescent dyes intercalating with any double-stranded DNA (i.e., SYBR Green), TaqMan probes and hybridization probes protocols.

9.3 High-throughput Arrays

Microarray technology

The ability to have true high-throughput testing became a reality with the development of microarrays. DNA microarrays integrate multiple simultaneous nucleic acid amplification reactions into a single test and subsequently discriminate the amplicons by hybridization capture onto a solid matrix. A solid matrix may be a glass slide with either spotted DNA probes or probe coated fluorescent-tagged latex beads. The analysis is performed using fluorescent capture microscopy or flow cytometry. The CE-IVD marked Human-erythrocyte-antigen (HEA) BeadChip™ with its FDA approved counterpart PreciseType™ HEA (Immucor, Warren, NJ, USA) and the CE IVD marked ID-Core XT (Grifols, Bizkaia, Spain) are two of the commonly used commercial microarray platforms.

It has the capacity to discriminate DNA fragments that differ in a single nucleotide. It consists of two parts:

1. Laser-induced desorption/ionization of analyte/matrix molecules
2. Separation and analysis of different biomolecules based on intrinsic physical properties.

It is a high-throughput, qualitative, and quantitative method that can analyse 36–40 multiple SNPs in a single reaction that takes approximately 8 hours. It has been used for the genotyping of some of the Kell system genes, platelet antigens and a prenatal screening test for fetal RHD.

Next-Generation Sequencing (NGS)

A major limitation of all the methodologies described earlier is that they are only capable of identifying known alleles, as primer and probe designs are based on existing allele information. NGS offers an unbiased approach to sequencing and can sequence large numbers of genes from multiple patients in parallel. This allows sequencing of millions of reads in one run. Single, long-read sequencing, particularly nanopore sequencing, will allow resolution of polymorphisms that were previously difficult to resolve, particularly when allelic ambiguities are present.

9.4 Applications of molecular blood grouping:

9.4.1 Patient typing:

1. After a recent transfusion.
   a. Help in antibody identification and RBC unit selection.
   b. Select RBCs for adsorption.
2. Identify if a fetus is or is not at risk for haemolytic disease of the fetus and newborn by predicting the father is homozygous or heterozygous for *RHD* in mother with anti-D.
3. When the antibody is not available (eg, anti-Do^a, -Do^b, -Js^a, -V, -VS).

4. Help to distinguish an alloantibody from an autoantibody (eg, anti-e, anti-Kp^b).
5. Identify alloantibody when a patient's type is antigen-positive, and a variant phenotype is possible (eg, anti-D in a D-positive patient, anti-e in an e-positive patient).
6. When DAT is positive, and direct agglutinating antibodies are not available.
   - The antigen is sensitive to the IgG removal treatment (e.g., antigens in the Kell system are denatured by EDTA-glycine-acid elution).
   - Testing requires the indirect antiglobulin test, and IgG removal techniques are not effective at removing cell-bound immunoglobulin.
   - Antisera are weakly reactive, and reaction is difficult to interpret (e.g., anti-Do^a, anti-Do^b, anti-Fy^b).
7. If an antibody problem arises post allogeneic stem cell transplantation, test stored DNA samples from the patient and the donor(s).
8. To detect weakly expressed antigens (e.g., Fy^b with the Fy^x phenotype); where the patient is unlikely to make antibodies to transfused antigen-positive RBCs.
9. Identify molecular basis of unusual serological results, especially Rh variants.
10. Resolve discrepancies, e.g., A, B, and Rh.
11. Aid in the resolution of complex serologic investigations, especially those involving high-prevalence antigens when reagents are not available.
12. Chronically transfusion-dependent patients to reduce (further) alloimmunisation risk (e.g., sickle cell anaemia, thalassemia)
13. Identifying RhD variants that are at risk for anti-D alloimmunisation.

9.4.2 Donor typing:

1. Screen for antigen-negative donors.
2. When antibody is weak or not available (e.g., anti-Do^a, -Do^b; -Js^a, -Js^b; -V/VS).
3. Mass screening to increase antigen-negative inventory.
4. Find donors whose red cells lack a high-prevalence antigen.
5. Resolve blood group A, B, and Rh discrepancies.
6. Detect genes that encode weak antigens.
7. Type donor's red cells for antibody screening cells and antibody identification panels (e.g., Do^a, Do^b, Js^a, V, VS).
8. Determine zygosity of donors on antibody detection/identification reagent panels, especially D, S, Fy^a, and Fy^b.

9.4.3 Fetal typing.

1. The non-invasive method is based on the detection of RHD sequences from cell-free fetal DNA in the maternal plasma.
2. Invasive procedures, such as chorionic villus sampling, percutaneous amniotic fluid, or umbilical cord blood sampling for fetal genotyping

10. Automation in immunohaematology

Automated analysers have been used in many immunohaematology laboratories since their discovery in the mid-1960s. They have many advantages: improved quality of preanalytical steps, reduced error rates, and reduced individual exposure to biohazardous materials.

The most commonly employed method for conventional testing in immunohaematology laboratory is the tube technique. Though it is still considered a gold standard, the methodology is quite cumbersome, demands technical expertise and has some inherited limitations. Elution of low-affinity antibodies during washing, variability in the red cell concentrations, improper cell serum ratio, and lack of consistency in reporting the

results due to inter-observer variability are a few of them. In addition, the blood centre needs quality systems and infrastructure to maintain good laboratory practices in spite of the heavy workload. Automation also addresses the issues related to traceability and reproducibility of the test results as well as documentation of the reports.

Newer techniques such as column agglutination technique (CAT), solid-phase red cell adherence assay (SPRCA) and erythrocyte-magnetized technology (EMT) are being adapted in immunohaematology. These technologies are suitable for automation, and manufacturers are coming up with semi- and fully automated equipment. Several instruments are now available for full automation from vein to vein in the transfusion service. However, the selection of an instrument is based on the facility's needs for testing and its resources. The following discussion introduces the reader to the technologies available for immunohaematology laboratory and aspects of automation.

10.1 Column agglutination technology (CAT):

The column agglutination test system consists of a plastic card with six to eight inbuilt microtubes. The microtubes have a broad reaction chamber in the upper part, while the lower part contains either a clear gel (BIO- RAD and Grifols) or a glass microbead matrix (Ortho-Clinical). Antihuman globulin (AHG) or other antisera may or may not be incorporated in the gel or microbead matrix as per requirements for a particular test. The required reagents (maybe a 0.8-1% reagent red cell suspension or antisera) and samples (maybe patient's plasma or 1% red cell suspension) are dispensed to the reaction chamber followed by a recommended incubation period (if required) and the suspension is forced to pass from the gel/microbead chamber using centrifugation. The sensitized red cells agglutinate in the presence of AHG in the gel/bead matrix and get trapped, while unsensitized cells form a button at the bottom of the microtube. The reactions may be graded from 1+ to 4+, and a card reader if used, provides objectivity to the grading. Omission of the washing steps during testing reduces the turnaround time and elution of antibodies. The tests results may be preserved for up to 24 h in the testing card. However, they can be stored for longer periods in electronic formats. The column agglutination system is an open system and can fulfil a variety of red cell serology testing requirements.

10.2 Solid-phase red cell adherence assay (SPRCA):

This is a technique in which one of the components of an antigen-antibody reaction is immobilized onto a solid medium and after reaction with a free antigen/antibody, the endpoint of the reaction is indicated by use of red cells, which may be a part of the antigen-antibody reaction or may be added as indicator cells. The same equipment also utilizes the principle of haemagglutination for blood grouping. In forward grouping, U shaped microplate wells are coated with Anti-A antiserum, Anti-B antiserum, and Anti-D antiserum, A drop of 0.5% bromelin-treated red cells are added to the well. On centrifugation antigen positive cells spread out while antigen negative cells form a button at the bottom of the well. In case of reverse grouping a monolayer of RBC membrane is attached to the bottom of the well and plasma to be tested is added after incubation for 5 min, the excess plasma is blotted, and anti-IgG bound indicator red cells are added to give a visible reaction. SPRCA may be adapted to other red cell serology tests such as antibody screening, identification, and cross matching. It may also be adapted to platelet serology.

10.3 Erythro-Magnetic technology:

This technology is based on the magnetisation of RBCs. Paramagnetic particles are adsorbed on to the surface of RBCs. Once antibodies in plasma/antisera react with antigens on RBCs in a micro plate well, a magnetic force is applied at the bottom of the microplate using a magnetic plate, this causes the RBCs to be pulled toward the bottom of the microplate. In this manner the magnetic force replaces the centrifugation step. On shaking/resuspension the reactions may be deciphered. In forward grouping, the test RBCs are suspended in a solution of iron chloride and bromelin, then the RBC suspension is dispensed into the microplate well precoated with antisera. This is followed by gentle shaking and incubation for 10 min, and then the microplate is put on a magnetic plate. The magnetised RBCs gather at the bottom of the plate. On shaking after this step, the free RBCs are resuspended while agglutinated RBCs form a button at the bottom of the well. In case of reverse grouping pre-magnetised RBCs are mixed with test plasma in the microplate wells followed by the same steps as above. This technology may also be adapted to antibody screening and identification.

Table 21: Comparison of various techniques

Technology	Column agglutination technology	Solid phase red cell adherence assay	Erythro-magnetic technology	Conventional tube testing
Number of steps required	8–12	13–15	8–14	14–19
Washing step	Omitted	One washing step	Omitted	Multiple washing step
Advantages/Disadvantages				
Sample volume	Small	Small	Small	Large
Uniformity of testing in repeat testing	Yes	Yes	Yes	Subjective/ technical skill
Clear and easily readable results	Yes	Yes	Yes	Variability
Detection of IgG antibodies	Yes	Yes	Yes	Yes
Detection of IgM antibodies	Yes	No	No	Yes
Detection of weaker expression of blood groups	Yes	Yes	No	May detect
Suitable for lipemic / haemolysed samples	Yes, up to 75 mg/dl of free haemoglobin (Hb)	Yes	False positives with lipemic/ samples with fibrin	Difficult in haemolyzed samples
Amenable to all modifications of RBC and serum during testing	Some modifications may be possible	No (Bromelin treated cells are used for testing)	No	Yes
Time taken to do ABO/D grouping (manual method)	It takes a minimum of 20 min		It takes a minimum of 20 min	Fastest method to do grouping
Batch testing	More suited to batch testing in terms of time efficiency	More suited to batch testing in terms of time efficiency	More suited to batch testing in terms of time efficiency	No
Antibody Screening				
Sensitivity for clinically significant antibodies	Better than CTT	Better than CTT	Better than CTT	Less than other methods
Sensitivity for CSAs antibodies	90–94%	Approx. 97%	83.3–90.4%	Approx. 43% (LISS–IAT)
Specificity	94.4%	94.3%	98.2%	98.6%

10.4 Potential benefits of implementing automation

- **Redesign work process and support systems:**
  Examine the work process and identify areas of improvement
  Assessing the workflow conducive for potential of batch testing
  Current staff configurations: Hands on time of staff, optimal usage of staff
- **Increased productivity:**
  Better testing capacity
  Improved turnaround time
  Reliable and consistent results
- **Enhance total quality**:
  Reduction of human errors
  Elimination of variability in testing process

The end product has better precision and accuracy
Compliance with regulatory bodies

10.5 Potential challenges

- **Concern among staff members:**
  Comparison of potential efficacy
  Elimination of staff
  Staff training for confidence and competence
- **Implementation issues:**
  Start-up time: Replacement of routine process
  Validated protocols and procedure in order.
- **Cost Justification**
  Concern about initial capital investment

10.6 Automated and semi-automated platforms available are manufactured by:

1. BIO-RAD (Switzerland) - IH-1000/Techno Twin Station/Saxo ID Reader.
2. DIAGAST (France) - Qwalys 3/ FREELYS Mini Lab.
3. Ortho-Clinical Diagnostics (Johnson & Johnson, USA) - Autovue Innova/ Biovue.
4. IMMUCOR (USA) - Galileo/ NEO.
5. Grifols (Singapore) - WADIANA

These systems differ in their technical specifications, throughput, turnaround time, sample loading operation etc. They use micro-plates or gel columns for testing. The tests performed on these systems include ABO-RH grouping, phenotype, irregular antibody screening and compatibility testing etc. Fully automated systems manage all the steps from the sample positioning on the carrier down to the final result (Autovue, Galileo, ID gel station, Qwalys, Tango, Techno) and the semi-automated systems require intervention of an operator for the phases of centrifugation, stirring and incubation, the reading being automated for all. All systems have connection to the central data processing system through an interphase. Biorad, Ortho and Grifols are based on column agglutination technology. Diagast uses the principle of erythrocyte magnetized technology (EMT). Immucor platform is based on principle of solid phase red cell adherence assay.

The newer methods have added to the quality of testing but none of the methods has been found to be unequivocally superior to others. Detection of all antibodies during antibody screening is not the goal of antibody screening; rather detecting all clinically significant antibodies should be the aim for any technology, as detection of insignificant antibodies adds to the burden of further work up and delay in providing blood to the patients. While selecting an appropriate technology for automation there are a number of other issues to be considered.

There are several fully automated immunohaematology workstations available at present which differ in the technology used, configuration of the immunohaematology tests, throughput, turnaround time, sample loading options, and priority sample facility. The decision to buy an automated system depends on the location of the blood centre, type of services provided, cost issues, space availability, staff competency, and feedback regarding the equipment and services of the vendor.

10.7 Characteristic of an ideal instrument for blood centre

1. Criteria important in automation of testing
   a. Random access operating mode (STAT)
   b. Multiple analysis to increase throughput
   c. Extensive testing menu
   d. Automated results reading

   e. Automatic regent dispensing and recognition
   f. Precision pipetting
   g. Options for quality control
2. Criteria for sample handling
   a. Clot detection
   b. Liquid detection
   c. Positive identification between sample and test results (barcode)
   d. Automated sampling of red cells and plasma and negligible carry over
3. Criteria for data handling
   a. Software for data
   b. Comparison for current and previous results
   c. Interfacing with LIS
   d. Updating of results on LIS

10.8 Assessment for incorporating an equipment

1. Vendor assessment
   a. Automation experience
   b. Record of installations
   c. Customer Service
   d. Training programs
2. Check list of base technology
   a. Sensitivity and specificity
   b. TAT
   c. Workload capacity
   d. Cost of reagent and controls
   e. Ease of operation
   f. Ability to cross train staff
3. Instrument assessment
   a. Installation
   b. Operations and specifications
   c. Test Repertoire: Various tests which can be performed
   d. Maintenance and reliability
   e. Data management

10.9 Semi-automated or fully automated

Blood centres depending on the workload and need, can choose a semi or a fully automated platform. In spite of reduction of many manual steps, semi-automated systems can be demanding. Steps such as preparation of samples and reagents, sample identification and loading and interpretation of results may add up to errors. Though there is improvement in the objectivity and reproducibility of results as well as the time taken to perform certain tests, lack of interfacing with hospital information systems (HIS) may lead to manual transcription errors while handling the data. In view of the above, semi-automated systems may be appropriate for small blood centres settings, however, for transfusion centres with high workload, a fully automated system is always a better option.

10.10 Cost issues

Automation in immunohaematology is expensive and usually requires a large initial investment and can be one of the major rate limiting factor. There are number of methods which can be put in place to tide over the initial

surge in the cost increment. Centres can adopt a 'reagent rental agreement' under which the equipment is placed maintenance free in return for a promised workload and reagents at a negotiated rate contract. Another approach could be PPP (Public Private Partnership) model. In this agreement the initial costing which could include structuring the lab, reagent storage facility (refrigerators, shelves), furniture, etc., is worked out on a mutual consent.

The cost should also include the cost of hardware and software required for interfacing the equipment with the HIS when introducing an automated system.

10.11 Feedback from users

Taking feedback from current users is one of the most important steps while deciding for equipment.

- Installation-related issues (time taken from completion of installation to actual regular use of equipment, additional requirement, and problems faced during installation such as air conditioning, electrical refitting requirement of a water purification plant, etc.).
- After sales service support (turnaround time of a service call, competency of vendors service staff, staff training, etc.).
- Reagents supply chain – (whether there is downtime due to delay in receiving reagents from the vendor, gap between the date of receiving reagent and expiry of reagent).
- Miscellaneous end-user problems

10.12 Back-Up for the automated equipment

Automated equipment will invariably have downtime at some point in time; therefore, it is always advisable to have a backup system. Most vendors provide semi-automated equipment along with fully automated equipment, which may be helpful for a moderate workload setting; however, fully automated standby equipment is a better option. The important issue here is to keep the backup equipment in working order, and the concerned staff should be trained to use both systems. This may be done by placing the semi-automated equipment at another place and assigning a certain category of tests to be done on that equipment or using the systems alternatively.

10.13 Staff training

Education, training, and proficiency testing of staff is an important part whenever any new method or technique is introduced. The introduction of automation brings a change in work practices; therefore, protocols for staff training must be discussed and well planned beforehand. A comprehensive training program is required to prevent any loss in routine patient care practices and to prevent falling back of staff to traditional ways after learning new technology.

10.14 Sample collection and sample flow

Most of the fully automated systems may have stringent sample requirements to be loaded on to the system. Uniformity in donor samples in terms of barcoding, type of sample (EDTA/plain), the volume of sample, storage time for units collected in the blood bank is easier to achieve, but bringing uniformity in inpatient bedside sample collection may be difficult. Therefore, new standard operating procedures (SOPs) and workflow charts should be designed with the requisite information to be circulated in the concerned areas.

10.15 Validation of equipment

The purpose of validation of an automated system is to test the competence of an automated system and demonstrate control over the processes executed by the automated system. It is also to ensure compliance with the accuracy and safety standards and enhance knowledge regarding maintenance and calibration of the equipment. A detail regarding validation can be seen at ISBT Guidelines for Validation of Automated Systems in Blood Establishments.

10.16 Continuous quality assurance

To continuously maintain the quality of work and improve performance, a continuous appraisal of the following should be done.

- Audit use of the equipment
- Optimization of reagent inventory
- Staff competence in performing the procedures.
- Audit of results and downtime

Key points

- Antigens may be proteins or polysaccharides and are capable of generating an immune response. Antibodies are glycoproteins that are formed in response to exposure to a foreign antigen.
- Red cell antibodies are generally immune antibodies that form after exposure to foreign red cell antigens after transfusion or pregnancy etc.
- IgG and IgM class of antibodies are of concern in immunohaematology, and their properties define how they interact in-vivo and in-vitro.
- A gene is considered to be a basic heritable unit that is responsible for the transmission of traits or characteristics from parents to offspring.
- Alleles are alternative forms of a gene that can occur at the same place or locus on a homologous chromosome.
- If a person inherits the same alleles from both parents, he/she will be called homozygous for that gene, and if he/she inherits different alleles from each parent, the individual will be called heterozygous for that gene
- A homozygous state as compared to a heterozygous state in some blood group antigens results in strong antigenic expression which is called as dosage. The phenomenon has implications in the interpretation of reactions in antigen screening and identification.
- Hardy Weinberg principle states that the frequency of alleles and genotype in a population will remain constant from one generation to the other if any evolutionary influences are absent.
- Hardy Weinberg principle can be used to calculate the genotype frequencies if the phenotype frequency of one of the alleles is known.
- Phenotype prevalence can be used to calculate an average number of blood units that need to be screened to find the requisite number of phenotype negative units for a patient with alloantibodies.
- Antiglobulin tests help in distinguishing the immune cause of haemolysis from non-immune cause.
- ABO blood group system is clinically the most significant system as ABO-incompatible transfusions can result in severe life-threatening intravascular haemolysis.
- Rh blood group system is regarded as the second most important blood group system after ABO, as some of the severe hemolytic transfusion reactions and most hemolytic disease of the fetus and newborn (HDFN) cases are associated with antibodies to the Rh group antigens.

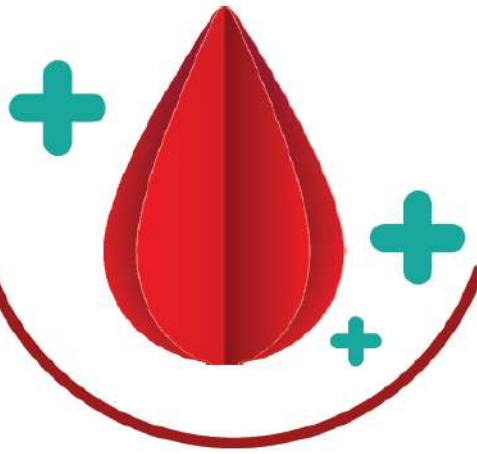
References

1. Coombs R.R.A. Historical Note: Past, Present and Future of the Antiglobulin Test. Vox Sang 1998;74:67–73.
2. Reverberi R, Reverberi L. Factors affecting the antigen-antibody reaction. Blood Transfus. 2007;5(4):227-240.
3. Armstrong B. Antigen–antibody reactions. ISBT Science Series (2008) 3, 21–32.
4. Storry JR, Olsson ML. The ABO blood group system revisited: a review and update. Immunohaematology. 2009;25(2):48-59.

5. Meny GM. Recognizing and resolving ABO discrepancies. Immunohaematology. 2017 Jun;33(2):76-81.
6. Issitt PD, Anstee DJ. Applied blood group serology, 4th edition (book). 1999. Montgomery Scientific Publication.
7. Connie M. Westhoff. The Rh blood group system in review: A new face for the next decade. Transfusion 2004;44: 1663-1673.
8. Curtis BR, McFarland JG. Human platelet antigens - 2013. Vox Sang. 2014 Feb;106(2):93-102.
9. Browne T, Dearman RJ, Poles A. Human neutrophil antigens: Nature, clinical significance and detection. Int J Immunogenet. 2020 Sep 24. doi:10.1111/iji.12514.
10. Daniels G. Molecular blood grouping. Vox Sang. 2004 Jul;87 Suppl1:63-6.
11. Harmening D. Modern blood banking and transfusion practices; 7th Ed., Davis Company, Philadelphia: F.A. (2019)
12. Klein HG, Anstee DJ. Mollison's Blood Transfusion in Clinical Medicine; 12th Ed. Wiley-Blackwell, John Wiley & Sons, Ltd. The Atrium, Chichester, West Sussex, PO19 8SQ, UK (2014)

Section 7

Compatibility testing



The term compatibility testing or pre-transfusion testing refers to a set of procedures required before blood is issued as being compatible. The purpose of pre-transfusion testing is to select blood and its components so that:

- They will have acceptable survival when transfused.
- They will not cause the destruction of the recipient's red cells.

The compatibility testing process starts with the reception of a complete and accurate blood requisition form with recipient sample at transfusion laboratory, accurate recipient identification, laboratory testing to determine the recipient's blood group and to identify the presence of red blood cell alloantibodies, followed by compatibility testing. Pre-transfusion testing is complete when a compatible blood component is identified and labelled for issue and transfusion to the intended recipient.

1. Steps in pre-transfusion testing

- Blood request form with sample
- Identification of recipient's (patient's) blood sample
- Testing of a recipient blood sample for
  - Sample acceptability (adequate volume, haemolysis etc.)
  - ABO and Rh D grouping of recipient
  - Screening for irregular antibodies
  - Comparison with records (blood group and antibody screen results)
- Selection of component and testing
- Compatibility testing (Cross-matching)
- Proper labelling of donor blood and issue.

2. Blood requisition form

A written request form with the information of the recipient for correct identification of the recipient and the blood component required with quantity duly signed by the ordering physician should accompany the sample for cross-matching. The form may also have non-mandatory fields for patient diagnosis, transfusion history, indication for transfusion (lab values) and any other special requirements such as leucoreduction or irradiation etc. This should be checked for completeness and accuracy, and incomplete/inaccurate requests should be rectified before accepting the request. Regulations in our country do not allow verbal/telephonic blood requests currently. A sample blood request form is provided in annexure VII. The blood request should accompany the recipient sample (either EDTA/plain/both as per hospital policy).

3. Identification of the recipient's blood sample

Blood centre staff should match the information in the blood request form with that on the sample received. The amount of information over the sample may vary. However, it must include two recipient identifiers in the form of the full name and hospital identification number of the recipient. Phlebotomist name or initials with his/her signature should also be present over the sample. It is also desirable to have the date and time of collection of the sample. The patient information should be written directly over the sample tube instead of being pasted over it.

Blood samples with inaccurate/incomplete information, including any overwriting or correction, are not acceptable, and a new sample should be asked before testing is started. If the patient information over the sample does not match that over the blood request form, then also a new sample should be asked.

A new blood sample is required if the earlier transfusion was given more than three days back in order to detect antibodies that may have developed in response to the transfusion.

4. Testing of recipient blood sample

Before testing could be initiated, check for the adequacy of the sample. A 3 ml EDTA sample for blood grouping and a 5 ml EDTA sample for both blood grouping and cross-matching is desirable. In addition, the sample should be checked for haemolysis. Grossly haemolysed samples should not be accepted, and a fresh sample should be requested. The recipient sample should be tested for ABO and Rh D grouping and antibody screening.

4.1 Determination of ABO type

Both cell grouping and serum grouping should be done on the recipient sample (see methods section for detailed procedure of blood grouping). In case of any discrepancy between the forward and the reverse group, blood should not be released until the discrepancy is resolved. Group O units may be cross-matched and released in emergency situations after proper communication with the treating clinician.

4.2 Determination of Rh D type

Determination of Rh D antigen should be done following the procedure in the methods section. Weak D testing is not required to be done on the recipient sample as a part of pre-transfusion testing. Because weak D testing of transfusion recipient samples is not performed, individuals with weak D are considered to be Rh-negative and can receive Rh-negative RBC units without risk of D alloimmunization. This practice permits some patients with partial D who can make alloanti-D to be treated as Rh-negative. It also avoids errors in Rh typing from a positive DAT result or mixed red-cell populations (D-positive and D-negative) following RBC transfusion, causing a positive weak D test result.

4.3 Checking recipient's records

If the patient has a history of blood transfusion, his /her previous transfusion records must be checked (if available) for:

- ABO and Rh (D) group
- Unexpected antibody /antibodies
- Any problem in cross-matching
- Any transfusion reaction

If any discrepancy is found between the current and historical blood groups, a fresh sample should be requested and tested to confirm the correct blood group. Similarly, if an antibody is identified in the records, then the patient should be provided antigen-negative blood for that antigen irrespective of the result status in the current sample.

In case where there are no records or the records are not available, a specimen collection or patient ID error may result in the wrong blood in the tube (WBIT), and this might not be detected by the blood bank. One approach to detect such specimen errors is to have a policy requiring a second specimen to be collected at a

different time or phlebotomy than the original specimen that was used to determine ABO/Rh status for patients. This will permit the identification of WBIT specimen errors and prevent ABO-incompatible transfusions

4.4 Antibody screening and identification

The recipient's serum should be tested for unexpected antibodies by indirect antiglobulin test with proper controls (positive, negative and endpoint using check cells). The antibody screen of the recipient should ideally be done using either a 2-cell or a 3-cell panel (for detail of cell panels, see section 6). If on screening, antibody/ies are detected, the antibody/ies should be identified by red cell panel if possible. Further, the corresponding antigen-negative red cell unit should be cross-matched in the indirect antiglobulin phase and compatible units be transfused, if possible.

In general, an antibody is considered to be potentially clinically significant if it has been demonstrated to cause haemolytic disease of the fetus and newborn (HDFN), haemolytic transfusion reaction, or with notably decreased survival of transfused red cells. Antibodies reactive at either 37^0C or in the AHG test phase are more likely to be clinically significant than those reactive at cold temperatures. Proper detection and identification of antibodies are important for the selection of appropriate blood for transfusion.

4.5 Prerequisite for antibody identification

A positive antibody screen is followed by antibody identification. The following patient-related information is of help in antibody identification:

- Medical history
- Age & sex
- Medical diagnosis
- History of transfusion
- In case of females- history of pregnancy, abortion, hydrops etc.
- History of drug therapy including RhIg, daratumumab, antibiotics
- Any other relevant information which may be helpful during an investigation, like previous serological testing results

4.6 Antibody identification

Identification of an antibody to red cell antigen(s) requires testing the recipient serum against a panel of reagent red cells with known antigenic composition.

Each reagent red cell of the panel is from a different donor. The reagent red cells are selected so that if one takes all of the examples of red cells into account, a distinctive pattern of positive and negative reaction exists for each of many antigens. The phenotypes of the reagent red cells should be distributed so that single specificities of the common alloantibodies can be clearly identified, and most others can be excluded.

Panel cells should not be used after the expiration date; however, this restriction is not always practical. Most of the time, in-date reagent cells can be used for initial antibody identification and, if necessary, expired reagent red cells can be used to exclude or confirm specificities. However, each blood centre should establish a policy for using expired reagent red cells and validate any procedures associated with this practice before starting this.

4.6.1 Sample requirement

Either serum or plasma may be used for antibody identification. Plasma is not suitable for the detection of antibodies where complement activation is a requirement for their detection. A 5-10 ml of whole blood in a plain tube usually contains sufficient serum to identify antibodies. When autologous red cells are studied, the use of cells anticoagulated with EDTA avoids problems associated with the in-vitro uptake of complement components by red cells.

4.6.2 Saline test at room temperature (20-25ºC)

The test is used to identify (IgM) cold reacting antibodies, e.g., anti-M, anti N, anti-Le^a, anti-Le^b anti I, anti P etc. This is done when running the test in a tube, and it enhances the detection of certain antibodies and may

help explain reactions detected in other phases. However, as the antibodies detected at this temperature are not clinically significant, they can be omitted.

4.6.3 Test at 37⁰ Celsius

Can detect the presence of potent anti-D, -E, or -K that may cause direct agglutination of red cells. The lysis of antigen-positive red cells may occasionally detect other antibodies (e.g., anti-Le^a or -Jk^a) during the 37^{0}C incubation if the serum is tested. Omitting centrifugation and the reading at 37^{0}C should lessen the detection of unwanted positive reactions caused by clinically insignificant cold-reactive autoantibodies and alloantibodies. However, in some instances, potentially clinically significant antibodies are detected only by their 37^{0}C reactivity.

4.6.4 Indirect antiglobulin test

The indirect antiglobulin test identifies the warm reacting IgG and complement binding allo- and auto-antibodies. Both saline & LISS indirect antiglobulin tests may be used simultaneously for the identification of weaker antibodies. Various enhancement media such as enzymes, LISS, PEG etc. may be used during the antibody identification to facilitate the identification of a complex mixture of antibodies or to enhance the reactivity of weaker antibodies. Detail of these enhancement media is available in section 6.

4.6.5 Automation

Pretransfusion tests may also be performed using column-agglutination technology, microplate solid-phase technology, or haemagglutination- microplate technology. Details available in section 6.

4.6.6 Interpreting results

Antibody-detection results are interpreted as positive or negative according to the presence or absence of reactivity (i.e., agglutination or haemolysis), respectively. Interpretation of panel results is a complex process combining technique, knowledge, and skills. Panel results generally include both positive and negative results, sometimes at different phases of testing. The patient's phenotype is also considered in the final interpretation wherever possible.

A single alloantibody usually produces a clear pattern with antigen-positive and antigen-negative panel cells. This is because the reactive panel cells express the antigen, and all nonreactive cells lack it. When there is no discernible pattern to explain the reactivity, the presence of multiple antibodies, dosage, and variations in antigen expression should be considered. The following rules are helpful in interpreting and reading out the reactions of panel cells on antigram.

"Rule Out," or "Cross Out."

The commonly used first approach to the interpretation of panel results is to exclude specificities based on non-reactivity of the patient's serum with panel cells that express the antigen. Suppose an antigen is present on the red cell, and the serum is not reactive with it, the presence of the corresponding antibody may tentatively be excluded or ruled out or crossed out on the antigram. However, antibodies to blood group antigens known to show dosage should not be ruled out on panel cells with heterozygous expression.

Next, the red cells that are reactive with the serum are considered. The pattern of reactivity for each specificity that is not excluded is compared to the pattern of reactivity obtained with the test serum. If an antigen pattern matches the test serum pattern exactly, this pattern identifies the specificity of the antibody in the serum. If there are remaining specificities that were not excluded, additional testing is needed to eliminate the possibilities and confirm the suspected specificity. This process requires testing of the serum with additional selected red cells.

Use of select red cells

Select red cells are red cells that have been chosen because they express some specific antigens and lack others. Select red cells with different antigen combinations can be used to confirm or rule out the presence of antibodies.

Three and three rules

To be sure that the reaction pattern suggesting an antibody is not by mere chance, it is required to test the test serum with a sufficient number of panel cells lacking and expressing the corresponding antigen. Based on Fisher's Exact method, it is required that three antigen-positive cells are reactive and three cells lacking the antigen are non-reactive. However, this is always not feasible. Therefore, in some cases, the use of two reactive and two nonreactive red cell samples is also an acceptable approach for antibody confirmation. The other few rules followed are two reactive and three nonreactive red cell samples or with one reactive and seven nonreactive red cell samples (or the reciprocal of either combination), based on calculations by Harris and Hochman.

Phenotyping autologous red cells

When an antibody is tentatively identified in the serum, the corresponding antigen is expected to be absent from the autologous red cells. So, phenotyping recipients red cells for the same antigen supports the specificity of the antibody identified. However, this cannot be performed if there is a history of transfusion in the previous 3-month duration.

Auto control

The recipient serum and autologous red cells are tested under the same conditions as serum and reagent red cells. If the auto-control is positive in the antiglobulin phase, a DAT should be performed. If the DAT result is negative, antibodies to an enhancement medium constituent or autoantibodies that are reactive only in the enhancement medium should be considered. If the DAT result is positive, it must be interpreted with careful attention to the transfusion and drug history. Elution and adsorption studies may be necessary to establish if any autoantibodies are present and if they are not masking alloantibodies.

Example of antibody screening and identification

Red blood cells are listed in the left-hand column as OI and OII and are known as screening cells (Table 1). The O means that they are group O as ABO antibodies would interfere with the test. Screening cells have antigens that correspond to antibodies that are most often a problem in transfusion.

Table 1: Antibody screening for the example

Screening Cells	Phenotype	AHG
OI	DCe, Kk, Fy^a Le^b, SsMN, P1	0
OII	DcE, k, Fy^a Jk^a, Le^b, Le^a, SsMN, P1	3+

In the AHG column, the serum reacts 3+ with OII but not with OI. The results indicate the presence of an antibody in the tested serum sample. Further, antibody to the antigens present in a homozygous state on OI can be ruled out at this stage.

We will now see with an example of how to proceed with the antibody identification with a simplified 8-cell antibody identification panel (Figure 1a-j).

This is how the typical antibody identification panel may appear after performing the identification in the AHG phase. As we see in the above example (Figure 2a), some of the panel cells react with the antibody giving a positive reaction of varying strengths; others give a negative reaction.

Proceeding further with identification (Figure 1b), beginning with the first cell, we note that the cell is homozygous for C antigen. So, since the cell failed to react with the patient's serum, as shown by the reaction interpretation in the right-hand column, we can infer that the antibody causing the positive antibody screen is not directed against the C antigen. We can thus, cross out the C antigen in the top row. We then proceed to look at other antigens for which the first cell's phenotype is positive.

Note that the cell is also positive for the D, e, s antigens and for both K and k antigens. Since E and e are antithetical antigens, the cell appears to be homozygous for antigen e and we infer that the serum antibody is not directed against e. Therefore, antigen e can also be crossed out. Here, we can cross out D as well.

Antigens S and s are antithetical, so by logic, we have been following, donor cell number one has a double dose of s antigen, and we can cross out little as well. On the other hand, the cell possesses both K and k antigens which are determined by antithetical alleles at the same genetic locus. Thus, there is only a single dose of each of these antigens, and we will not eliminate either of them as possibilities for the specificity of the unknown antibody.

Let us look at panel cell 2 (Figure 1c). This cell has a double dose of the D, C, e, and s antigens, but we gain no new information. We have already inferred that the antibody in question does not react with them and have crossed them out, as shown by the black slash marks. However, in contrast to the first cell, the k antigen is present, and the K antigen is absent. So, k must be present in double dose, and we can cross it out. This new information is shown in the form of a grey slash mark in the first row.

Let us move on to the third cell (Figure 1d). In this case, the patient's serum reacted strongly in the anti-human globulin phase of the test. This cell is positive for the D, c, E, K, k, and S antigens, so it might be directed against one or more of them. But we have already ruled out anti D and anti k. So, we suspect that the serum is probably reactive against one of the four remaining antigens circled in black.

We will now proceed with the crossing out. Here is another cell that is reactive in the anti-human globulin phase of testing (Figure 1e). Again, its reactivity could be explained if the antibody were anti c, anti E, or anti S. On the other hand, anti K could not explain the reactivity of this cell, although more than one antibody may be present. But we do not cross out anything when the cell is reactive.

Moving on to cell five (Figure 1f), we note that it does not react with the serum. In fact, this cell is C negative, c positive, so the c antigen must be in double dose, and we can cross out little c as one of the potential specificities for the unknown antibody.

Here is another non-reactive cell (Figure 1g). It is positive for antigens c, e, k, and s, all of which we have been ruled out previously. Note that it also has a single dose of the K antigen and note that K has been crossed out in the top row. This seems to be a violation of our rule on only crossing out antigens that are present on the cell in double dose. To understand this, we have to be aware of the fact that anti-K antisera rarely shows dosage effect. They typically react just as well with single-dose as with double dose K positive RBCs. So, we can assume that our unknown antibody is not anti-K. Hence, we make an exception to our rule in this case and cross out K.

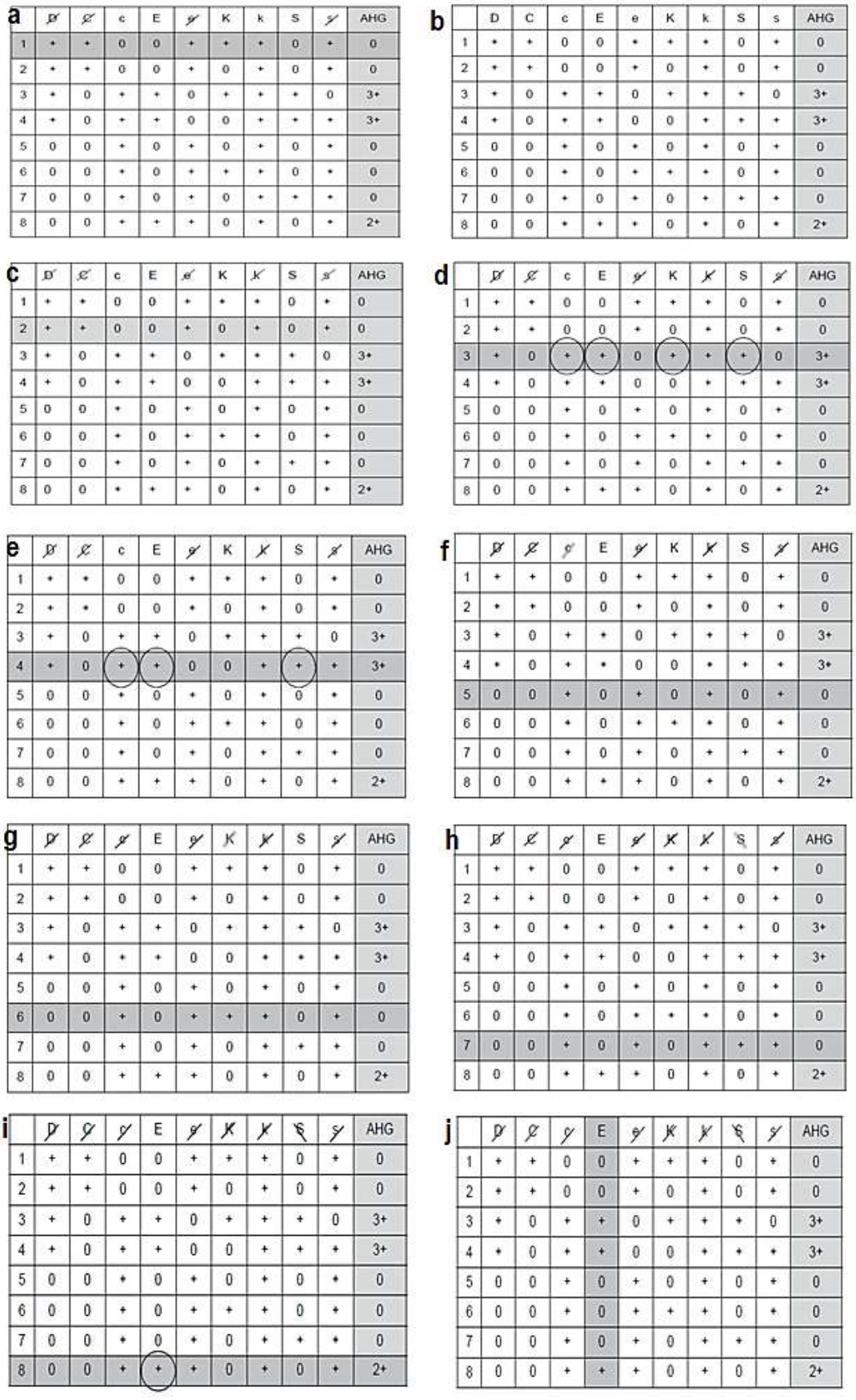
Cell number seven is again a non-reactive cell (Figure 1h), so we look for anything new that can be crossed out. Cell number seven has a double dose of the antigens c, e, and k, but we have already crossed these out. The cell expresses the antigen S, which we have not crossed out, but this antigen is only present in a single dose since little s, is present as well. So, unless the unknown antibody is weak, it is probably not directed against S. But we cannot be sure. This is indicated by a slash mark going in the opposite direction to show that we cannot absolutely rule out anti S, but that we do not really think that it is going to explain the reactivity of this patient's antibody.

Looking at the final cell (Figure 1i), note that it reacted with the antiserum. Cell eight possesses a number of blood groups that we have already crossed out but lacks S, which we are unable to rule out. But note that the E antigen is again present. We have no evidence to suggest that the antibody is not directed against E.

In fact, the pattern of reactivity follows the E antigen exactly (Figure 1j). All of the cells with the E antigen, numbers three, four and eight, are reactive. And all of the cells that lack it are non-reactive. So, all the phenomena we see would be explained if the antibody were anti E. We have not ruled out anti S to our satisfaction. So, we need to test the patient serum against a "rule out" cell that has the S antigen in double dose. It also has to lack big E, the antigen we hypothesize our unknown serum is directed against. (Figure 2) If our hypothesis is correct, it should not react.

The reaction is shown above now rules out anti S, and hence we can conclude that our serum has anti E antibody.

In this step, if the antibody screen is positive and the specificity of antibody could be identified, then a unit that is ABO compatible with the recipient and negative for the antigens against whom antibodies were identified should be selected and crossmatched.



a

	D	C	c	E	e	K	k	S	s	AHG
1	+	+	0	0	+	+	+	0	+	0
2	+	+	0	0	+	0	+	0	+	0
3	+	0	+	+	0	+	+	+	0	3+
4	+	0	+	+	0	0	+	+	+	3+
5	0	0	+	0	+	0	+	0	+	0
6	0	0	+	0	+	+	+	0	+	0
7	0	0	+	0	+	0	+	+	+	0
8	0	0	+	+	+	0	+	0	+	2+

b

	D	C	c	E	e	K	k	S	s	AHG
1	+	+	0	0	+	+	+	0	+	0
2	+	+	0	0	+	0	+	0	+	0
3	+	0	+	+	0	+	+	+	0	3+
4	+	0	+	+	0	0	+	+	+	3+
5	0	0	+	0	+	0	+	0	+	0
6	0	0	+	0	+	+	+	0	+	0
7	0	0	+	0	+	0	+	+	+	0
8	0	0	+	+	+	0	+	0	+	2+

c

	D	C	c	E	e	K	k	S	s	AHG
1	+	+	0	0	+	+	+	0	+	0
2	+	+	0	0	+	0	+	0	+	0
3	+	0	+	+	0	+	+	+	0	3+
4	+	0	+	+	0	0	+	+	+	3+
5	0	0	+	0	+	0	+	0	+	0
6	0	0	+	0	+	+	+	0	+	0
7	0	0	+	0	+	0	+	+	+	0
8	0	0	+	+	+	0	+	0	+	2+

d

	D	C	c	E	e	K	k	S	s	AHG
1	+	+	0	0	+	+	+	0	+	0
2	+	+	0	0	+	0	+	0	+	0
3	+	0	+	+	0	+	+	+	0	3+
4	+	0	+	+	0	0	+	+	+	3+
5	0	0	+	0	+	0	+	0	+	0
6	0	0	+	0	+	+	+	0	+	0
7	0	0	+	0	+	0	+	+	+	0
8	0	0	+	+	+	0	+	0	+	2+

e

	D	C	c	E	e	K	k	S	s	AHG
1	+	+	0	0	+	+	+	0	+	0
2	+	+	0	0	+	0	+	0	+	0
3	+	0	+	+	0	+	+	+	0	3+
4	+	0	+	+	0	0	+	+	+	3+
5	0	0	+	0	+	0	+	0	+	0
6	0	0	+	0	+	+	+	0	+	0
7	0	0	+	0	+	0	+	+	+	0
8	0	0	+	+	+	0	+	0	+	2+

f

	D	C	c	E	e	K	k	S	s	AHG
1	+	+	0	0	+	+	+	0	+	0
2	+	+	0	0	+	0	+	0	+	0
3	+	0	+	+	0	+	+	+	0	3+
4	+	0	+	+	0	0	+	+	+	3+
5	0	0	+	0	+	0	+	0	+	0
6	0	0	+	0	+	+	+	0	+	0
7	0	0	+	0	+	0	+	+	+	0
8	0	0	+	+	+	0	+	0	+	2+

g

	D	C	c	E	e	K	k	S	s	AHG
1	+	+	0	0	+	+	+	0	+	0
2	+	+	0	0	+	0	+	0	+	0
3	+	0	+	+	0	+	+	+	0	3+
4	+	0	+	+	0	0	+	+	+	3+
5	0	0	+	0	+	0	+	0	+	0
6	0	0	+	0	+	+	+	0	+	0
7	0	0	+	0	+	0	+	+	+	0
8	0	0	+	+	+	0	+	0	+	2+

h

	D	C	c	E	e	K	k	S	s	AHG
1	+	+	0	0	+	+	+	0	+	0
2	+	+	0	0	+	0	+	0	+	0
3	+	0	+	+	0	+	+	+	0	3+
4	+	0	+	+	0	0	+	+	+	3+
5	0	0	+	0	+	0	+	0	+	0
6	0	0	+	0	+	+	+	0	+	0
7	0	0	+	0	+	0	+	+	+	0
8	0	0	+	+	+	0	+	0	+	2+

i

	D	C	c	E	e	K	k	S	s	AHG
1	+	+	0	0	+	+	+	0	+	0
2	+	+	0	0	+	0	+	0	+	0
3	+	0	+	+	0	+	+	+	0	3+
4	+	0	+	+	0	0	+	+	+	3+
5	0	0	+	0	+	0	+	0	+	0
6	0	0	+	0	+	+	+	0	+	0
7	0	0	+	0	+	0	+	+	+	0
8	0	0	+	+	+	0	+	0	+	2+

j

	D	C	c	E	e	K	k	S	s	AHG
1	+	+	0	0	+	+	+	0	+	0
2	+	+	0	0	+	0	+	0	+	0
3	+	0	+	+	0	+	+	+	0	3+
4	+	0	+	+	0	0	+	+	+	3+
5	0	0	+	0	+	0	+	0	+	0
6	0	0	+	0	+	+	+	0	+	0
7	0	0	+	0	+	0	+	+	+	0
8	0	0	+	+	+	0	+	0	+	2+

Figure 1 a-j: Antigram of 8-cell identification panel showing steps of antibody identification using an example (details in text)

D	C	c	E	e	K	k	S	s	AHG
+	+	0	0	+	+	+	+	0	0

Figure 2: A select cell or "rule out" cell

5. Selection of component and testing

Once the recipient testing is complete, a suitable unit needs to be selected for crossmatching as per the requirement of the component. In addition to the ABO and Rh D blood group, the selected unit should be checked for its TTI screening status, expiry date and any visible haemolysis, colour change, clots, or any leakage. After selection, the unit should be rechecked for ABO, and Rh D blood group and the same should match over the donor label pasted over the unit. The tubing segment attached with the blood unit should be used for repeat testing.

5.1 Whole blood, red cell components

The following points are to be kept in mind while selecting blood for transfusion

- It is preferable to select ABO identical blood for the recipient as far as possible. If any antibodies were identified, the unit should be negative for corresponding antigens as well.
- When group-specific blood is not available, use alternate ABO compatible blood (Figure 3).

Donor grp Patient grp	A	B	O	AB	Rh D pos	Rh D neg
A	Option 1		Option 2			
B		Option 1	Option 2			
O			Option 1			
AB	Option 3	Option 2	Option 4	Option 1		
Rh D pos					Option 1	Option 2
Rh D neg						Option 1

Figure 3: Selection of blood group of the donor unit with alternative options

In group AB patient, it is advisable not to change from group A to group B blood or vice –versa, when more than one unit is given in a continuous transfusion. The decision to change back to group-specific blood should be based on the presence or absence of anti A/anti B in a subsequent sample of the patient.

In the case of a neonate less than 3-4 months of age, use blood according to Figure 4. Mother's sample is also required till the age of 4 months. If the mother's sample is not available, group O unit Rh D compatible with the infant should be selected.

Baby blood group	Mothers blood group	Blood to be issued
O	O	O
	A	O
	B	O
	AB	O
A	O	O
	A	A, O
	B	O
	AB	A, O
B	O	O
	A	O
	B	B, O
	AB	B, O
AB	O	O
	A	A, O
	B	B, O
	AB	AB, B, A, O

Deciding Rh Group

Rh Pos	Rh Pos	Rh pos
	Rh Neg	Rh Neg
Rh Neg	Rh Pos	Rh Neg
	Rh Neg	Rh Neg

Figure 4: Selection of donor unit in neonates and infants (till 4 months of age)

5.1.1 General considerations for selecting a red cell unit

- Select the blood of the same Rh (D) type as that of the patient, particularly in female patients who are of childbearing age.
- If Rh D negative blood is not available in emergency situations, RhD positive blood can be given to male patients & to female patients beyond menopause, provided no preformed anti-D is demonstrable in their sera. If inadvertently given to women of childbearing age, consider RhIg immunoprophylaxis.
- Suppose it is impossible to identify the specificity for an irregular antibody either because of the lack of a cell panel or an emergency transfusion requirement, the patient's serum should be cross-matched with several units of the same ABO and Rh (D) type blood to select the compatible units of blood.
- In some cases, when it is not possible to find compatible units of blood, the patient's relatives, especially siblings, should be tested as they have a higher probability of being compatible.
- If the patient is known to have irregular antibody(ies), autologous transfusion may be considered in case it is practicable.
- In general, the oldest units should be used first, but there are the following exceptions where relatively fresh units should be selected:
  - Intrauterine transfusion & exchange transfusion in neonates (units preferably less than 5 days old).
  - Patients with thalassemia & sickle cell anaemia
- In ABO haemolytic disease of the newborn, group O red cells of the same RhD type as that of the baby should be selected.

- In Rh haemolytic disease of the newborn, RhD negative blood of the same ABO group as that of the baby is used if it is the same as that of the mother or if it is compatible with the mother's blood. If the baby's ABO group is not compatible with mother's ABO group, then O RhD negative blood is selected and matched with the mother's serum.

  Note: Antigens other than ABO and Rh D are not routinely considered in the selection of units of blood for transfusion to non-alloimmunized patients. However, for patients with certain medical conditions, such as sickle cell disease, thalassemia, and other frequently transfused population, some institutions may select to transfuse Rh (D, C, c, E, e) and Kell phenotypically matched RBC units to prevent alloimmunization.

5.2 Fresh frozen plasma

Fresh frozen plasma should be ABO identical/compatible with the recipient's red blood cells. In neonates, ABO identical plasma should be preferred. No considerations for matching RhD should be made as far as plasma component selection is concerned. Cryoprecipitate does not require ABO/ Rh grouping except in neonates.

5.3 Platelet concentrate

Platelet concentrate should be ABO and Rh (D) type-specific with the recipient blood as far as possible. In case of shortage of ABO and Rh (D) type-specific whole blood-derived / random donor platelets, any ABO/ Rh group should be used provided there is no visual red cell contamination of the platelet concentrate. In the case of single donor platelets prepared by apheresis, plasma should be reduced, or the use of platelet additive solutions should be considered if the component is used across the blood group barrier (e.g., use of 'O' group SDP to B patient).

Infants should be provided ABO identical platelets (both whole blood-derived and single donor) till the age of one year.

5.4 Granulocyte concentrate

As leucocyte concentrate is known to contain high amounts of red cells, it should be ABO and Rh(D) type specific and should be compatible after cross-matching at indirect antiglobulin phase with the recipient blood.

6. Compatibility testing

The crossmatch test is carried out to ensure that there are no antibodies present in the patient's serum that will react with donor cells when transfused. The method used should demonstrate ABO incompatibility and clinically significant unexpected antibodies and should include an indirect antiglobulin test (IAT). In certain clinical conditions, where autoantibodies are present, the least incompatible unit should be issued with specified instructions.

Historically crossmatch testing procedures have been divided into two parts

- Major cross-match test
- Minor cross-match test

Major cross-match consists of testing donor's red cells with recipient's (patient's) serum/plasma, while minor crossmatch consists of testing patient's red cells with donor's plasma. As donor samples are screened beforehand for the common irregular type of antibodies, a minor cross-match is not practised routinely.

Crossmatch may be done at different phases depending on temperature and the use of AHG for testing.

6.1 Immediate spin crossmatch

It is performed to detect ABO incompatibilities between the donor red cells and patient's serum.

6.2 Antiglobulin crossmatch

It is performed to detect incomplete or IgG type of antibodies in recipient serum against the antigens on donor red cells. It can be performed either by tube technique, column agglutination method or on solid phase

systems. If antibody screening is performed before this, it may help detect some antibodies against low-frequency antigens present over donor red cells that were not present in the panel cells.

6.3 Compatibility testing in emergencies

Blood or blood components should be issued before completion of routine cross-matching tests in the case where delay in providing blood may jeopardize the patient's life, on receipt of a signed written request of the treating physician stating that the clinical condition of the patient is sufficiently urgent to require the release of blood before completing ABO and Rh(D) tests and compatibility testing.

Under such circumstances, recipients whose ABO and Rh(D) type is not known should receive red cells of group O Rh(D) negative if available, otherwise, O Rh(D) positive blood should be used. A recipient whose ABO, Rh(D) type has been determined should receive ABO and Rh(D) specific blood group whole blood or red cells before the tests for compatibility have been completed. The donor tag or label on the blood container and the crossmatch report form should indicate that compatibility testing has not been completed at the time of issue. However, standard compatibility test should be completed promptly. If incompatibility is detected in further testing, the concerned clinician should be informed immediately to discontinue the transfusion.

7. Interpretation of antibody screening and crossmatch results

- **Incompatible immediate spin crossmatch with negative antibody screen**
  - Incorrect ABO grouping of patient or donor
  - Weak expression of the antigen which does not get detected on routine serological testing
  - A_2 or A_2B individual with anti-A_1 antibody
  - Polyagglutinable donor cells
  - Other alloantibodies which react at room temperature such as anti M, anti N etc.
  - Cold autoantibodies
  - Rouleaux formation
- **Positive antibody screen with incompatible AHG crossmatch**
  - If red cells of all donors tested are incompatible with the patient's serum and also antibody screen is positive, a suspect antibody directed against an antigen of high incidence of multiple antibodies.
- **Antibody screen negative with incompatible antiglobulin crossmatch**:
  - Donor red cells with a positive DAT
  - The antibody reacts only with cells with strong expression of a particular antigen which may either be because of dosage such as with Rh, Kidd, Duffy, MN antigens or because of intrinsic variation in antigen strength such as with P1.
  - The antibody reacts with an antigen present on the indigenous donor cells but not on the screening cell panel.
  - If the antibody screen is negative and only one donor unit is incompatible, then the antibody in the patient's serum is likely to be directed against an antigen of low incidence.
  - Passive transfer of antibody: significant levels of circulating anti A or -B may be present after an infusion of group O platelets/plasma to a non-group O recipient.
  - The patient's serum may contain an antibody i.e., anti-A1- Check the serum grouping of the patient to confirm the presence of anti-A_1 with known A_1 cells.
- **Antibody screen is positive crossmatch is compatible**
  - Auto-anti H (-IH)
  - Anti Le^{bH}
  - Antibodies dependent on reagent cell diluent
  - Antibodies demonstrating dosage and donor red cells are from heterozygotes (i.e., expressing a single dose of antigen).

  - The donor unit lacks a corresponding antigen.
  - An autoantibody in patient's serum reacting with corresponding antigens on donor red cells. This can be solved by putting auto control which will be positive.
- **Positive antibody screen result, incompatible crossmatch, positive auto control, and negative direct antiglobulin test result**
  - An antibody is present to an ingredient in the enhancement media or enhancement-dependent autoantibody.
  - Rouleaux have formed.
- **Positive antibody screen result, incompatible crossmatch, positive auto control, and positive direct antiglobulin test result**
  - Alloantibody causes a delayed serologic or haemolytic transfusion reaction.
  - Passively acquired autoantibody (e.g., intravenous immune globulin) is present.
  - Cold- or warm-reactive autoantibody is present.
  - Problems in a patient's serum, i.e., multiple myeloma and macroglobulinemia can result in rouleaux formation. This is resolved by saline replacement technique.

8. Labelling and issue of blood

Blood unit found to be crossmatch compatible with the patient should be labelled before being reserved or issued to the recipient. The label or tag should have the patient's full name, hospital, identification number, blood unit number, blood group of the patient and unit, the result of the compatibility testing and signature of the technician who has performed the testing.

Blood should be issued only after receiving a written issue request from the ward mentioning the patient name and hospital identification number. A crossmatch report or compatibility report should accompany the blood unit with detail of the patient and the unit and compatibility testing results. A portion of the integral tube with at least one numbered segment should remain attached with the blood bag being issued.

The cross-matching report should have the patient's first name with surname, age, sex, identification number, ward, bed number, ABO and Rh(D) type. The form should have donor unit identification number, segment number, ABO and Rh(D) type and expiry date of the blood. Interpretation of cross-matching report and the name of the person performing the test and issuing the blood should be recorded.

Each unit of blood should be visually inspected before issue. It should not be issued if there is any evidence of leakage, haemolysis or suspicion of microbial contamination such as unusual turbidity or change of colour.

9. Return of issued blood units

It is recommended that blood, once issued, should not be taken back by the Blood Centre, especially if the cold chain is broken and the blood is returned to the Blood Centre after 30 minutes.

The Blood Centre should have a SOP for acceptance and reissue of blood unit returned within 30 minutes or after 30 minutes when the cold chain has not been broken

Key Points:

- Pre-transfusion testing involves procedures and testing which ensure that transfused blood components survive in-vivo and produce intended effects.
- Identifying the recipient and matching the patient details on the sample and blood requisition is an important step to prevent identification errors.
- Recipient testing for blood group and antibody screen is another important element of pre-transfusion testing

- Selected donor units should be tested again to confirm the blood group on the label and should be ABO and Rh D identical or compatible.
- Compatibility testing can be done by both the immediate spin technique and IAT technique and detect incompatibilities due to IgM (mostly ABO) and IgG antibodies, respectively.
- Emergency situations require that blood be released with incomplete testing. However, compatibility workup should be completed after the units have been issued; in case of discrepancy, the clinician needs to be informed.
- Blood should be issued from the Blood Bank/ Blood Centre and the blood cross-matching report or compatibility report.

Reference

1. Makroo R. Principles and practices of transfusion medicine. 2nd edition. New Delhi: Kongposh publications Pvt. Ltd.;2018.

Section 8

Transfusion of blood components

1. Good clinical transfusion practices

Blood transfusion is one of the most frequently performed procedures in the hospital. Yet, ironically, blood transfusion has also been identified as one of the commonly overused interventions. What makes these two already risky combination of high frequency and overuse further dangerous is an overall complex process involving a multidisciplinary group of healthcare professionals with varying levels of understanding of blood transfusion practice. Thus, good clinical transfusion practices cannot be overemphasized so far as the patient safety is concerned.

Clinical transfusion comprises the appropriate use of blood/ components, safe transfusion practices and all the clinical aspects of blood transfusion. Thus, it includes both the risks and benefits of transfusion as well as close monitoring of the patients. Wherever feasible transfusion alternatives should be considered, and patients should be transfused only when there is evidence for potential benefit outweighing the risk.

2. Haemolytic disease of the fetus and newborn (HDFN)

HDFN is a condition where the life span of the fetus/ neonate's red cells is decreased due to the action of the antibodies derived from the mother by placental transfer.

2.1. Etiopathogenesis

Haemolytic disease of the newborn and fetus is caused by the destruction of the red cells of the fetus or newborn by IgG antibodies produced by the mother. Only antibodies of the immunoglobulin IgG class actively transfer across the placenta. The subclasses IgG1 and IgG3 are more notorious for causing haemolysis than IgG2 and IgG4. This haemolysis leads to an accelerated bone marrow production of red cells, resulting in the spilling of erythroblasts (immature red cells) into the peripheral circulation. The term erythroblastosis fetalis derives its origin from this finding. When red cell production rate cannot match the red cell destruction rate, erythropoiesis starts happening in the spleen and the liver resulting in its enlargement, portal hypertension and liver damage. Severe anaemia and decreased albumin (liver damage) lead to cardiac failure and generalized oedema, ascites and third space collections causing the appearance of hydrops fetalis.

Hyperbilirubinemia is due to the breakdown of the haemoglobin released from the destroyed red cells before and even after the delivery. This is indirect bilirubin which crosses the placenta and is converted (conjugated) in the maternal liver to form direct bilirubin. The mother excretes this conjugated bilirubin. At birth, the infants are anaemic but may not be jaundiced. However, after birth, the infant is unable to convert (conjugate) indirect bilirubin efficiently, especially in premature infants, due to the lack of an enzyme glucuronyl transferase, and jaundice develops. When the level or the rate of production of bilirubin exceeds the binding capacity of the albumin, the unconjugated bilirubin crosses into the infant's brain. If untreated, it can cause kernicterus or permanent damage to the brain. The maternal antibody decreases in the infant over 10-12 weeks.

2.2. Maternal alloimmunization

The maternal antibodies are produced due to stimulation in a previous pregnancy, past blood transfusions or sometimes in the current pregnancy itself. Small (0.1 mL) quantities of fetal RBCs gain access to the maternal blood circulation in nearly all pregnancies, as shown by studies using flow cytometry. Red cell membrane/ enzyme abnormalities and extreme prematurity are less common causes of HDFN. Blood centres in India deal with HDFN mostly due to the maternal antibodies in the Rh system (anti-D is still the most common cause in India and the first pregnancy usually remains unaffected) or due to ABO antibodies (e.g., O group mother with A/ B group fetus). The introduction of antenatal and postnatal Rh(D) immune globulin prophylaxis has reduced alloimmune sensitization in pregnant women who are Rh(D) negative and thus a lower than before incidence of HDFN due to anti-D antibodies.

About 70% of Rh HDFN cases are seen in 2nd and 3rd pregnancy. Usually, the first pregnancy is unaffected if there is no previous sensitization through blood transfusion, etc. ABO incompatibility between mother and fetus provides some protection to the fetus from Rh-HDN. It is believed that naturally occurring maternal ABO allo-antibodies destroy the Rh(D) positive fetal red cells immediately as they enter the maternal circulation before they can sensitize the mother for Rh antigen. Once the mother becomes immunized to Rh antigen, this protective effect due to ABO incompatibility is lost. Antibodies against Kell, Kidd and Duffy blood groups systems have also been rarely found.

2.3. Diagnosis

Past and present obstetric history, ABO and Rh(D) typing and AHG phase antibody screening can help in suspecting and establishing the diagnosis.

2.4. Investigations

2.4.1. Mother's testing:

ABO & Rh(D) typing and indirect coombs test for irregular antibody detection at 37^{0}C in AHG phase. For all antibody-positive patients, antibody identification and titration should be done. In addition, tests to determine the type of antibody (IgM or IgG or both) should be done (e.g., DTT treatment). The critical titre of all immune anti-D below which severe disease in the fetus is unlikely, is usually considered as 16-32 in the AHG phase. However, a rising titre even below this value should be considered significant and followed closely. If the antibody is detected at any stage of pregnancy, the titre should be repeated every 4 weeks.

2.4.2. Fetal testing:

(a) Fetal middle cerebral artery (MCA) blood flow, determined by Doppler ultrasound, is a non-invasive technique to determine the fetal anaemia. An increased MCA flow of more than 1.5 times of mean denotes moderate to severe fetal anaemia, and intervention can be planned. (b) Fetal DNA typing using cell-free fetal DNA in mother's blood or by blood sample obtained through amniocentesis.

2.4.3. Biological father's testing:

Antigen typing to rule-in or rule out the irregular antibody should be done. To determine the fetal risk, father's homozygous/ heterozygous state for the involved antigen and DNA typing can be done if facilities are available for such testing. Homozygous expression of the implicated antigen in the father would put the fetus at 100% risk and 50% in the case of heterozygous expression.

2.4.4. Newborn testing:

Cord blood sample obtained from the maternal side of the umbilical cord is tested for:

- **ABO and Rh typing:** Only forward/ cell typing is done on the washed cells. If Rh(D) is negative, weak D testing (Du) should be done. A heavy coating of fetal Rh(D) positive cells with mother derived anti-D antibody may paradoxically give a negative Rh(D) test – called the blocked D phenomenon. Strong result of DCT/ DAT of fetal cells should raise the suspicion of blocked D phenomenon, especially if fetal cells are typing as Rh (D) negative despite strong clinical suspicion of HDFN.

- **Direct Coombs test (DCT/ DAT)**: It should be done on the washed cells. A positive result is indicative of HDFN. However, a negative result may not rule out HDFN. Reactions are stronger in the case of anti-D and other clinically significant alloantibodies in the mother. However, the much weaker or negative DAT in HDFN is seen due to ABO antibodies. Suppose the DAT is positive and the maternal serum has a negative antibody screening test. In that case, suspicion falls on ABO-HDN or HDN due to antibody against a low incidence antigen which is not present on the screening cell panel.
- **Eluate testing** (only if necessary): In case of unconfirmed specificity of the antibody, eluate obtained from the fetal cells can be used to determine the specificity of the coating antibody.

Washing of the cord cells is necessary to remove the Wharton's jelly coating the fetal cells.

2.5. Monitoring

Antibodies like anti-I, -P1, -Le^a, - Le^b can be ignored since these antigens are poorly developed at birth.

2.6. Treatment

2.6.1. Fetal transfusion – Intra uterine transfusion (IUT):

IUT is a procedure undertaken only in specialized centres with the requisite skill and experience to correct severe fetal anaemia (common) or thrombocytopenia. High-resolution sonography is used to transfuse blood components in the umbilical vein. IUT can be performed from 16 weeks of gestation to as late as 35 weeks of gestation. Complications of IUT include miscarriage, preterm labour, fetal bradycardia, fetomaternal haemorrhage, vessel spasm, cord haematoma, infection, bleeding, and death (risk of death is 1-3% per procedure). Interventions (IUT) done at later weeks of gestation increases the risk of complications. A pre-transfusion sample of fetal blood is obtained to determine the fetal blood group, direct antiglobulin test, haemoglobin level, antigen typing, haematocrit, platelet count, bilirubin level and even DNA typing.

- **Intrauterine red blood cell transfusion:** This procedure is undertaken to treat fetal anaemia, most commonly due to HDFN or fetal parvovirus infection. This procedure may be required every 2-3 weeks and thus the need to accurately calculate the volume and haematocrit of the transfused RBC unit. The formula to calculate the volume of blood transfused is as follows:

  Volume to be transfused (ml) = Fetal and placental blood volume *X* desired increase in

  haematocrit / haematocrit of the donor RBC unit

  Where fetal and placental blood volume (ml) = ultrasound estimated fetal weight (grams) X 0.14 ml/ gm

The goal is to raise the fetal haematocrit to 0.45, and usually, an RBC unit with a haematocrit of 85% is used. For fetuses requiring an IUT, the timing of delivery depends on the rate of fall in fetal Hb, gestation age, degree of fetal anaemia and time from last IUT.

RBC units should be irradiated, negative for antigens for which maternal antibodies are present, CMV negative/leucocyte reduced, HbS negative and freshly collected (<5 days). Donor group O RBC compatible with maternal plasma should be selected, and if these are negative for maternal antigens, further maternal sensitization can be decreased. When maternal antibodies are directed against the high-frequency antigens and no compatible unit is available, mother's washed or frozen-deglycerolized RBCs can be used for IUT. Mother's sibling or rare donor registry may also be helpful in such cases.

- **Intrauterine platelet transfusion:** IUT of platelets is done to correct fetal thrombocytopenia, most commonly caused due to platelet alloimmunization. The most common antibodies are anti-HPA-1a, and transfused platelets should be compatible with alloantibodies. Platelets should be irradiated, and volume transfused is calculated based on the desired increase in the fetus.

2.6.2. Neonatal treatment

Phototherapy, IVIG, top-up transfusion, exchange transfusion (see below) and a combination of these is usually used. About 40% of infants born with DAT positive require no treatment. The safety of PRBCs in

additive solutions (e.g., SAGM/ ADSOL) for exchange transfusion have not been proved yet in any randomized clinical trial.

- **Exchange transfusion (ET):** An exchange transfusion, also referred to as blood exchange transfusion or exchange blood transfusion, involves gradual removal of patient's blood and simultaneous isovolumetric replacement with allogenic donor blood.[2] Over the past few decades, widespread use of rhesus immunoglobulin, intrauterine transfusion, improvement in diagnostic prenatal ultrasound, intensive phototherapy and intravenous immunoglobulin has resulted in a steady decline in the incidence of severe neonatal jaundice requiring ET. Still, it is an effective intervention to lower the bilirubin in at-risk infants. Besides hyperbilirubinemia, the use of ET has been extended in the management of conditions like nonimmune hydrops fetalis, congenital leukaemia, disseminated intravascular coagulation, sclerema neonatorum, hyperammonaemia, polycythaemia, fluid and electrolyte imbalance, and severe neonatal sepsis.

  The aim of ET is to:

  - Lower serum bilirubin level to reduce the risk of kernicterus (most common indication) in patients not responsive to phototherapy and/ or IVIG. This is achieved through the removal of unconjugated bilirubin and facilitating albumin binding of residual bilirubin. In sick and very low birth weight infants, kernicterus can develop at a bilirubin level of 8-12 mg/dl; however, it rarely develops at a bilirubin level in full-term infants less than 25 mg/dl.
  - To remove infants affected red blood cells and circulating maternal antibodies to reduce red cell destruction, e.g., in HDFN.
  - To correct anaemia,
  - Remove bacteria, bacterial toxins, and circulating pro-inflammatory cytokines (e.g., in sepsis)
- **Component selection for exchange transfusion:** Select less than 5-7 days old and preferably non-additive solution RBCs to get maximum red blood cell survival and low extracellular potassium in the unit. The donor unit can be group O or any other group compatible with maternal antibody. Donor RBC unit should be haemoglobin S and cytomegalovirus seronegative (or at least leucodepleted/ leucoreduced when CMV seronegative blood is not available). The selected unit should preferably be irradiated. Suspend it in ABO compatible or neutral (AB group) FFP and mix thoroughly. The final haematocrit of the "reconstituted whole blood" should be between 50 – 60%. A single-volume ET using 80 -100ml/ kg of infant's weight removes nearly 75% of neonatal cells. For a double volume ET, approximately 160ml/ kg for full-term infants and 200 ml/ kg for very low birth weight infants is required. A double volume exchange transfusion removes approximately 85 – 90% of the patient's red cells and nearly half of the total bilirubin.

  An ET should be performed in an intensive care setting, and usually, an umbilical catheter (placed in an umbilical vein) is used as a means of vascular access. A standard blood filter and blood warmer is recommended. Typically, 5% of an infant's blood volume is removed and replaced in one cycle. Either of the techniques – automated or manual pull-push, can be used as per the available resources. Careful monitoring for metabolic disturbances, hypothermia, thrombocytopenia, and hypoglycaemia should be done. Continuous mixing of the reconstituted whole blood during the procedure should be done. The last aliquot of the exchanged blood can be used to measure the infant's haematocrit, platelet count and bilirubin level at the end of the exchange transfusion.
- **Partial exchange transfusion:** The use of haemodilution (partial exchange transfusion) for treatment of polycythaemia (e.g., in chronic fetal hypoxia) is not supported by evidence and not recommended in asymptomatic patients (BCSH 2016 recommendations). In symptomatic patients, clinical decision making should be based on the risk-benefit analysis. There is a negligible difference in the effectiveness of plasma, crystalloid or 5% albumin in decreasing the haematocrit and no difference in relief from symptoms due to hyperviscosity.

2.6.3. Maternal treatment

At gestational age, when IUT cannot be given, plasma exchange and IVIG can be used in the cases of severe HDFN.

2.7. Prevention

All Rh (D) negative (and some variant D positive) mothers should receive Rh immunoglobulin (RhIg)at 28 weeks and/ or immediate postpartum (within 72 hours) if the biological father and/ or the newborn is Rh (D) positive. Besides routine prophylaxis, RhIg is recommended after any event that increases the risk of feto-maternal haemorrhage (FMH). RhIg is not required for females who have already produced anti-D, Rh (D) positive females and Rh (D) negative females with known Rh (D) negative infant.

RhIg contains IgG anti-D and is commonly available now in most pharmacies as a 150-300 microgram dose (e.g., RhoGAM™ or Rhoclone™, etc. injections). The appropriate dose and time of immunization decrease the immunisation rate of Rh (D) negative mother to <0.1%.

Following are the key recommendations by the British Committee for Standardization in Haematology (BCSH) for the use of anti-D immunoglobulin for the prevention of HDFN in Rh (D) negative mothers (test for FMH are not routinely available in India and therefore should be done wherever possible):

a. Following potentially sensitizing events, anti-D Ig should be administered as soon as possible and always within 72 h of the event. If, exceptionally, this deadline has not been met, some protection may be offered if anti-D Ig is given up to 10 days after the sensitising event.
b. Recommendations for RhIg prophylaxis during pregnancy are mentioned in Table 1:

Table 1: RhIg prophylaxis during pregnancy

Gestational age (in weeks)	Indication for RhIg	Minimum dose	FMH estimation required
< 12	Ectopic pregnancy, molar pregnancy, therapeutic termination of pregnancy, repeated and heavy uterine bleeding with abdominal pain	250 IU	No
12-20	Any potentially sensitising event like: Amniocentesis, chorionic villus biopsy, cordocentesis, antepartum haemorrhage/Uterine (PV) bleeding in pregnancy, external cephalic version, abdominal trauma (sharp/blunt, open/ closed), ectopic pregnancy, evacuation of molar pregnancy, intrauterine death and stillbirth, in-utero therapeutic interventions (transfusion, surgery, insertion of shunts, laser), miscarriage, threatened miscarriage, therapeutic termination of pregnancy, delivery – normal, instrumental or caesarean section, intraoperative cell salvage	250 IU within 72 hours of the event	No
>20		500 IU within 72 hours of the event	Yes. A further dose of RhIg if FMH is >30ml of whole blood.

*FMH: feto-maternal haemorrhage

c. It is important that the 28-week sample for the blood group and antibody screen is taken prior to the administration of the first routine prophylactic RhIg injection.
d. Routine antenatal RhIg prophylaxis should be regarded as a separate entity and administered regardless of, and in addition to, any RhIg that may have been given for a potentially sensitising event.
e. Following birth, ABO and Rh D typing should be performed on cord blood, and if the baby is confirmed to be D positive, all D negative, previously non-sensitized women should be offered at least 500 IU of anti-D Ig within 72 h following delivery. Maternal samples should be tested for FMH and additional dose(s) given as guided by FMH tests.
f. In the event of intrauterine death (IUD), where no sample can be obtained from the baby, an appropriate dose of prophylactic anti-D Ig should be administered to D negative, previously non-sensitized women

within 72 h of the diagnosis of IUD, irrespective of the time of subsequent delivery.

Where anti-D is detected in a blood sample of a pregnant woman, further history should be taken, and investigation should be undertaken to establish whether this is immune or passive. The outcome will inform clinical decisions regarding Anti-D prophylaxis and antenatal follow-up. In cases where no clear conclusion can be reached as to the origin of the anti-D, prophylaxis should continue to be administered in accordance with the guidelines for D negative women.

3. Neonatal transfusion

Due to their unique physiology, blood volume, immature immune system and variable response to hypoxia, blood transfusion practices in neonates differ significantly from those in adults.[1] Owing to their longer life expectancy, lower absolute blood volume and immature organ systems, it is essential that blood transfusion benefits outweigh the risks. Preterm and sick neonates receive the largest number of transfusions among all hospitalized patients. Therefore, the blood centre should try to provide repeated aliquots from the same unit to avoid donor exposures. Hospitals and institutions with a significant number of neonatal transfusions should have the technology to provide smaller aliquots of blood for these categories of patients.

3.1. Top up transfusion in neonates and paediatric patients

Age-appropriate haemoglobin levels, the ability to tolerate blood volume loss and total blood volume differ in paediatric patients compared to adults.[2] Indications of RBC transfusion in paediatric patients are mentioned in Table 2 and 3:

Table 2: Indication of RBC transfusion in infants less than 4 months of age:

Category	Recommendation for RBC transfusion @10-15ml/ kg
Symptomatic anaemia (tachycardia, tachypnoea, poor feeding)	Maintain Hb above 7 gm/dl
On oxygen (cannula/ hood) or mechanical ventilation, significant tachycardia (>180 beats/ min) or tachypnoea (>80 breaths/ min) or apnoea or bradycardia	Keep Hb > 10 gm/dl
On >35% oxygen by a hood or on CPAP/ IMV* with mean airway pressure ≥ 6-8cm of water	Keep Hb > 12gm/ dl
Congenital cyanotic heart disease or on ECMO	Keep Hb > 15 gm/ dl

*CPAP continuous positive airway pressure

IMV intermittent mandatory ventilation

Table 3: Indication of RBC transfusion in infants older than 4 months and children

Category	Recommendation for RBC transfusion
Intraoperative blood loss > 15% of blood volume, perioperative anaemia, chemotherapy, radiotherapy, chronic congenital or acquired symptomatic anaemia	Maintain Hb above 8gm/ dl
Severe pulmonary disease, ECMO	Keep Hb > 13 gm/ dl
Sickle cell disease for surgery under general anaesthesia	Keep Hb > 10gm/ dl
Acute blood loss with unresponsive hypovolemia	Any Hb level

4. Thalassemia major and sickle cell anaemia

Thalassemia and sickle cell diseases are two common haemoglobinopathies in India that require long and specialized treatment.[3] Despite many advancements in the diagnosis and management of these diseases, it is

unfortunate that most of the patients in India are dependent mainly upon repeated blood transfusions.

4.1. Blood transfusion in thalassemia patients

In thalassemia, excess iron due to these repeated blood transfusions needs to be removed by using the expensive chelation treatment.

Thalassemia intermedia and Hb E thalassemia patients may not need regular red cell transfusions. Even in thalassemia major patients, regular transfusions are not justified only on the basis of Hb levels. Clinical parameters should be assessed before advising a chronic transfusion therapy because of grave risks with chronic BT therapy. The following parameters suggest that the patient will need chronic red cell transfusions.

- Hb level <7 g/dl on two successive occasions separated by at least 2 weeks (the patient should be on folic acid replacement, and there should be no other aggravating cause, i.e., infection, bleeding, etc.)
- Patient's growth, activity, academic performance, zeal, etc., are hampered
- Unnatural bony growth due to marrow expansion
- Development of organ failure such as cardiac failure, oedema.
- Even if the Hb level is >7 g/dl and <10 g/dl, and the above clinical features are present, the patient may need chronic transfusion therapy.

The objective of chronic red cell transfusion is to ensure adequate Hb level so that O_2 delivery to the tissues is not hampered. This will be indicated by:

- Normal growth spurt.
- Increased zeal, energy, enthusiasm, and improved academic performance.
- Improved appetite.
- Suppressed overactive erythropoiesis leading to bone deformities.

Red cell transfusions should be given at an interval of 2-5 weeks. This interval is optimized based on:

- The volume of red cells transfused so that pre-transfusion Hb remains >9 g/dl, but post-transfusion Hb does not go above 12 g/dl
- There is no fluid overload.
- The transfusion process is over within a reasonable time (within 4 hours).
- The frequency of transfusions is not such that it interferes with the patient's normal activities.
- Reducing the number of venepuncture (as lifelong transfusion is needed, peripheral veins need to be preserved well).

Suitable blood components for transfusion:

- Red cell concentrate (haematocrit around 0.65) is suitable
- Leucodepleted (pre-storage) blood is desirable.

Amount of PRBC to be transfused:

- Pretransfusion Hb is to be estimated along with the weight of the patient and recorded.
- If the haematocrit of the red cell concentrate used is 0.65, then 3-4 ml/kg will raise the Hb by 1 g/dl in the absence of hypersplenism.
- Generally, in a single transfusion, an attempt is made to raise the Hb by 4 g/dl if transfusions are scheduled at 3-5 weekly intervals.
- The volume of transfusion based on the desired Hb increase in the patient and the haematocrit of the blood bag[4] is mentioned in Table 4:

Table 4: Volume of transfusion for a desired increase of Hb

Target increase in Hb level	Haematocrit of the blood bag			
	50%	60%	75%	80%
1 g/dl	4.2 ml/kg	3.5 ml/kg	2.8 ml/kg	2.6 ml/kg
2 g/dl	8.4 ml/kg	7.0 ml/kg	5.6 ml/kg	5.2 ml/kg
3 g/dl	12.6 ml/kg	10.5 ml/kg	8.4 ml/kg	7.8 ml/kg
4 g/dl	16.8 ml/kg	14.0 ml/kg	11.2 ml/kg	10.4 ml/kg

Pre-transfusion testing should be done before starting chronic transfusion therapy as follows:

- Irregular antibody screening at regular intervals is necessary and should be done during pre-transfusion testing. Once an alloantibody is detected, it should be identified, and antibody-negative blood should be crossmatched.
- Extended phenotyping of patient's red cells is desirable. If possible, an extended phenotype of the patient should be done, and a record kept for any future need.
- If the family is interested in stem cell transplantation, then counselling and early referral to such a centre should be done.
- Serum ferritin level is not needed if the child is <2 years.
- Serum ferritin levels should be recorded at regular intervals (3 months) after the first 10 units of red cells have been transfused, and iron chelation should be started, and the dose should be adjusted so as to maintain serum ferritin between 500 and 1000 ng/ml.
- Before starting chronic red cell transfusions, hepatitis B vaccination should be completed.
- Every 3 months, virus serology should be done to detect viral infection at the earliest.
- The patient should receive NAT tested blood components as far as possible.
- A detailed record of red cell transfusions, complication, management, etc., should be kept.
- Close relative's blood should not be transfused.

4.2. Blood transfusion in sickle cell disease patients:

Transfusion requirement in sickle cell disease can be managed as following[3]:

- PRBC transfusions play an important role in the treatment of some acute illnesses in patients with sickle cell disease. In addition, a timely blood transfusion may be lifesaving in severe complications.
- PRBC should be transfused if the Hb is ≥1-2 g/dl, below the baseline, and the patient shows any signs of cardiovascular compromise.
- Indications for red cell transfusions include acute exacerbation of the patient's baseline anaemia (e.g., hyperhaemolysis, hepatic sequestration, splenic sequestration, aplastic crisis) that requires increased oxygen carrying capacity, acute life, or organ-threatening vaso-occlusive episodes (e.g., stroke, acute chest syndrome, severe infection, multiorgan failure, etc.) and preparation for surgical or radiographic procedures.
- Leucocyte-depleted, packed RBCs are recommended and where available, Rh, Kell antigen matched, sickle-negative cells are preferred.
- For example, slow correction of the anaemia, 4-5 ml/kg PRBC over 4 h, often with furosemide or isovolumic partial exchange transfusion, may be needed to prevent precipitation of heart failure.
- Simple transfusion with 10 ml/kg of PRBC typically raises the Hb by about 2 g/dl.
- PRBC transfusion at a dose of 10 ml/kg for Hb <4-5 g/dl and signs of cardiovascular compromise should be done. Transfusion may be needed for Hb <7-8 g/dl for patients with relatively high baseline Hb Sickle levels. In severe cases, urgent initiation of transfusion prior to inpatient admission may be life-saving. A post-transfusion Hb level <8-9 g/dl is generally recommended to avoid the risk of hyperviscosity that may

occur several days later when RBCs sequestered in the spleen may return to the circulation and increase the Hb 1-2 g/dl above the post-transfusion levels.

- Since sickle red cells are poorly deformable, simple red cell transfusions that increase the Hb levels to >10-11 g/dl may cause hyperviscosity in patients not receiving chronic transfusions and should be avoided.
- Partial exchange transfusion, generally by erythrocytapheresis (using apheresis technology), may be needed for a severe life-threatening illness or in situations where a relatively high baseline Hb precludes a simple transfusion that would lead to the risk of hyperviscosity.
- Partial exchange transfusion or erythrocytapheresis to achieve Hb 10 g/dl and keep Hb Sickle (patient's RBC) <30%. Remove femoral or central venous catheter as soon as possible after exchange transfusion to reduce the risk of thrombosis.
- Simple transfusion with PRBCs to achieve post-transfusion Hb approximately 10 g/dl may be considered as an alternative to partial exchange transfusion for stable patients with Hb 6-7 g/dl (do not transfuse acutely to Hb >10 g/dl, Hct >30%).

5. Transfusion support in obstetrics and gynaecology patients

The WHO guidelines for blood transfusion in obstetric patients published more than 15 years back recommended that blood transfusion should not be based on the Hb levels alone. There was no specific recommendation regarding the trigger for blood transfusion in these patients. In view of the absence of any specific recommendations/ guidelines from the Indian obstetrics and gynaecology bodies/ societies (e.g., FOGSI, ICOG) till December 2019, the below-mentioned practice points have been taken from the Royal College of Obstetricians and Gynaecologists (RCOG), UK guidelines (2018).[5]

- For normocytic or microcytic anaemia, a trial of oral iron should be considered as the first step and further tests should be undertaken if there is no demonstrable rise in Hb at 2 weeks and compliance has been checked.
- Pregnant women should be offered screening for anaemia at booking and at 28 weeks. Women with multiple pregnancies should have an additional full blood count done at 20–24 weeks.
- Parenteral iron is indicated when oral iron is not tolerated or absorbed, or patient compliance is in doubt or if the woman is approaching term as there is insufficient time for oral supplementation to be effective.
- All women should have their blood group, and antibody status checked at booking and at 28 weeks of gestation.
- Pre-delivery autologous blood deposit is not recommended.
- There should be a clear local protocol on how to manage major obstetric haemorrhage.
- There are no firm criteria for initiating red cell transfusion. The decision to provide blood transfusion should be made on clinical and haematological grounds. FFP at a dose of 12–15 ml/kg should be administered for every 6 units of red cells during major obstetric haemorrhage. The results of clotting tests should guide subsequent FFP transfusion. Aim to maintain the platelet count above 50,000/ microliter in the acutely bleeding patient. A platelet transfusion trigger of 75,000/ microliter is recommended to provide a margin of safety.
- In anaemic women who are not actively bleeding intrapartum or immediate post-partum, a Hb less than 7gm/dl indicates PRBC transfusion. For actively bleeding patients, follow major obstetric protocols or massive transfusion protocol developed locally by the Hospital Transfusion Committee.
- The use of rFVIIa may be considered as a treatment for life-threatening postpartum haemorrhage (PPH). However, it should not delay or be considered a substitute for a life-saving procedure such as embolization or surgery or transfer to a referral centre.
- During major obstetric haemorrhage or with clinically diagnosed PPH, in addition to the standard care, early (within 3 hours of birth) use of intravenous Tranexamic acid is recommended (WHO 2017).

6. Transfusion support in cardiac surgery patients

Cardiac surgeries have traditionally been considered as big consumers of blood and blood components, with the incidence of peri-operative blood transfusion ranging from 40-90%.[6,7] However, with increased awareness of adverse outcomes associated with BT and additional cost, it is now realized that transfusions should be done optimally. However, Indian professional bodies like ICCA and IACTA have not yet officially endorsed (till Jan 2020) any guidelines for blood use in cardiac surgery in India.

6.1. Pre-operative transfusion

Preoperative erythrocyte transfusion is not routinely recommended in preoperatively anaemic patients to prevent postoperative acute kidney injury. However, in emergency surgery and life-threatening anaemia, it is legitimate to use preoperative blood transfusions to increase the Hb levels.

Oral or intravenous iron alone may be considered in mildly anaemic patients (women, Hb 10–12 g/dl; men, Hb 10–13 g/dl) or in severely anaemic patients (both genders, Hb $\leq$ 10 g/dl) to improve erythropoiesis prior to cardiac surgery. Erythropoietin with iron supplementation should be considered to reduce postoperative transfusions in patients with non-iron deficiency (e.g., EPO, vitamin D or folate acid deficiency) anaemia, undergoing elective surgery.

6.2 Pre-operative autologous blood donation

In patients posted for elective surgery with Hb > 11 gm/dl and without severe aortic stenosis or an acute coronary syndrome within 4 weeks, pre-operative autologous blood donation (PABD) may decrease the number of postoperative BT. Acute normovolemic haemodilution (ANH) has not shown many advantages in cardiac surgery patients.

6.3. Quality of blood

The use of PRBCs of all ages is recommended because the storage time of the PRBCs does not affect the outcomes (Class I, level A evidence). The use of leucocyte depleted PRBCs is recommended to reduce infectious complications (Class I, level B evidence).

6.4. Haemoglobin trigger

The Society of Thoracic Surgeons and Society of Cardiovascular 2011 guidelines recommended[7] RBC transfusion for Hb <6 g/dL during cardiopulmonary bypass and <7 g/dL post-operatively, except in patients at risk for decreased cerebral oxygen delivery, for whom a higher Hb level is recommended. However, instead of a fixed Hb threshold, BT should be based on the patient's clinical condition. A restrictive Hb of 7-8 gm/dl (Hct 21-24%) with a patient maintaining an adequate DO_2 (> 273 ml O_2/min/m^2) level can be considered during cardiopulmonary bypass.

Most cardiac anaesthesiologists now agree that it is reasonable to transfuse blood with Hb <7 g/dl, and transfusion is unnecessary when Hb is >10 g/dl. The individualized approach used in between these two triggers (Hb between 7-10 g/dl) should be based on a restrictive strategy, with a focus on the improved clinical outcome along with the additional cost and risk of a BT.

7. Transfusion support in autoimmune haemolytic anaemia (AIHA) patients

AIHA is autoimmune-mediated destruction of a patient's RBCs (haemolysis) by autoantibodies bound to the RBC membrane and activated complement proteins.[8] The autoantibodies are formed against various target specificities and may not be identifiable in most cases. However, in cases where specificity can be determined, it is most commonly against Rh-antigens, anti-i/I or anti-P. RBC destruction can occur directly in the circulation (intravascular haemolysis) or by the macrophages of the reticuloendothelial system (extravascular haemolysis).

AIHA can be classified as primary AIHA (idiopathic) and secondary AIHA due to infection, drugs, immune diseases, malignancy, transplantation, and congenital disabilities. Approximately half of the AIHA cases are secondary. (Table 5)

Table 5: Classification of AIHA:

Type of AIHA	Antibody type	Usual immunoglobulin subclass	Place of haemolysis	Direct Coombs test positive for
Warm AIHA and drug-induced AIHA	Incomplete	IgG (IgG1 to IgG 4)	Extravascular	IgG alone (20%) or IgG + C3d (67%)
Cold AIHA	Complete	IgM (anti-I common)	Intravascular	Only C3d
Mixed AIHA	Both complete and incomplete	IgG and IgM	Mostly extravascular	IgG + C3d
Paroxysmal cold/ nocturnal haemoglobinuria (PCH or PNH)	Donath- Landsteiner antibody	IgG (anti-P common)	Intravascular	Only C3d

7.1. Clinical features in AIHA

The mode of onset, type of antibody and severity of anaemia determine the symptoms in AIHA. Usually, presentation is with acute severe symptoms like haemoglobinuria, dyspnoea, abdominal pain, fever, malaise, and jaundice in patients with post-infectious and drug-induced AIHA as well as PCH. Patients with cold AIHA or cold agglutinin disease have a chronic indolent course with symptoms of cold dependent acrocyanosis, acral numbness or cold sensitivity. Anaemia in these patients worsens after cold exposure. Similarly, other warm AIHA may run a chronic course with symptoms mainly attributed to anaemia like malaise, dyspnoea on exertion, etc.

The presence of lymphadenopathy, splenomegaly or any organomegaly points more towards secondary AIHA and is rarely seen in primary AIHA.

On investigation, normocytic/ macrocytic anaemia, reticulocytosis, low serum haptoglobin, elevated lactate dehydrogenase, increased unconjugated bilirubin and a positive direct Coombs test points towards AIHA. Bone marrow examination and RBC survival study are not always recommended.

7.2. Blood transfusion support in AIHA

It may not be possible to find compatible donor units for RBC transfusion in AIHA. However, in critical cases, transfusion should not be avoided or delayed because of uncertainty in cross-matching, and incompatible (earlier called 'least incompatible') units may have to be transfused. Nonetheless, the possibility of alloantibody masked by the autoantibody must be ruled out by the clinical history (e.g., alloantibody is unlikely in the previously non-transfused male) and laboratory testing (warm autoadsorption or allogenic adsorption). RBC transfusion can be safely given if alloantibodies can be ruled out, although crossmatch in such cases is incompatible. ABO and Rh(D) matched blood must be given, and if possible extended phenotype matched blood for common Rh antigens, Kell, Kidd and S/s antigen should be transfused. In cold AIHA, RBCs must be warmed ideally using in-line filters or at least pre-warmed. Biologic in-vivo compatibility test, though rarely done these days, entails rapid infusion of 20 ml of RBCs followed by 20 minutes of observation and if no reaction, further transfusion at the usual rate.

The decision to transfuse should be individualized depending upon the age, symptoms, clinical signs, rate of development and severity of anaemia in the patient. Type and cause of haemolytic anaemia at times also determine the morbidity and mortality, for e.g., IgM warm AIHA, fludarabine related AIHA and PCH are associated with the highest acute death rates.

The goal of blood transfusion should be to alleviate acute symptoms due to the severity of anaemia rather than achieve a target haemoglobin. Complete clinical and laboratory remission of AIHA may not be possible

in primary AIHA but can be achieved in secondary AIHA. It usually means a Hb above 11gm/ dl without signs of haemolysis irrespective of direct Coombs test status. Since this complete remission is usually difficult to achieve or, even if possible, maybe much delayed, a practical definition of remission (partial) is the absence of blood transfusion requirement and a satisfactory clinical status with Hb more than 9 gm/dl. However, the treatment goals must be defined individually and customized to suit the patient's requirements.

7.3.Drug treatment in AIHA

Steroids form the first line of treatment in AIHA with or without blood transfusion. In refractory cases, escalation of steroid dose after 3-4 weeks of treatment may be needed. Rituximab and splenectomy form the second line of treatment in refractory patients to high dose steroid therapy. Immunosuppressants like azathioprine, mycophenolate mofetil, cyclosporine and cyclophosphamide may have to be added in patients not responding or not willing for the second-line treatment

8. Transfusion support in intensive care unit patients [9] (Table 6)

Table 6: Transfusion support in ICU patients

SN	Clinical condition	Transfusion trigger	Strength of recommendation	Quality of evidence
1	Haemodynamically stable adult or paediatric patient	Hb ≤ 7gm/ dl	Strong	High
2	Post-operative surgical patients	Hb ≤ 8 gm/ dl or symptoms like chest pain, orthostatic hypotension, tachycardia unresponsive to fluid resuscitation or congestive heart failure	Strong	High
3	Haemodynamically stable patients with pre-existing cardiovascular disease		Weak	Moderate
4	Haemodynamically stable patients with acute coronary syndrome	No recommendation for or against a liberal or restrictive RBC transfusion threshold	Uncertain	Very low

9. Platelet transfusion

Platelets may be derived from the whole blood collection or using the apheresis technique. The transfusion recommendation of platelet concentrates is as following[10]:

Dosage: One unit of platelet concentrate/10 kg; for an adult of 60-70 kg, 4-6 single donor units or 1 single donor platelet (adult dose) should raise the platelet count by 20-40 thousand per microliter. The increment will be less if there is splenomegaly, disseminated intravascular coagulation (DIC), active bleeding, fever, or septicaemia.

Table 7: WHO bleeding score

Grade of bleeding	Type of bleeding
1	• Petechiae/purpura that is localized to 1 or 2 dependent sites or is sparse/non-confluent • Oropharyngeal bleeding, epistaxis of < 30 min duration
2	• Melaena, haematemesis, haemoptysis, fresh blood in stool, musculoskeletal bleeding, or soft tissue bleeding not requiring RBC transfusion within 24 h of onset and without haemodynamic instability • Profuse epistaxis or oropharyngeal bleeding >30 min • Symptomatic oral bleeding or the one causing major discomfort

	• Multiple bruises, each >2 cm or anyone >10 cm • Petechiae/purpura that is diffuse • Visible blood in the urine • Abnormal bleeding from invasive or procedure sites • Unexpected vaginal bleeding saturating more than 2 pads with blood in a 24 h period • Bleeding in cavity fluids evident microscopically • Retinal haemorrhage without visual impairment
3	• Bleeding requiring RBC transfusion specifically for support of bleeding within 24 h of onset and without haemodynamic instability • Bleeding in body cavity fluids grossly visible • Cerebral bleeding noted on computed tomography (CT) without neurological signs and symptoms
4	• Debilitating bleeding, including retinal bleeding and visual impairment • Non–fatal cerebral bleeding with neurological signs and symptoms • Bleeding associated with haemodynamic instability (hypotension, >30 mmHg change in systolic or diastolic blood pressure) • Fatal bleeding from any source

Indications: Platelet transfusion is therapeutic to treat bleeding or prophylactic to prevent bleeding. For patients with a WHO bleeding score of ≤1, usually, a prophylactic transfusion is given, and with bleeding scores of ≥2, therapeutic transfusions may be given. (Table 7, 8, 9 and 10)

Table 8: Prophylactic transfusion of platelets in patients with thrombocytopenia with reversible or chronic bone marrow failure

Indication	Transfuse platelet at
WHO score 0 or 1 – no clinically significant bleeding and no invasive procedure planned and on intensive chemotherapy or undergoing allogeneic HSCT	≤ 10,000/ μl
Bone marrow aspirate or trephine biopsy, cataract surgery	≤ 10,000/ μl
As above but with additional risk factors for bleeding e.g., venous central lines (tunnelled/ untunnelled)	≤ 20,000/ μl
Lumbar puncture	≤ 40,000/ μl
Insertion/ removal of epidural catheter	≤ 80,000/ μl
Major surgery, percutaneous liver biopsy, renal biopsy (provided anaemia and uraemia are corrected)	≤ 50,000/ μl
Neurosurgery/ ophthalmic surgery involving posterior segment of the eye	≤ 100,000/ μl
WHO bleeding score >2	No trigger, manage individually according to the symptoms

*WHO: World health organisation

Table 9: Indications of therapeutic platelet transfusion

Indication	Transfuse platelet at
WHO bleeding score >2 and severe life-threatening bleeding	No trigger, manage individually according to the symptoms and maintain platelet count ≥50,000/ μl
Multiple trauma, traumatic brain injury or spontaneous intracerebral haemorrhage,	≤100,000/ μl
Non severe and/ or non-life-threatening bleed	≤30,000/ μl

Table 10: Indications of platelet transfusion in immune thrombocytopenia

Indication	Transfuse platelet at
No bleeding	$\leq$5,000/ μl
Invasive procedure or surgery	Transfuse 1 adult/ paediatric dose prior to the intervention or peri-interventional period. Any threshold count may not be achievable and is unnecessary
Serious and or life-threatening bleeding	$\geq$2 adults/ paediatric dose of platelets $\pm$ co-administration of IVIG

10. Massive transfusion

Definition:

Massive blood transfusion is traditionally defined as the replacement of one or more blood volumes in 24 hours. More practical definitions can be[8]:

- $\geq$10 blood unit transfusion within 24 hours; or
- Transfusion of $\geq$4 blood units in 1 hour; or
- Replacement of 50% of blood volume in 3-4 hours; or
- A rate of loss of blood $\geq$ 150 ml/hour.

Massive haemorrhage is a major cause of mortality and morbidity in several clinical settings like trauma, cardiovascular procedures, obstetric bleeds, and gastrointestinal bleeds—early identification and aggressive, ratio-based blood component resuscitation promises improved patient outcomes. Various international scientific bodies have published guidelines for massive transfusion protocol. However, these guidelines need to be adapted to suit the local needs and resources to improve survival in the conditions of massive blood loss.

The goals of the massive blood transfusion protocol are:

- Maintenance of tissue perfusion
- Oxygenation by restoration of blood volume and Hb
- Early recognition of blood loss and activation of a massive transfusion protocol
- Cessation of bleeding by means like early surgical or radiological intervention
- Judicious use of blood component therapy to correct coagulopathy.

Complications of massive transfusion : Massive transfusion (MT) is a lifesaving treatment but can be associated with significant complications. The lethal triad of acidosis, hypothermia, and coagulopathy associated with MT is associated with a high mortality rate.

- Acidosis: Acidosis in a patient receiving a large volume transfusion is more likely to be the result of inadequate treatment of hypovolaemia than due to the effects of transfusion. Under normal circumstances, the body can readily neutralize this acid load from transfusion. The routine use of bicarbonate or other alkalizing agents, based on the number of units transfused, is unnecessary.
- Coagulopathy: Dilution of the platelets and coagulation factor with initial resuscitation of a bleeding patient is done with excessive crystalloids and red cell transfusion. Coagulopathy is ascribed to loss of active haemostatic blood, dilution factor, acidosis and hypothermia, which reduces the enzymatic activity leading to destabilization of coagulation complexes. Hence, it becomes a vicious circle where acidosis and hypothermia cause coagulopathy, and coagulopathy leads to increased bleeding, causing tissue hypo-transfusion leading to acidosis and hypothermia. Hence, active initial management with ratio-based transfusion therapy avoiding blood dilution and providing extracorporeal blood warming allows many trauma patients to survive.
- Hypothermia

- Hyperkalaemia/Hypokalaemia: The storage of blood results in a small increase in extracellular potassium concentration, which will increase the longer it is stored. This rise is rarely of clinical significance, other than in neonatal exchange transfusions. Hypokalaemia is more frequent than hyperkalaemia due to the inward shift of potassium ions in red cells due to citrate toxicity and aldosterone-induced urinary loss.
- Citrate toxicity and hypocalcaemia: Citrate toxicity is rare but is most likely to occur during the course of a large volume transfusion of whole blood. Hypocalcaemia, particularly in combination with hypothermia and acidosis, can cause a reduction in cardiac output, bradycardia, and other dysrhythmias. In addition, citrate is usually rapidly metabolized to bicarbonate. It is, therefore, unnecessary to attempt to neutralize the acid load of transfusion.
- Air embolism

10.1. Massive transfusion protocol:

Massive transfusion protocols (MTPs) are established to provide rapid blood replacement for patients with massive blood loss. Early optimal blood transfusion is essential to sustain organ perfusion and oxygenation. There are many variables to consider when establishing an MTP, and studies have prospectively evaluated different scenarios and patient populations to establish the best practices to attain improved patient outcomes. The establishment and utilization of an optimal MTP are challenging given the ever-changing patient status during resuscitation efforts. Initial resuscitation should be started immediately in a ratio-based blood component therapy. As given by BCSH guidelines, the most accepted ratio-based protocol recommends blood components to be transfused in equal ratios.

After initial resuscitation, the following parameters should be evaluated while guiding further therapy:

- Temperature $>35^{o}C$
- Acid-base status: pH >7.2, base excess < -6, lactate <4 mmol/L
- Ionised calcium (Ca) >1.1 mmol/L
- Haemoglobin (Hb): This should not be used alone as a transfusion trigger; and should be interpreted in context with haemodynamic status, organ, and tissue perfusion
- Platelets $\geq 50 \times 10^{9}$ /L
- PT/APTT (activated partial thromboplastin time) ≥ 1.5 x of normal
- Fibrinogen > 1.0 g/dl
- Viscoelastic testing to guide the component therapy including status of platelet function and fibrinolysis at the patient bed side.

The best practice for massive transfusion (MT) includes an established institutional definition of massive transfusion protocol, an accurate method for predicting which patients will require MT so therapy can be promptly initiated. Over-utilization can be avoided, and finally, an established MT protocol with a clear plan for activation and use of appropriate blood products to maintain haemostasis. Adherence to the established protocol is critical to extract the full clinical benefit of an MTP for treating either trauma or non-trauma patients.

10.2. Massive transfusion in trauma:

Adult trauma patients with, or at risk of, massive haemorrhage should initially be transfused empirically with a 1:1:1 ratio of plasma: red cells: platelets. These patients with or at risk of major haemorrhage should be given tranexamic acid as soon as possible after injury.

10.3. Massive transfusion in obstetric patients:

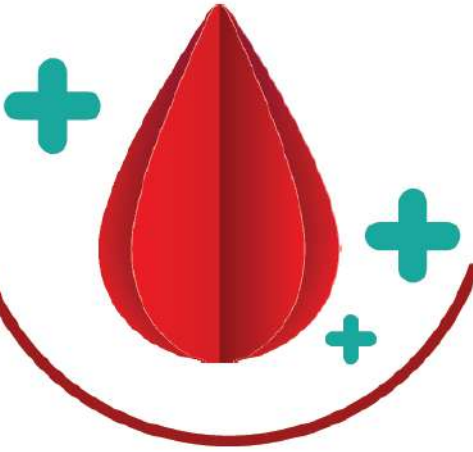
Blood component therapy should be same as in non-pregnant patients except that fibrinogen supplementation with cryoprecipitate should be considered at fibrinogen levels <2.0 gm/dl. Tranexamic acid may also be considered post-partum.

The latest recommendations advise uses of viscoelastic testing like thromboelastography (TEG), ROTEM, Sonoclot in the settings of massive blood loss in situations of trauma, obstetric haemorrhage, liver, and cardiac surgery

References

1. New HV, Berryman J, Bolton-Maggs PH, Cantwell C, Chalmers EA, Davies T et al; British Committee for Standards in Haematology. Guidelines on transfusion for fetuses, neonates and older children. Br J Haematol. 2016; 175:784-828
2. Kapoor D, Singh P and Seth A. Current Perspective on Exchange Transfusion. Indian Pediatr 2017; 54:961-962.
3. Ghosh K, Colah R, Manglani M, Choudhry VP, Verma I, Madan N et al. Guidelines for screening, diagnosis, and management of Hemoglobinopathies. Indian J Hum Genet. 2014; 20:101-19.
4. Guidelines for the Clinical Management of Thalassemia. Thalassemia International Federation, April 2000.
5. Royal college of obstetricians and gynaecologists. Blood transfusion in obstetrics [Internet]. 2nd ed. London: Royal college of obstetricians and gynaecologists; 2015 [accessed on 2020 Jan 18] 23 p. Available from: https://www.rcog.org.uk/globalassets/documents/guidelines/gtg-47.pdf
6. 2017 EACTS/EACTA Guidelines on patient blood management for adult cardiac surgery. The Task Force on Patient Blood Management for Adult Cardiac Surgery of the European Association for Cardio-Thoracic Surgery (EACTS) and the European Association of Cardiothoracic Anaesthesiology (EACTA). Journal of Cardiothoracic and Vascular Anesthesia. 2018; 32: 88–120.
7. 2011 update to the Society of Thoracic Surgeons and the Society of Cardiovascular Anesthesiologists blood conservation clinical practice guidelines. Society of Thoracic Surgeons Blood Conservation Guideline Task Force. Ann Thorac Surg. 2011; 91(3): 944-82.
8. Kaufman RM, Shehata N. Hemotherapy decisions and their outcomes. In: Mark K Fug editor. Technical Manual. 19th ed. Bethesda, Maryland: AABB; 2017. p. 505 – 26.
9. Tobian AA, Heddle NM, Wiegmann TL, Carson JL. Red blood cell transfusion: 2016 clinical practice guidelines from AABB. Transfusion. 2016 Oct;56(10):2627-2630
10. Estcourt LJ, Birchall J, Allard S, Bassey SJ, Hersey P, Kerr JP et al on behalf of the British Committee for Standards in Haematology. Guidelines for the use of platelet transfusions. Br J Haematol. 2017; 176(3): 365-394.

Section 9



Apheresis

1. Introduction

Apheresis is a Greek word that means to separate or remove. In apheresis, blood is withdrawn from a donor or patient in an anticoagulant solution and separated into components. One (or more) component is/are retained, and the remaining constituents are returned to the individual.[1]

Any one of the components of blood can be removed with the currently available apheresis devices according to the specified procedure for the same.[2] These are as following:

- Plasmapheresis: to remove plasma
- Plateletpheresis: to remove platelets
- Erythrocytapheresis: to remove red blood cells
- Leucapheresis: to remove leucocytes
- Granulocytapheresis: to remove granulocytes

Besides blood component harvesting, apheresis technology can also be utilized for therapeutic purpose in various clinical indications.

The issues of safety and quality, which are the basic requirements in blood banking, are met by the use of single-donor apheresis components. The advantages of single-donor component separation by apheresis are:

a. Reduced multiple donor exposure- reduced risk of alloimmunisation and transfusion-transmitted infections
b. Better quality product and adequate therapeutic dose
c. Leucoreduced blood components- reduced risk of febrile non-haemolytic transfusion reaction, alloimmunization and cytomegalovirus transmission.
d. Reduced donor adverse reactions- fluid replacement option and fluid shift from interstitial space to intravascular compartment.
e. Smaller needle technology- lesser venous injuries.

2. Principle of blood component separation by apheresis:

This is based on the following principles:

- Centrifugation
- Membrane filtration
- Combination of centrifugation and membrane filtration

2.1. Centrifugation:

In most apheresis instruments, centrifugal force separates the blood into its components based on differences in specific gravity (Table 1). Blood is drawn from an individual with the assistance of a pump. Anticoagulant is

added to the tubing to prevent clotting. Anticoagulated blood from a donor or patient is pumped into a rotating bowl, chamber, or tubular rotor in which layering of components occurs based on their density. The desired component is retained, and the remaining elements are returned to the donor or patient by intermittent or continuous flow.[3]

Table 1: Specific gravity of the blood component

Type of component to be separated	Specific gravity of the component
1. Plasma	1.02
2. Platelet	1.03
3. Lymphocyte	1.07
4. Granulocyte	1.08
5. Red cells	1.085

2.2. Membrane filtration:

Membrane filtration separate blood components based on their size. Filtration of whole blood by a membrane allows the collection of plasma or other blood components from healthy donors or therapeutic removal of abnormal plasma constituents. Membrane filtration is mostly using for plasma collections. Most of the instruments have the membrane arranged as hollow fibres, but some have flat plates. Inner membrane surface repels cellular elements in the flow of blood to prevent platelet activation and red cell membrane damage due to high shear forces during the filtration process.

2.3. Combination of centrifugation and membrane filtration:

Both of these techniques used in combination in the rotating filter. In this centrally located rotating filter separate whole blood into plasma and cellular components, which push cellular component away from filter surface and collect plasma more efficiently. In this technology, lower g force and smaller filter surface required as compared to both of these technologies use independently.

3. Currently available equipment and technologies:

All currently available automated apheresis systems based on the centrifugation principle require pre-packaged disposable sets of sterile bags, tubing, and centrifugal devices unique to the instrument. Each has a mechanism to rotate the separation device without twisting the attached tubings. The separation process devices either can be intermittent or continuous flow type. The centrifuge container is alternately filled and emptied during the component separation process in the intermittent flow method. However, currently, most of the equipment's in use are continuous flow type. With both methods, single- or dual-vein access techniques are possible. The duration of the procedures varies according to the component harvested and the device used. Comparisons of different types of commonly using apheresis equipment[4,5] is mentioned in Table 2.

Table 2: Comparisons of different types of commonly using apheresis equipment

S No	Manufacture	Instrument	Venous access	ECV (ml)	Centrifugation /Filtration	Channel type	Modalities available
1	Hemonetics	MCS+ LN9000	SN	359-480	IF	Single stage	Platelet, WBC, Plasma, RBC, TPE
		MCS+ LN8150	SN	391-542	IF	Single stage	RBC, cPlasma
		PCS-2	SN	385-491	IF + MF	Single stage	Plasma

S No	Manufacture	Instrument	Venous access	ECV (ml)	Centrifugation /Filtration	Channel type	Modalities available
2	Terumo BCT	COBE spectra	DN/ SN	272 (DN) 361 (SN)	CF	Dual/single stage	Platelet, WBC Plasma, RBC, TPE
		Trima Accel	SN	182-196	CF	Single-stage	Platelet, Plasma, RBC
		Optia	SN	141-185 (TPE) 253-297 (PLT) 191 (WBC)	CF	Single stage	Platelet, WBC Plasma, RBC, TPE
3	Fresenius Kabi	Amicus	SN /DN	210(DN) 209(SN)	CF	Dual-stage	Platelets, WBC, RBC, TPE
		COM.TEC	SN /DN	175(D)-285(S)	CF	Dual stage	Platelet, WBC, Plasma, TPE
		Amicore	SN	205	CF	Dual stage	Platelet
		ALYX	SN	110	CF	Dual stage	RBC (Double), cPlasma
		Auto pheresis C	SN	200	MF	MF	Plasma

ECV: Extra corpuscular volume, SN: Single needle, DN: Double-needle, IF: Intermittent flow, CF: Continuous flow, MF; Membrane filtration, RBC; Red blood cells, WBC; While blood cells, TPE; Therapeutic plasma exchange, cPlasma: Concurrent plasma

3.1. Hemonetics cell separators:

Three types of Hemonetics cell separators MCS + LN9000, LN8150 and PCS-2 are available nowadays. All of these are based on the intermittent-flow-centrifugation (IFC) principle. These cell separators use conical shape Latham's bowl to separate blood components (Figure 1). Whole blood enters from one side. It separates into different components into Latham's bowl so that red cells move to the periphery and plasma to the inside of

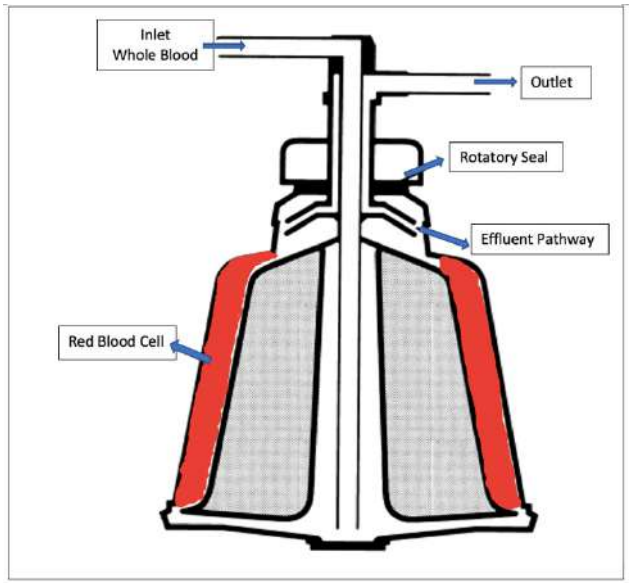


Figure 1: Cross-section of Latham's bowl

the rotating bowl, and white cells and platelets layer between the red cells and plasma. Using optical detectors and a fluid surge elutriation process, desired blood component is retained, and the remaining constituents of blood are returned to the donor or patient. The leucoreduction of blood components and addition of additive solutions can be performed either concurrently or at the end of the procedure. The extracorporeal blood volume (ECV) in Hemonetics cell separators is varied according to a haematocrit of donor or patients; the lesser the haematocrit, the higher the ECV or vice versa.

However, it has a larger extracorporeal volume. It takes longer time for component harvesting due to its single venous access for (both blood draw & return) in comparison to the continuous-flow centrifugation devices utilizing double venous access.

3.2. Terumo BCT cell separators:

Currently, three cell separators Cobe Spectra, Trima Accel, and Optia, from Terumo BCT manufacturer, are available. The Cobe Spectra device uses continuous-flow centrifugation technology and a single or double arm venous access for a blood draw and return. Cobe Spectra uses a dual-stage channel for leucoreduction of platelet apheresis (Figure 2). Nowadays, the leucocyte reduction system (LRS) is also used in plateletpheresis to improve the leucoreduction efficacy of Cobe Spectra. Leucoreduction efficacy is also improved with a software update of the instrument. Cobe Spectra is also useful for therapeutic plasma exchange, red cell exchange, granulocytapheresis and stem cell collection.

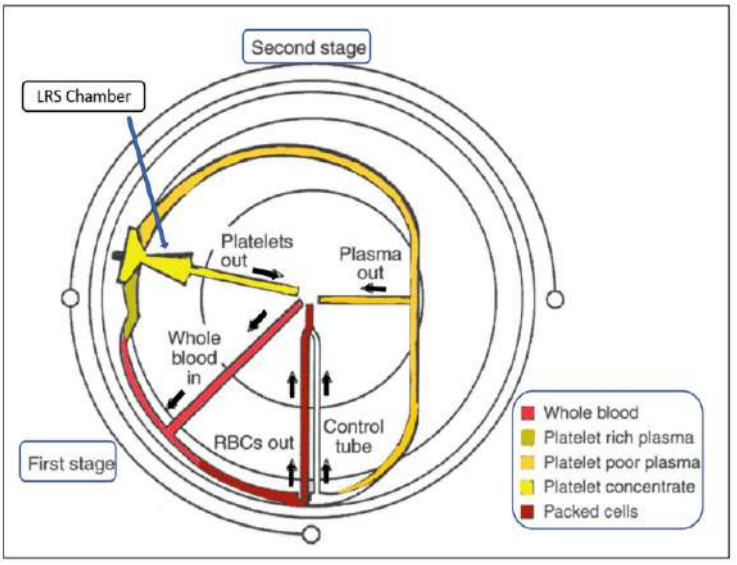


Figure 2: Dual-stage channel with LRS chamber

The Trima Accel cell separator is an updated and more efficient component separator. It uses a single needle venous access, which provides continuous flow to the centrifuge but intermittent flow to the donor. To increase platelet yield and better leucoreduction Trima Accel uses a single stage, doughnut-shaped channel and larger LRS chamber. The LRS chamber uses saturation, fluidization and particle bed filtration technology which leads to enhanced separation of platelets from leucocytes. Platelets and leucocytes enter through the small opening of the conical LRS chamber at high speed. But as they enter into a large diameter of the conical chamber, peripheral leucocytes stay at the chamber entrance while centrally placed platelets enter rapidly into the chamber and fill it rapidly. Leucocytes are blocked at the entrance because of higher *g* force in the outer portion, and less dense platelets easily drain through to exit the chamber.

The latest Optia Spectra cell separator of Terumo BCT is a multi-component cell separator. It has an Automated Interface Management (AIM) system. The AIM system regulates the plasma pump flow rate to control the concentration of cells flowing through the collection port. It determines the concentration by evaluating how much light penetrates the contents in the collect port (cannot identify the type of cells). Optia Spectra is a

highly mobile and transportable instrument with a data management facility with the help of external storage and historical data retrieval option as well. It has a touch graphical user interface display which is very user friendly. Various protocols can collect multiple components, i.e., platelets, granulocytes, donor lymphocytes, plasma, RBCs and PBSC/MNC. Two tubing set types, i.e., exchange set and IDL set can be used for multiple procedure types with minimal steps required to load these tubing sets.

3.3. Fresenius Kabi cell separators:

Fresenius Kabi also has three kinds of cell separators called Amicus, COM.TECH, and Amicore. Fenwal uses continuous-flow technology and a seal-less system. The Fresenius Kabi devices are fully automated and limited manual interruptions.

Amicus has both single or double needle procedure capability, adequate anticoagulant delivery, lesser processing time, and efficient platelet collection with consistent leucoreduction. It is a mobile equipment with low ECV and user-friendly with easy kit loading and on-screen prompts. Restricted manual control and selected procedural capability like a platelet, concurrent plasma, and red blood cells and MNC collection are major limitations of this equipment. The centrifuge of Amicus is seal-less. The rotating belt has two containers, separation and collection chambers, which are wrapped around the spool. In this first, platelet-rich plasma separates from red blood cells in the separation chamber, then PRP again separates into platelet concentrate and plasma into collection chambers. Leucoreduction in Amicus is achieved through the elutriation principle.

Amicore is a dedicated automated plateletpheresis collection system with an advanced alarm management system and intelligent flow control technology to automatically establish required donor flow rates for an enhanced donor experience. Leucoreduced platelets are obtained automatically with the facility for automatic addition of PAS (platelet additive solution). It takes complete care of donor comfort and safety.

COM.TECH is a continuous flow cell separator that collects components outside the centrifuge and is based on either single or dual needle protocol. It also has automatic process management for all protocols with an option to shift to manual mode as well. A wide range of therapeutic and collection procedures can be done on this separator, including plateletpheresis (leucoreduced platelet collection and plasma collection option); therapeutic exchange, and depletion procedures for platelets and leucocytes; granulocyte collection and donor lymphocyte collection as well as peripheral blood stem cell collection.

Fresenius Kabi also has equipment for a collection of plasma called Autopheresis-C. It uses a spinning membrane, thus incorporating both membrane and centrifugation technology. It can be used for the collection of 500-990 ml plasma. This system is an intermittent-flow system and usually a one-arm procedure. This collects the blood in a small rotating cylinder, forcing plasma through a polycarbonate membrane.

Equipment for double red blood cell collection of Fenwal is ALYX. It uses a pneumatic pressure pump system in place of a roller pump to move the fluid. It also uses a single needle venous access that gives intermittent flow to doors but continuous flow to centrifuge. As a result, ALYX can collect two units of packed red blood cells (PRBCs) or one-unit PRBC and one unit of fresh frozen plasma (FFP).

4. Blood component separation by apheresis

4.1. Plateletpheresis:

In a plateletpheresis procedure, donor's platelet are removed from whole blood, and the remaining components are returned back to the donor. In this procedure, large amount of platelets can be collected from a single donor, which leads to reduced donor exposure to the patient. With newer instruments and advanced technologies, leucoreduced, higher yield platelet concentrate with automatic addition of platelet additive solution is possible. Platelets can be prepared with or without concurrent plasma or concurred in a separate bag which can be transfused or stored as FFP. If extra plasma is collected during plateletpheresis, all precautions are taken, as in the plasmapheresis.

Besides the general donor selection criteria for whole blood donors, plateletpheresis donor's donor should also fulfil the following specific requirements:

1. Donor weight should be more than 50 Kg.
2. The interval between procedures should be at least 48 hours. A donor should not undergo the procedure more than 2 times a week or 24 times a year. After the whole blood donation plateletpheresis, the donor should be accepted only after 28 days of interval.
3. Platelets may be collected from donors who do not meet the requirement if the component is of particular value to the patient - HLA matched donors.
4. Donors who have taken aspirin-containing medication within 3 days / 72 hours are deferred.
5. A complete haematological profile, including platelet count, should be done before all plateletpheresis procedures, and platelet count and haemoglobin must be more than 150,000/μl and $>$ 12.5 g/dl before starting the procedure.
6. If extra plasma is collected and if the procedure is performed more than once every 4 weeks, the procedure should not be done if the total serum protein is less than 6.0 g/dl or if there has been an unexplained weight loss.
7. Double unit apheresis can be taken in donors whose platelet count is more than 250,000/ul and weight more than 60kg and those who are not first-time apheresis donors.

Platelet additive solution:

Platelet additive solution (PAS) is a synthetic storage solution that is used to substitute plasma in an apheresis platelet product. 60 to 70% of the plasma in the orignal platelet product is subsituted by PAS. The replacement of a portion of plasma helps in the reduction of allergic and febrile non-haemolytic transfusion reactions (FNHTR) and reduction in ABO isoagglutinin-mediated haemolysis of antibody-mediated transfusion-related acute lung injury (TRALI). Furthermore, it helps to use pathogen reduction technologies to inactivate pathogens in platelet components as plasma may interfere with the functioning of such technologies. The additional advantage is in providing out of group (ABO incompatible) apheresis products for transfusion if PAS is used as a storage medium.

The common components used in PAS are citrate, acetate, phosphate, gluconate, magnesium, and potassium. Acetate is used for providing free fatty acids. Acetate is used as a source of free fatty acids for oxidative phosphorylation in the citric acid cycle and produces bicarbonate which helps in buffering the pH. Phosphate also serves as a buffer and increases glycolysis—magnesium and potassium help in buffering the pH and prevent activation of the platelets. Glucose is a source of energy molecules for metabolism. Citrate is an anticoagulant, chelates calcium, is metabolized in the citric acid cycle and helps in decreased activation of platelets.

Some commercially available PAS are Intersol/PASIII (Fresenius Kabi AG, Germany), Composol/ PAS-G (Fresenius Kabi AG, Germany), PAS-IIIM or SSP+ (Macopharma, France).

4.2. Leucapheresis or Granulocytapheresis:

Occasionally, granulocytes are needed in neonates and adults with neutropenia and sepsis, not responding to antibiotics. The apheresis technique is applied to collect an adequate number of granulocytes - usually, 1.0 - 3.0 x 10^{10}. A daily dose of at least 1 x 10^{10} granulocytes and HLA matching may be necessary to achieve a therapeutic effect. Granulocytes concentrates can induce HLA immunization, may transmit cytomegalovirus infection, and if not irradiated, may cause graft-vs-host disease in susceptible recipients.

The collection of adequate doses of granulocytes needs administration of drugs or infusion of certain drugs during the collection process. These drugs are corticosteroids, colony-stimulating factors and hydroxyethyl starch. Corticosteroids are used to increase the mobilization of granulocytes from the pool, and that increases the circulating granulocytes up to two times. Routinely 60 mg oral prednisone or 8 mg oral dexamethasone are used in single or divided doses before donation. Before giving steroids to the donor, it would be better to take the donor's history of hypertension, diabetes, cataracts, or peptic ulcer as these can be a relative or absolute contraindication to corticosteroid use.

Granulocyte colony-stimulating factor (G-CSF) can also increase granulocyte yield up to 4 to 8 x 10^{10} granulocytes per apheresis procedure. Doses of G-CSF for granulocytapheresis are 5 to 10 μg/kg given 8 to 12 hours before the procedure. Donors who receive G-CSF may experience dyspnoea, chest pain, nausea, hypoxemia, diaphoresis, anaphylaxis, syncope and flushing shortly after subcutaneous injection.

Hydroxyethyl starch (HES), in low- or high- molecular weight form, is infused during the collection procedure to increase RBC sedimentation and to facilitate the separation and result in better granulocyte collection yield. HES should not be used in donors with a prior history of increased risk of bleeding. It can cause the development of a vWD like state and prolongation of aPTT. Donors who receive HES may also experience headaches or peripheral oedema because of expanded circulatory volume. Skin deposition with pruritus is reported in some donors. It can also lead to anaphylactoid reaction and tissue deposition with organ failure in rare cases.

All laboratory tests, ABO and Rh D grouping, antibody screening and infectious disease markers are tested before phlebotomy. Red cells in granulocyte concentrates are always present, red cells should be compatible with the recipient's plasma, and the granulocyte concentrate should always be cross-matched with the patient's plasma before issue.

Granulocytes should be stored at a storage temperature of 20 to 24^{0}C for no longer than 24 hours. Agitation during storage is not recommended, while irradiation is required. Leucoreduction is also contraindicated because it removes the collected granulocytes.

Frequency of donation: Maybe donated after a gap of 48 hours but not more than twice a month. Whole blood donation after granulocyte donation can only be made after a gap of 8 weeks.

Indications:

1. Severe bacterial and fungal infections with persistent neutropenia and absolute neutrophil count (ANC) $\leq$ 200/ul.
2. Patients undergoing intensive chemotherapy and/or haematopoietic stem cell transplantation with severe neutropenia and absolute neutrophil count (ANC) $\leq$ 200/ul.

However, prophylactic transfusions may be given at ANC $\leq$ 500/ul.

4.3. Erythrocytapheresis:

Recent advancements in apheresis enable the collection of RBCs by automated apheresis devices. Many of the automated apheresis machines have been improved and have facility for the collection of single or double red cell units. It can be collected alone or along with platelet or plasma. Double unit red blood cells can be collected by an ALYX apheresis cell separator. For the collection of double units, PRBC haematocrit of the donor should be more than 40% with a weight of around 60 kg and 5'1" height, while for female donors, it should be 70 Kg weight and 5'5" height.

Advantages of two RBC unit collection:

1. Receiving two RBC units from the same donor potentially reduces the risks for transfusion-transmitted infections.
2. Restricting exposure to a different allogeneic donor is beneficial in the prevention of alloimmunization in multi-transfused patients like thalassemia major and sickle cell disease or females of childbearing age.
3. Good quality RBC units through apheresis.

4.4. Plasmapheresis:

In the plasmapheresis procedure, plasma separates from other cellular blood components by centrifugation and membrane filtration techniques. In plasmapheresis large amount of plasma, 500-600 ml plasma, can be collected in one procedure. It is safe to accept 600 ml plasma per session with an interval of two weeks between the donations or 24 times a year. Selection criteria for plasmapheresis donor are almost the same as whole blood donors except few specific criteria like: -

1. Age should be between 18- 50 years.
2. Weight should be 60 Kg or more.

3. Preferably donor should have given whole blood 1 -2 times earlier.
4. Total blood count and serum proteins (> 6.0 g/dl) should be within normal limits and tested periodically.

Indications of plasmapheresis:

1. To increase plasma inventory of FFP for transfusion.
2. To collect plasma from IgA deficient donor for transfusion.
3. To obtain plasma to prepare immunoglobulins to Rh, tetanus or HBsAg etc.
4. To collect plasma for preparing albumin, plasma protein factor (PPF) and other plasma components.
5. To prepare coagulation factors like Factor VIII, Factor IX complex etc.

5. Anticoagulants for apheresis:

All apheresis procedures use anticoagulant to prevent clotting of blood. The most commonly used anticoagulant is ACD-A. Normal saline is used to prime the system, keep the line open, and help in maintaining fluid volume.[5]

Citrate exerts its anticoagulant effect through reversible chelation of circulating divalent cations, including Ca^{2+} and Mg^{2+}, and sequestration of these ions from their normal physiological function. Heparin is not routinely used as an anticoagulant for plateletpheresis procedure as it causes an increase in adhesiveness and the tendency of platelets to clump.

6. Therapeutic apheresis-

The removal of pathogenic substances from a patient using apheresis technology either in the form of infected and dysfunctional cells or depleting a disease mediator like immunoglobulin causing hyper viscosity, auto-allo-antibodies, protein bound toxins, immune complexes, lipoproteins etc. The efficacy of the procedure is enhanced by concomitant drug therapy, particularly immunosuppressive therapy in immune-mediated disorders. The apheresis procedures are classified by the component removed – cytapheresis if the cellular component is being removed and plasma exchange if plasma having offending substance is removed from the patient's body.

Principal of therapeutic apheresis majorly based on four goals:

1. To remove pathological substances
2. To replace deficient substances
3. To modify diseased cells
4. To collect normal autologous cells and use them for therapeutic purpose

There are evidence-based guidelines for therapeutic apheresis published by the American Society for Apheresis (ASFA).[6] These guidelines are based upon a systematic review of available scientific literature. Clinical utility for a given disease is denoted by the assignment of an ASFA category (Category I to IV) (Table 3). Standard GRADE recommendations denote the quality and strength of evidence. ASFA categories are defined as follows[6]:

Table 3: ASFA categories for therapeutic plasma exchange

S. No	Category	Criteria	Example
1.	Category I	Disorders for which apheresis is accepted as first-line therapy, either as primary stand-alone treatment or in conjunction with other modes of treatment.	Guillain-Barré syndrome and Thrombotic Thrombocytopenic Purpura (TTP)

S. No	Category	Criteria	Example
2.	Category II	Disorders for which apheresis is accepted as second-line therapy, either as a stand-alone treatment or in conjunction with other modes of treatment.	Haemolytic anaemia for cold agglutinin disease and Lambert-Eaton myasthenic syndrome.
3.	Category III	Optimum role of apheresis therapy is not established, and decision making should be individualized.	Hypertriglyceridemia pancreatitis.
4.	Category IV	Disorders in which published evidence demonstrates or suggests apheresis to be ineffective or harmful. Institutional Review Board (IRB) approval is desirable if apheresis treatment is undertaken in these circumstances.	Active rheumatoid arthritis.

6.1. Therapeutic plasma exchange:

During therapeutic plasma exchange, pathological substances like auto, alloantibodies, immune complexes, drugs, or toxins bound to proteins, high cholesterol or triglycerides in plasma are removed and replaced with suitable replacement fluid. The replacement fluid may be the donor's plasma, albumin, saline or a combination of albumin and saline and is done continuously to maintain an isovolumic state so that patients' blood volume does not change. The efficiency of plasma exchange is related to the amount of plasma removed. A procedure that removes the plasma equal to the patient's plasma volume is called one-volume plasma exchange. A one-volume plasma exchange reduces 30% of unwanted constituents of plasma. The subsequent plasma removal only removes 10% of unwanted constituents on 2nd and 3rd volume exchanges. Thus, it is recommended that approximately 1 to 1.5 L plasma may be exchanged per procedure.[7]

Pathological constituents present in intravascular and extravascular spaces affect the outcome of plasma exchange. If the antibody is IgM type, apheresis may be quite effective as IgM is primarily in intravascular whereas IgG is equally redistributed both in intravascular and extravascular spaces; thus, repeated procedures are required for desired clinical benefit. Generally, a minimum of five procedures on an alternate day basis is recommended for most of the conditions in which IgG is an offending antibody as it is synthesized and reappears quickly in the circulation. Removal of IgG antibodies is more effective when combined with immunosuppressive drugs.

6.1.1. Calculations in therapeutic apheresis procedures-

a. Total blood volume (TBV)

TBV varies with body composition and with other clinical factors. In general, TBV is related to lean body mass and is, therefore, greater in males than in females of the same weight. Moreover, weight-based calculations overestimate TBV in extremely obese individuals and underestimate it in muscular individuals. TBV can be calculated by multiplying an age-based estimate of blood volume in mL/kg by the patient's weight in kg. Estimated TBVs for different age groups are as follows: the TBV of a child more than 3 months old is 65 to 75 mL/kg; the TBV is larger in infants younger than 3 months of age, ranging from approximately 80 to 100 mL/kg; and in adult males, it may be estimated as 75 to 80 mL/kg.

Total blood volume (TBV) calculations[3]:

i. Nadler's formula:

It overestimates obese patient's blood volume and underestimates muscular patient's blood volume

- For male: $(0.006012 \times ht^3) / (14.6 \times wt) + 604 = TBV$ (ml)
- For female: $(0.005835 \times ht^3) / (15 \times wt) + 183 = TBV$ (ml)

ii. Gilcher's rule of 5:

As mentioned in Table 4

Table 4: Blood volume (ml/kg) of body weight as per Gilcher's rule of 5

Blood volume (ml/kg) of body weight				
	Fat	Thin	Normal	Muscular
Male	60	65	70	75
Female	55	60	65	70
Infant / child	-	-	80/70	-

b. Plasma volume and red cell volume:

Simple formulae for estimation of plasma volume (PV) and red cell volume (RCV) are shown below, with haematocrit (Hct) expressed as a decimal fraction[16]:

PV=TBV x (1-HcT) or TBV-RCV

RCV = TBV x Hct or TBV – PV

Haematocrit (Hct) expressed as a decimal fraction

c. Extracorporeal blood volume (ECV).

This varies depending on the type of equipment and, even with the same equipment, may depend on the type of procedure and haematocrit of the patient. For example, intermittent-flow type instruments have significantly large ECV than continuous-flow type instruments for therapeutic apheresis procedures. (Table 1)

Percentage extracorporeal blood volume (EBV)= (EBV/TBV) X100

(EBV depends on the extracorporeal volume of the cell separator in use and should not exceed 12-15% of TBV)

d. Extracorporeal RBC volume (ECRV)

ECRV is RBC volume required to fill the bowl or channel and all the tubing, which depends on the patient's haematocrit. It also varies according to the system (continuous or intermittent flow), type of procedure, haematocrit of the patient, and ancillary equipment such as blood warmer or single needle device.

ECRV= ECV x Hct

Percentage Extracorporeal Red Cell volume (ERCV)= ERCV/RCV X100

Management of extracorporeal volume: ECV and ECRV should not exceed 15% of the total patient blood volume and red cell volume.

- If ECV is 15-20% but ECRV is less than 15% (patients with high haematocrit), a saline bolus or colloid prime may be required to prevent hypotension due to intravascular hypovolemia.
- If ECV is less than 15% and ECRV is more than 15%, the patient should be monitored for signs of hypoxia.

Here, priming of the extracorporeal circuit with RBCs should be considered in patients with a history of cardiac or pulmonary problems.

e. Intraprocedural haematocrit-

Initial RBC volume (RCV) – Extracorporeal RBC volume (ERCV) x 100/

Total blood volume (TBV)

It should be ≥ 24% in an asymptomatic patient

6.1.2. Vascular access:

TPE procedures require high blood flow rates, which can only be achieved by good peripheral venous access using one or two large-bore needles (16-18 gauge). In children and sick adults who do not have prominent peripheral venous access, central venous catheters (CVC) are needed. CVC should be rigid so that they should not collapse under negative pressure exerted by cell separators during withdrawal or during inspiration. The choice of CVC is the subclavian vein, internal jugular vein, and femoral vein. It is influenced by the duration of treatment as a femoral vein for a shorter time while subclavian and internal jugular is for long term treatment. CVCs need to be flushed regularly with heparin or 4% trisodium citrate. These catheters need proper care as they can be associated with risk of infection (especially femoral vein CVC), bleeding, pneumothorax or haemothorax, and air emboli.

6.1.3. Anticoagulants:

Citrate, heparin, or a combination of both is used during apheresis to prevent coagulation in the extracorporeal circuit. Citrate is available in three forms- ACD-A, ACD-B, and concentrate of trisodium citrate. ACD-A is commonly used. Citrate prevents coagulation by binding to the ionised calcium, which is required in the coagulation cascade. The liver metabolises citrate; if liver functions are deranged or if citrate infusion exceeds the metabolic rate, transient hypocalcaemia may occur, which may manifest as mild paraesthesia (perioral, distal extremities), gastrointestinal symptoms, hypotension or in most extreme cases, cardiac dysrhythmias can occur. The risk of citrate toxicity is more in patients where FFP is used as replacement fluid as it has four times higher citrate than 5% albumin. Calcium supplementation can reduce the chances of hypocalcaemia. So, the addition of 10% calcium gluconate (10 ml per litre of return fluid) should be considered in TPE either as a separate infusion (1% calcium gluconate infusion) or 5ml of 10% calcium gluconate can be added directly to 500ml of 5% albumin.[8,9]

Heparin has some undesirable side effects like bleeding, thrombocytopenia, osteoporosis, etc. But heparin alone may be considered in patients with contraindications to citrate infusion, including those with hypoxemia, insufficient tissue perfusion, severe liver or renal dysfunction, or receiving long-duration TPE or LDL apheresis. A combination of heparin and citrate is also preferred in some patients.

6.1.4. Replacement fluid:

In TPE procedure large volume of the patient's plasma has to be replaced with suitable replacement fluids to maintain adequate intravascular volume and oncotic pressure. Commonly used replacement fluids in therapeutic plasma exchange procedure are mentioned in Table 5:

Table 5: Advantages and disadvantages commonly used replacement fluids in therapeutic plasma exchange procedure

Replacement Fluid	Advantages	Disadvantages
Crystalloid - Normal Saline	Least expensive Hypo-allergenic No risk of transfusion transmitted infection	2-3 volumes required Hypo-oncotic No coagulation factors
Albumin in 5 % solution	Iso-oncotic Used in 1:1 ratio of the plasma removed	High cost No coagulation factors No immunoglobulins
Fresh Frozen Plasma (FFP)	Iso-oncotic Replace coagulation factors, immunoglobulins ADAMTS 13 enzymes, VWF	Risk of allergic reactions ABO compatibilities required Risk of transfusion transmitted infection

6.1.5. Indications of plasma exchange

There are several indications of therapeutic plasma exchange as per ASFA-2019[6,7] as mentioned in Table 6.

Table 6: Indications of therapeutic plasma exchange according to ASFA guidelines 2019

Neurological disorders	Haematological disorders	Renal disorders	Connective tissue disorders	Other disorders
• Acute inflammatory demyelinating polyradiculoneuro-pathy • Chronic inflammatory demyelinating polyradiculoneuro-pathy • Myasthenia gravis • Paraproteinemic demyelinating neuropathies with IgG/IgA/IgM • Sydenham's chorea • Progressive multifocal leucoencephalo-pathy associated with natalizumab • N-Methyl-D-Aspartate receptor antibody encephalitis • Acute disseminated encephalomyelitis • Rasmussen's encephalitis • Neuromyelitis optica spectrum disorders • Lambert-Eaton myasthenic syndrome • Multiple sclerosis	• Thrombotic microangiopathy thrombocytopenia purpura • Factor –H autoantibody • Complement factor gene mutation • Drug associated (Ticlopidine, Clopidogrel) • Hyper viscosity in monoclonal gammopathies • Severe cold agglutinin disease • Cryoglobulinemia • Haematopoietic stem cell transplantation, (ABO incompatible) and for HLA desensitisation • Autoimmune haemolytic anaemia • Immune thrombocytopenia • Heparin-induced thrombocytopenia • Post transfusion purpura • Haemophagocytic syndrome	• Anti-glomerular basement membrane antibody disease • Recurrent focal glomerulosclerosis • Renal transplantation, ABO compatible (Antibody-mediated rejection desensitisation, LD) • Renal transplantation, ABO incompatible (desensitisation, LD) • ANCA associated rapidly progressive glomerulonephritis • Goodpasture's syndrome • Immunoglobulin A nephropathy • Focal segmental glomerulosclerosis • Myeloma cast nephropathy	• Vasculitis –ANCA associated • Henoch – Schoenlein purpura • Scleroderma (systemic sclerosis)	• PANDAS (paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections) • Wilson's disease • Familial hypercholesterolemia • Phytanic acid storage disease • Hashimoto's encephalopathy • Voltage-gated potassium channel antibodies • Systemic lupus erythematosus • Acute hepatic failure • Atopic (neuro-) dermatitis • Cardiac neonatal lupus • Erythropoietic porphyria • Hypertriglyceridemic pancreatitis • Pemphigus vulgaris • Sepsis with multi-organ failure • Psoriasis • Stiff-man syndrome

6.1.6. Effect of plasma exchange on clotting factors and drugs:

Removal of large quantities of plasma during TPE is generally associated with depletion of various plasma coagulation proteins and clotting factors varying from 40% to 70% and can be observed immediately after the exchange that manifests as prolonged prothrombin time and activated partial thromboplastin time and generally recovers after 48 hours of the exchange. Fibrinogen which is distributed in the vascular compartment and is depleted more and is recovered to 65% of baseline after 48 hours post exchange. Another unintentional effect of TPE is removing platelets from 9.4% to 52.6%, with recovery up to 75 to 100% after 48 hours.[10,11]

The drugs having an affinity for lipids and proteins with long half-lives and a small volume of distribution are removed by TPE more effectively. Thus, the dosage of such drugs has to be titrated in patients underway of regular therapeutic plasma exchange. Plasma exchange has been used to improve clearance of barbiturates, theophylline, vincristine, cisplatin, digoxin-anti digoxin antibody complexes, paraquat, quinidine, tricyclic antidepressants, acetaminophen, and phenytoin.[12]

6.2. Therapeutic leucapheresis

It is indicated in patients with clinical signs and symptoms of hyperleucocytosis which is defined as a circulating white blood cell or leukemic blast cell count >100 x 10^9/L.[6] Procedure is done to provide symptomatic relief in these patients with hyperleucocytosis in acute and chronic leukaemia by quickly decreasing the burden of white blood cell. In addition, S and G2/M phase of leukaemia cells is prolonged; hence it improves the sensitivity of proliferating leukemic cells to chemotherapy.

However, no significant advantage has been observed in the patient with acute lymphoblastic leukaemia (ALL) as the currently available aggressive induction chemotherapy is quite effective in reducing the white cell burden. The clinical signs and symptoms of leucocytosis develop in less than 10 per cent of ALL patients with a WBC count of ≥ 400 x 10^9/L, which at times can lead to pulmonary and central nervous system complications in about 50% of the cases.

The duration and number of cytapheresis procedures depend upon improvement in clinical signs and symptoms and patient's response to the concomitant chemotherapy, which prevents the re-accumulation of circulating blasts.

6.3. Thrombocytapheresis:

Common clinical signs and symptoms of thrombocytosis are headache, dizziness or light-headedness, chest pain, fainting, temporary vision changes, numbness or tingling of the hands and feet, redness, throbbing and burning pain in the hands and feet (erythromelalgia). Less commonly, bleeding may occur, which may take the form of nosebleeds, bruising, bleeding from mouth or gums, and bloody stool.

Symptoms in the affected site due to thrombocytosis include neurologic deficits with stroke or transient ischemic attack (TIA), which may manifest as weakness or numbness of the face, arm, or leg, usually on one side of the body, difficulty in speaking or understanding speech (aphasia), blurred, double or decreased vision, leg pain, swelling, or both with lower extremity thrombosis; chest pain and dyspnoea with pulmonary embolism and rarely gout or ocular migraines. Therapeutic thrombocytapheresis provides immediate symptomatic relief and is an efficient, helpful emergency lifesaving procedure in cases of symptomatic thrombocytosis. This condition has been reported in patients with myeloproliferative disorders like accelerated phase of CML and polycythaemia vera. Patients having counts of more than 1,000,000/μL may develop thrombotic or haemorrhagic complications. During the apheresis procedure, the platelet count can be brought down to as much as one third to one-half the initial value. The procedure can be repeated as frequently as necessary until drug therapy becomes effective and symptoms of microvascular occlusion disappear.

6.4. Red cell exchange:

A therapeutic procedure in which a patient's red blood cells are removed and replaced with donor's red blood cells and colloid solution. The clinical conditions for red cell exchange as per the ASFA guidelines are as shown in Table 7.[6]

Table 7: Indications of red cell exchange according to ASFA guidelines 2019

Disease	Indication	Category	
Sickle cell disease (Acute)	Acute stroke Severe acute chest syndrome Other complications	I II III	RBC exchange offers rapid reduction of HbS RBCs (Target to achieved HbS <30%)
Sickle cell disease (non-acute)	Stroke prophylaxis Pregnancy, Recurrent vaso-occlusive pain crisis Pre-operative management	I II III	
Babesiosis	Severe	II	The target of RBC exchange is to lower the level of parasitaemia to <5% in babesiosis and <1% in malaria. This improves the microcirculation and removes haemolytic metabolites and cytokines.
Malaria	Severe	III	
Red cell alloimmunization, Prevention and treatment		III	The procedure should be considered when the quantity of circulating RhD+ RBCs ≥ 20%. It reduces the volume of RhD+ RBCs allowing for safe RhIg administration
Erythropoietic protoporphyria, Liver disease		III	RBCs exchange is done to reduce plasma protoporphyrin levels. The benefit of the procedure is uncertain.
Transplantation Haematopoietic stem cell, ABO Incompatible (ABOi)	Minor ABOi transplant	III	Prophylactic RBC exchange can effectively reduce the host RBCs that are the target of passenger lymphocytes, however, the evidence for the same is weak based on very few case series.

7. Special considerations of therapeutic apheresis in paediatric patients:

The principles of apheresis are the same in children as in adults; however, available apheresis equipment is designed for adults rather than infants and young children.[13] To perform paediatric procedures safely, one must be familiar with the physical characteristics of apheresis instruments, including extra-corporeal blood volume (ECV) and patient's clinical condition and total blood volume (TBV). Regardless of the type of instrument, the ECV will represent a more significant fraction of TBV in a child than an adult, resulting in a greater volume shift in children. To estimate the degree of volume shift, the TBV of the child should also be estimated.

Before planning therapeutic apheresis in children, ECV of cell separator should be considered as ECV will represent a larger fraction of TBV of a child resulting in greater volume shift. In children, ECV should not exceed 15% of TBV, if so, priming saline may be infused into the patient without diverting. For the children who have low haematocrit level, red cell priming may be considered with undiluted packed red blood cells (PRBC's). Children have thin peripheral veins so, central catheters with two ports, one for inlet and the second for the return, are preferred.

Patient's co-operation is essential during the procedure, so the use of distraction techniques like electronic media, the help of a child life specialist and parental/family support is required to make the child comfortable throughout the procedure.

8. Extracorporeal photopheresis:

This procedure is a leucapheresis based therapy in which mononuclear cells of the patient are collected and exposed to ultraviolet-A (UVA) irradiation of specific wavelengths in the presence of a photoactivation agent, 8-methoxypsoralen before reinfusion to the patient. Once photoactivated, methoxypsoralen reversibly intercalates between DNA strands and leads to DNA crosslinking. This inhibits the proliferation of treated lymphocytes and leads to DNA strand breaks and apoptosis of the treated cells.[14]

Depending on the instrument used, two different methods of performing ECP have been described. A closed system in which the cells are collected, treated, photoactivated and reinfused in a closed system, in one sitting. Other is the open system in which cells are collected using a cell separator, and the photoactivation step is done offline later infusing the treated cells.[15]

Photopheresis is indicated in the treatment of cutaneous T-cell lymphomas, scleroderma, autoimmune diseases, Graft vs Host Disease (GVHD) in allogeneic haematopoietic stem cell transplantation and solid organ rejection.

9. Immunoadsorption:

In Immunoadsorption (IA), the patient's plasma is first separated from whole blood and then passed through the matrix of a column for adsorption of immunoglobulin or other pathogenic substances present in the plasma. The matrix of the column contains the molecules used to bind required immunoglobulins or pathogenic substances. Depending on the adsorbed molecule, columns can be classified as[16]:

a. **Selective**-Like ABO columns (Glycosorb ABO), IgE columns (IgEnio), columns are having binding material as cholesterol (DALI), lipoprotein(a) (Procard Lp(a) Lipopack).
b. **Semi–Selective-** Columns have binding material as Staphylococcal protein A (Immunosorba), sheep anti-human Ig (Therasorb and Ig-Adsopak), peptide GAM (Globaffin and Ligasorb)
c. **Non-selective**- These types of columns have a binding material in the form of phenylalanine (Immunosorba PH), tryptophan (Immunosorba TR-350), dextran sulphate (Selesorb).

For the patients on angiotensin-converting enzyme inhibitors (ACEi), the use of columns using tryptophan molecule as adsorbed material is contraindicated as ACEi induces reduction of bradykinin metabolism following release during IA and can lead to a hypotensive crisis.

Key points

- Apheresis is a procedure in which blood is withdrawn from a donor or patient mixed with an anticoagulant solution and separated into components manually or with the help of automated cell separator devices. One (or more) component is/are retained, and the remaining constituents are returned to the individual.
- Apheresis procedure is based on the principle of centrifugation, membrane filtration or a combination of both.
- In a plateletpheresis procedure, a portion of the donor's platelets and some plasma is removed with the return of the donor's RBCs, WBCs, and remaining plasma and platelets.
- The interval between two plateletpheresis procedures should be at least 48 hours (not more than 2 times in a week or 24 times in a year).
- A single donor apheresis platelet unit has platelets as $\geq 3.0 \times 10^{11}$ in 180-200 ml plasma.
- Platelet additive solution can be used as a storage medium to enhance the efficiency of apheresis platelet product.
- Removal of plasma from the donor/patient without any replacement solution is called plasmapheresis. It can be done manually as well with the help of automated systems.
- Apheresis procedures use anticoagulant to prevent clotting in the extra-corporeal circuit in the machine. The most commonly used anticoagulant is acid citrate dextrose (ACD).

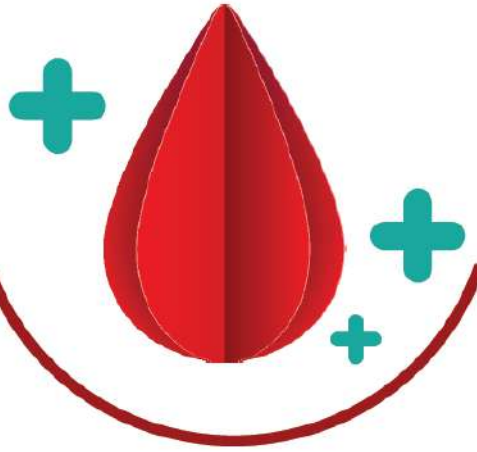
- Leucapheresis is an apheresis procedure in which white blood cells are harvested (granulocytes in granulocytapheresis procedure and mononuclear cells in peripheral stem cell harvest), returning other blood components back to the donor.
- Erythrocytapheresis enables the collection of RBCs by automated apheresis devices and provides a facility for collecting single or double red cell units.
- Therapeutic apheresis is the removal of pathogenic substances from a patient using apheresis technology either in infected and dysfunctional cells or depleting a disease mediator like immunoglobulin causing hyperviscosity, auto-alloantibodies, protein-bound toxins, immune complexes, lipoproteins etc.
- American Society for Apheresis (ASFA)-2019 has categorized various indications for therapeutic apheresis into four categories as Category I for disorders where therapeutic apheresis is accepted as a first-line treatment, Category II for disorders where therapeutic apheresis is accepted as a second-line treatment, Category III for disorders where the optimal role of therapeutic apheresis not clearly established and Category IV for disorders where therapeutic apheresis is considered ineffective or harmful.
- Therapeutic plasma exchange is the procedure in which pathological substances like auto, alloantibodies, immune complexes, drugs, or toxins bound to proteins, high cholesterol or triglycerides in plasma are removed and replaced with suitable replacement fluid. The replacement fluid may be the donor's plasma, albumin, saline or a combination of both.
- Therapeutic leucapheresis is indicated in patients with clinical signs and symptoms of hyperleukocytosis, which is defined as a circulating white blood cells or leukemic blast cell count $>100 \times 10^9$/L.
- Red cell exchange is a therapeutic procedure wherein the patient's red blood cells are replaced with the donor's red blood cells and the suitable colloid solution.
- Extracorporeal photopheresis is a leucapheresis based therapy in which mononuclear cells of the patient are collected and exposed to ultraviolet-A (UVA) irradiation of specific wavelengths in the presence of a photoactivation agent, 8-methoxypsoralen, before reinfusion to the patient.
- In immunoadsorption (IA), the patient's plasma is first separated from whole blood and then passed through the matrix of a column for adsorption of immunoglobulin or other pathogenic substances present in the plasma.

References:

1. Francis R Rodwig. Apheresis. In: Denise M Harmening, Editor. Modern Blood Banking 5th edition. Philadelphia, FA Davis Company; 2005. p. 332-4.
2. Wagner SJ. Whole blood collection and component processing. In: Fung MK, Eder AF, Spitalnik SL, Westhoff CM, editors. Technical manual American Association of Blood Banks. 19th Edition. AABB press.2017: 125-60.
3. Crookston KP and Novak DJ. Physiology of Apheresis. In: McLeod BC, Szczepiorkowski ZM, Weinstein R, eds. Apheresis: Principles and practice. 3rd ed. Bethesda, MD: AABB Press, 2010: 45-69.
4. Burgstaler EA, Winters JL. Apheresis: Principles and Technology of Hemapheresis. In: Simon TL, McCullough J, Snyder EL, Solheim BG, Strauss RG, editors. Rossi's Principles of Transfusion Medicine. 5th edition. John Wiley & Sons, Ltd; 2016.p.648-54.
5. Hess, J.R. and Solheim, B.G. (2016). Red blood cell metabolism, preservation, and oxygen delivery. In Rossi's Principles of Transfusion Medicine (eds T.L. Simon, J. McCullough, E.L. Snyder, B.G. Solheim and R.G. trauss). doi:10.1002/9781119013020.ch09
6. Padmanabhan A, Connelly-Smith L, Aqui N, Balogun RA, Klingel R, Meyer E et al. Guidelines on the use of therapeutic apheresis in clinical practiced evidence-based approach from the writing committee of the American Society for Apheresis: the eighth special issue. J Clin Apher 2019; 34:131-354.
7. Hans R, Prakash S, Sharma R R, Marwaha N. Role of therapeutic apheresis in pediatric disorders. PHOJ 2016;1: 63-68.doi-10.1016/j.phoj.2016.11.002.

8. Weinstein R. Prevention of citrate reactions during TPE by constant infusion of calcium gluconate with the return fluid. J Clin Apher 1996; 11:204-10.
9. Couriel D, Weinstein R. Complications of therapeutic plasma exchange: a recent assessment. J Clin Apher 1994; 9:1-5.
10. Chirnside A, Urbaniak SJ, Prowse CV, Keller AJ. Coagulation abnormalities following intensive plasma exchange on the cell separator. II. Effects on factors I, II, V, VII, VIII, IX, X and antithrombin III. Br J Haematol 1981; 48:627-34.
11. Orlin JB, Berkman EM. Partial plasma exchange using albumin replacement: Removal and recovery of normal plasma constituents. Blood 1980; 19:1055-9.
12. Ibrahim RB, Liu C, Cronin SM, Murphy BC, Cha R, Swerdlow P, et al. Drug removal by plasmapheresis: An evidence-based review. Pharmacotherapy 2007; 27:1529-49.
13. Kim HC. Therapeutic apheresis in pediatric patients. In: McLeod BC, Weinstein R, Winters JL, Szczepiorkowski ZM, editors. Apheresis principles and practice. third ed. AABB press; 2010. p. 446-57.
14. Choi J, Foss FM. Photopheresis. In: McLeod BC, Weinstein R, Winters JL, Szczepiorkowski ZM, editors. Apheresis principles and practice. third ed. AABB press; 2010. P 615-34.
15. Ara Cho, Christian Jantschitsch, Robert Knobler Extracorporeal Photopheresis—An Overview Front Med (Lausanne) 2018; 5: 236.
16. Hamilton P, Harris R, Mitra S. Immunoadsorption Techniques, and Its Current Role in the Intensive Care Unit. In Aspects in Continuous Renal Replacement Therapy. http://dx.doi.org/10.5772/intechopen.848

Section 10



Adverse effects of blood transfusion

1. Introduction

Blood transfusions are lifesaving but may also be associated with adverse effects. Hence, appropriate use and careful monitoring for early diagnosis and management of adverse effects are essential for patient safety. It is equally important to report the complications of transfusions to identify risk factors for preventive actions.

2. Definitions

International Society of Blood Transfusion (ISBT) working party on Haemovigilance proposed standard definitions for surveillance of non-infectious adverse transfusion reaction.[1]

Adverse event: It is an undesirable and unintended occurrence before, during or after transfusion. It may be the result of an error or an incident, and it may or may not result in a reaction. It includes incidents, near misses and reactions.

Incident: Where the patient is transfused with a blood component which did not meet all the requirements for a suitable transfusion for that patient, or that were intended for another patient. It may or may not lead to an adverse reaction.

Near miss: Error or deviation from standard operating procedures (SOPs) or policies that are discovered before the start of the transfusion and could have led to a wrongful transfusion or to a reaction in a recipient.

Adverse reaction: An undesirable response or effect in a patient temporally associated with the administration of blood or blood component.

Serious adverse reaction: An unintended response in a donor or in a patient associated with the collection or transfusion of blood or blood components that is fatal, life-threatening, disabling or incapacitating or which results in or prolongs hospitalisation or morbidity.

Sentinel events: Errors and incidents which are wholly preventable but unintentionally result in serious harm to the patient and require immediate attention. These include the following for transfusion related events.[2]

- Incorrect blood component transfused
- ABO mismatched transfusion
- Wrong name on the tube (wrong blood in a tube, WBIT)

3. Classification of transfusion reactions:

Transfusion reactions are usually classified on the basis of time of onset and the mechanisms (immune or non-immune) leading to the reaction. Reactions occurring within 24 hours of transfusion are termed acute, rest are delayed.

Acute transfusion reactions

a. Immune-mediated

i. Haemolytic transfusion reactions (HTR)
ii. Febrile non-haemolytic transfusion reactions (FNHTR)
iii. Allergic reactions
iv. Anaphylaxis
v. Transfusion related acute lung injury (TRALI)

b. Non-Immune mediated

i. Transfusion associated circulatory overload (TACO)
ii. Transfusion associated dyspnoea (TAD)
iii. Transfusion associated hypotension (TAH)
iv. Metabolic complications
v. Transfusion transmitted bacterial infections (TTBI)

Delayed transfusion reactions

c. Immune mediated

i. Delayed haemolytic transfusion reactions (DHTR)
ii. Delayed serological transfusion reaction (DSTR)
iii. Post-transfusion purpura (PTP)
iv. Transfusion associated graft vs. host disease (TA-GvHD)
v. Transfusion related immunomodulation (TRIM)

d. Non-Immune mediated

i. Transfusion transmitted viral infection
ii. Transfusion transmitted malaria
iii. Transfusion transmitted prion disease
iv. Iron overload

Table 1: Transfusion reactions based on their time of onset

Acute transfusion reactions	**Onset during or within**	**Type of reaction**
	1 hour	TAH
	4 hours	FNHTR, Allergic reaction
	6 hours/12 hours	TRALI, TACO
	24 hours	HTR, TAD
Delayed transfusion reactions	**Onset between**	**Type of reaction**
	24 hrs-28 days	DHTR, DSTR
	5-12 days	PTP
	7-42 days	TA-GvHD

4. Haemolytic transfusion reactions

Definition: A haemolytic transfusion reaction is one in which symptoms and clinical or laboratory signs of increased red cell destruction are produced by transfusion. Haemolysis can occur intravascularly or extra-vascularly and can be immediate (acute) or delayed.[1] It is usually a result of red blood cell (RBC) transfusion, however, it may also result from transfusion of plasma-containing blood components, such as fresh frozen plasma (FFP) or platelet concentrates (PC) containing red cell antibodies.

- Acute haemolytic transfusion reaction (AHTR): It is characterised by the onset of clinical or laboratory features of haemolysis within 24 hours of a transfusion.
- Delayed haemolytic transfusion reaction (DHTR): It is characterised by clinical or laboratory features of haemolysis which usually manifest between 24 hours and 28 days after a transfusion.

Mechanism:

AHTR usually occurs when the recipient has pre-existing antibodies against the transfused RBCs in the form of IgM antibodies and sometimes the haemolytic IgG antibodies against the A or B antigen, or the RBC alloantibodies, or sometimes due to autoantibodies. It may also occur due to non-immunological factors like mechanical factors inducing haemolysis (malfunction of a pump, blood warmer, use of hypotonic solutions, etc.). Usually, the IgM antibody in the recipient binds to foreign RBC antigen resulting in complement activation and subsequent formation of membrane attack complex (MAC), which eventually causes intravascular RBC lysis and release of free haemoglobin (haemglobinemia) in the circulation and then leading to acute tubular necrosis and renal failure, as haemoglobinuria also ensues. The early products (C3a, C5a: anaphylatoxins) of complement cascade cause activation of mast cells, leucocytes, monocytes and endothelium and subsequent release of cytokines and chemokines (TNF-α, IL-1, Il-6, IL-8), leading to increased capillary permeability, endothelial damage, vasodilatation and subsequent clinical manifestations of systemic inflammatory response syndrome. In DHTR, predominantly the immunoglobulin (IgG) and complement (C3b) coated cells interact with phagocytes in the liver and spleen, resulting in clearance of RBCs and activation of phagocytes, thus causing extravascular haemolysis, which also cause a rise in bilirubin levels. The peripheral blood film then shows spherocytes and microspherocytes. DHTR is more common with non-ABO alloantibodies and is frequently the outcome of an anamnestic (secondary) immune response in previously sensitized patients.

Signs and symptoms:

- Fever
- Chills/rigors
- Facial flushing
- Chest pain
- Abdominal pain
- Back/flank pain
- Nausea/vomiting
- Diarrhoea
- Hypotension
- Pallor
- Jaundice
- Oligo/anuria
- Diffuse bleeding
- Dark urine

In AHTR, the passage of reddish urine, hypotension and flank pain are usually the prominent signs and symptoms which usually occur within minutes to a few hours of the transfusion. Signs and symptoms of DHTR are usually less severe and manifest as fatigue, pallor and jaundice after 2-28 days of transfusion. It may sometimes manifest as an inadequate rise in of post-transfusion haemoglobin or an unexplained fall in haemoglobin after a transfusion. Blood group serology usually shows abnormal results. There is an unexpected degree of anaemia from the loss of transfused red blood cells. In some cases, particularly with extravascular haemolysis, this is the only clue to HTR as some reactions are asymptomatic.[2]

Differential diagnosis[3]:

- Alloantibody-induced haemolysis
- Delayed serologic transfusion reaction

- Autoimmune haemolytic anaemia
- Cold haemagglutinin disease
- Non-immune haemolysis
  - Incompatible fluids
  - Improper storage
  - Malfunctioning blood warmers
  - Small needles
  - Bacterial contamination

Investigations:

Common laboratory features are:

- Haemoglobinemia
- Haemoglobinuria
- Decreased serum haptoglobin
- Unconjugated hyperbilirubinemia
- Increased LDH and AST levels
- Decreased haemoglobin levels

Not all clinical or laboratory features are present in AHTR.

Management:

The most important role is careful observation. However, early vigorous intervention in severe reactions can save lives by maintaining hydration, inducing diuresis and vasodilation, and using, anticoagulants and, if needed, IV immunoglobulins and /or red cell exchange.

Prevention:

Haemolytic transfusion reactions can be prevented by proper donor unit typing, meticulous pretransfusion testing, antibody identification, crossmatching and by providing antigen-negative blood and good blood administration practices.

5. Febrile non-haemolytic transfusion reactions (FNHTR)

Definition:

FNHTR is defined as[1] the presence of one or more of:

- Fever ($\geq$38 ^{0}C oral or equivalent and a change of $\geq$1 ^{0}C from pretransfusion value)
- Chills/rigours

Mechanism:

Human leucocyte antigen **(**HLA), granulocyte, and platelet-specific antibodies have been implicated in the pathogenesis of FNHTRs. The recipient's antibodies, reacting with transfused antigens, elicit the release of cytokines (e.g., interleukin 1) that are capable of causing fever. [4]

Signs and symptoms:

Fever, chills and rigours may be accompanied by headache and nausea during or within four hours following transfusion without any other cause such as haemolytic transfusion reaction, bacterial contamination or underlying condition. FNHTR could be present in the absence of fever (if chills or rigours without fever).[1]

Differential diagnosis:

- Haemolytic transfusion reactions
- Sepsis
- Transfusion related acute lung injury

Investigations:

Blood cultures may be performed on the transfusion recipient's post transfusion blood specimen. Most blood centres do not test for HLA-specific, platelet-specific, or granulocyte-specific antibodies in the recipient's serum as possible causes of an FNHTR as identification of these antibodies and pyrogenic cytokines does not play a role in the immediate evaluation of most reactions.[2] Laboratory workup must be performed to exclude haemolysis.

Management:

Transfusion should be discontinued, and antipyretics should be administered.

Prevention:

Pre-storage leucocyte reduction, especially if performed at the time of collection, significantly decreases the frequency of FNHTRs.

6. Allergic reactions and anaphylaxis

Definition:

Allergic reaction may range from minor to life-threatening depending on the course and outcome of the reaction.

Mechanism:

An allergic reaction usually occurs due to hypersensitivity to an allergen and preformed IgE antibodies in the recipient. Mast cell activation (Type I hypersensitivity) occurs which results in degranulation and release of allergic mediators. Secondary mediators, including cytokines and lipid mediators, are also generated and released. IgA deficiency and/or anti-IgA in the recipient may be associated with severe allergic reactions.

Signs and symptoms:

An allergic reaction may present with mucocutaneous signs and symptoms occurring during or within 4 hours of transfusion:

- Morbilliform rash with pruritus
- Urticaria (hives)
- Localized angioedema
- Oedema of lips, tongue and uvula
- Periorbital pruritus, erythema and oedema
- Conjunctival edema

An allergic reaction can also involve respiratory and/or cardiovascular systems and present like an anaphylactic reaction when, in addition to mucocutaneous systems, airway compromise or severe hypotension requires vasopressor treatment (or associated symptoms like hypotonia, syncope). The respiratory signs and symptoms may be laryngeal (tightness in the throat, dysphagia, dysphonia, hoarseness, stridor) or pulmonary (dyspnoea, cough, wheezing/bronchospasm, hypoxemia). Such a reaction usually occurs during or very shortly after transfusion.[1]

Management:

Minor allergic reaction responds quickly to symptomatic treatment. Transfusion should be stopped, and an antihistaminic may be administered. If the symptoms do not subside or in a severe reaction, the patient may require treatment with steroid.

Prevention:

The use of washed red cells or platelets may be considered to prevent these reactions. Plasma reduction or replacement in platelet products may also be beneficial. The plasma used for transfusion for patients diagnosed with IgA deficiency who produce anti-IgA should be from IgA-deficient donors (<0.05 mg/dL).[3]

7. Pulmonary adverse transfusion reactions

A. Transfusion-associated circulatory overload (TACO) and transfusion-related acute lung injury (TRALI): Both these pulmonary adverse events are characterized by respiratory distress and pulmonary oedema. However, separate clinical definitions have been assigned to both these conditions depending on their pathophysiology and clinical spectrum.

- **TACO definition:** It is characterized by the presence of a total of 3 or more of the following criteria occurring during or upto 12 hours after transfusion.[1]
  - o Acute or worsening respiratory compromise: It is manifested by tachypnoea, shortness of breath, cyanosis and decreased oxygen saturation values. The clinical finding could include crackles on lung auscultation, orthopnoea, cough, a third heart sound and pinkish frothy sputum in severe cases.
  - o Radiographic chest imaging and/or other non-invasive assessment of cardiac function, e.g., echocardiogram. The findings suggest pulmonary oedema from circulatory overload includes the presence of new or worsening pleural effusions, widened vascular pedicle, progressive lobar vessel enlargement, peribronchial cuffing, bilateral Kerley lines, alveolar oedema with nodular areas of increased opacity and/or cardiac silhouette enlargement.
  - o Evidence of cardiovascular system changes not explained by the patient's underlying medical condition, including the development of tachycardia, hypertension, widened pulse pressure, jugular venous distension, enlarged cardiac silhouette and/or peripheral oedema.
  - o Evidence of fluid overload including any of the following: a positive fluid balance; response to diuretic therapy, e.g., from diuretic therapy or dialysis combined with clinical improvement; and change in the patient's weight in the peri-transfusion period.
  - o Supportive result of a relevant biomarker, e.g., an increase of B type natriuretic peptide level (e.g., BNP or NT-pro BNP) above the age group-specific reference range and greater than 1.5 times the pre-transfusion value. A normal post-transfusion NP level is not consistent with a diagnosis of TACO; serial testing of NP levels in the peri-transfusion period may help identify TACO.
- **TRALI definition:** TRALI can be defined as type 1 and type 2 as following[5]:
  - o TRALI Type - 1: Patients who have no risk factors for ARDS and meet the following criteria:
    a. The development of respiratory distress is characterized by:
      i. Acute onset
      ii. Hypoxaemia (P/F ≤ 300 or SpO2 < 90% on room air)
      iii. Clear evidence of bilateral pulmonary oedema on imaging (e.g., chest radiograph, chest CT, or ultrasound)
      iv. No evidence of left atrial hypertension (LAH) or, if LAH is present, it is judged to not be the main contributor to hypoxaemia
    b. Onset during or within 6 hours of transfusion
    c. No temporal relationship to an alternative risk factor for ARDS.

- **TRALI type 2** (earlier known as possible TRALI): Patients who have risk factors for ARDS (but who have not been diagnosed with ARDS) like direct lung injury due to aspiration of gastric contents, pneumonia, inhalational injury, lung contusion, near drowning, pulmonary vasculitis and indirect lung injury due to non-pulmonary sepsis, non-cardiogenic shock, major burns, severe burns, acute pancreatitis, cardiopulmonary bypass and drug overdose or who have existing mild ARDS (P/F of 200-300), but their respiratory status deteriorates and is judged to be due to transfusion based on:
  a. Findings as described in categories a and b of TRALI Type 1, and
  b. Stable respiratory status in the 12 hrs before transfusion

- **Mechanism**: Acute respiratory distress in TACO is due to cardiogenic oedema, which develops due to circulatory overload and increases central venous pressure. It causes pulmonary vascular congestion and decreased lung compliance presenting as dyspnoea, hypertension and tachycardia. In severe cases, it may even lead to cardiac failure. However, in TRALI, the pulmonary oedema is non-cardiogenic, which occurs as a result of capillary endothelial leak leading to the exudative fluid passing from pulmonary vessels to lung alveoli, causing dyspnoea.
- **Two hit model for TACO and TRALI**: In both these conditions, the first hit is provided by the patient's underlying comorbid condition, and the blood products convey the second hit.[6] Second hit, if strong enough is capable of inducing pulmonary oedema in the absence of the first hit, this explains TACO and TRALI in healthy individuals also.

Table 2: Two hit model for TACO and TRALI

	TACO	TRALI
First Hit	• Poor adaptability for volume overload • Pre-existing renal, cardiac insufficiency with hypertension	• Systemic inflammatory conditions like surgery, terminally ill patients and active infection causing neutrophil activation with increased sequestration in the pulmonary vessels. • Activation of pulmonary endothelial cells and monocyte also contribute towards the first hit.
Second Hit	• Suboptimal fluid management and rapid transfusion leading to positive fluid balance. • Increase in intravascular osmotic pressure due to transfused product leading to fluid shift. • Various immune mechanisms similar to TRALI have been hypothesised to cause mechano-transduction and endothelial barrier dysfunction leading to TACO like clinical picture.	• Antibody-mediated TRALI: o The presence of anti HLA I & anti-neutrophil antibodies (HNA 2, 3a, 4a) against neutrophils cause their degranulation and release of reactive oxygen species, granular enzymes and neutrophil extracellular traps (NETs), leading to endothelial damage and leakage of intravascular fluid leading to oedema. o Similarly, anti-HLA-II antibodies binding to monocytes induces inflammation in the lungs, causing oedema • Non antibody-mediated TRALI: Metabolic products present in blood products like bioactive lipids, CD40 ligand, and immunoglobulin can induce leakage in primed pulmonary endothelial surfaces.

- **Signs and symptoms**: Clinical presentation of TACO and TRALI are similar presenting with dyspnoea and hypoxaemia. However, it can be differentiated as shown in the table below:

Table 3: Clinical parameters in TACO and TRALI

Parameters	TACO	TRALI
Diagnosis clinically supported if	Orthopnoea raised jugular venous pressure (distended neck veins) and frothy sputum in severe cases	Copious frothy sputum (typically pinkish)
Auscultation	Rales (crackles), S3	Rales, No S3

Parameters	TACO	TRALI
Blood pressure	High	Normal or low
Tachycardia	Present	Present
Widened pulse pressure	Likely	No
Fever	Possible	Possible
X-Ray Chest	White lung fields (infiltrates) with enlarged cardiac silhouette (C/T ratio>0.55), Kerley B lines, peribronchial cuffing and may be pleural fluid	White lung fields (infiltrates) with typically no pleural fluid.
Diuretic response	Yes	No
Increased pulmonary artery occlusion pressure > 18mm Hg	Yes	No
B-type natriuretic peptide >250 or pre-post transfusion BNP > 1.5	Yes	No
Leucopenia	No	Yes

- **Management:** TACO and TRALI are similar clinically, but their management is completely different. Hence, early recognition and making a diagnosis is important in the management of both these conditions.

Table 4: Management in TACO and TRALI

Clinical signs	Treatment option in TACO	Treatment option in TRALI
Hypoxia	Oxygen therapy, mechanical ventilation may be required	Oxygen therapy, mechanical ventilation is required in most cases.
Upright posture for orthopnoea	Yes	No
Maintenance of haemodynamics	Diuretics. phlebotomy may be required in severe cases	Inotropic support, if required. Diuretics are contraindicated
Response	Rapid	It may take upto 24 hours

- **Prevention:**
  - **TACO:**
    - Avoidance of unnecessary transfusions.
    - In at-risk patients, the volume to be transfused should be restricted to a single unit. If multiple units are required, there should be an appropriate gap between consecutive transfusions. Similarly, in paediatric patients, blood volume to be transfused can be divided into aliquots. Diuretics can also be given if indicated.
    - Few studies have shown benefits of leucoreduction in the prevention of TACO, supporting immune mechanism of TACO.
  - **TRALI:**
    - Plasma rich products from multi gravida female donors should be avoided.
    - Directed donation from wife to a husband and children is known to cause TRALI.
    - Donor with known antibodies implicated in TRALI should be deferred from donating blood.
    - Proper storage of blood components to minimise metabolic product levels.

B. Transfusion-associated dyspnoea (TAD): It is characterised by respiratory distress within 24 hours of transfusion that does not meet the criteria of TRALI, TACO, or allergic reaction.[1] Respiratory distress should be the most prominent clinical feature and should not be explained by the patient's underlying condition or any other known cause. The pathophysiology of TAD is not clear as it is intermediate between TACO and TRALI. It is self-limiting and may require supportive management in few cases.

8. Transfusion-associated hypotension (TAH):

This reaction is characterized by hypotension defined as a drop in systolic blood pressure of more than or equal to 30 mm Hg occurring during or within one hour of completing transfusion and systolic blood pressure less than or equal to 80 mm Hg and all other transfusion reactions presenting with hypotension are excluded.[1]

Mechanism:[7]

Exposure to filter surfaces mainly negatively charged, which mimics subendothelium in bedside leucocyte filters, leads to activation of factor XII. It converts prekallikrein to kallikrein which cleaves kinonogen to bradykinin. This bradykinin causes vasodilation causing hypotension. Stagnation of bradykinin catabolism in patients on ACE inhibitors predispose them to hypotensive reactions. Hypotensive reactions are also seen without bedside leucoreduction filters. In these cases, patient's underlying condition and any extra corporeal circuits have been implicated in hypotensive reactions.

Signs and symptoms:

Hypotension is usually the sole manifestation, but facial flushing and gastrointestinal symptoms may occur.

Differential diagnosis:

Other transfusion reactions like HTR, allergic, anaphylaxis, TTBI and TRALI should be excluded as these reactions can also present with hypotension.

Management:

Most reactions do occur very rapidly after the start of the transfusion (within minutes) and respond rapidly to the cessation of transfusion. Inotropic support may be required in severe cases.

Prevention:

Pre-storage leucoreduced blood component can be used in place of bedside leucofilters. Discontinuation of ACE inhibitors 24-48 hours before transfusion depending on the half-life of ACE inhibitors can also help in the prevention of TAH.

9. Metabolic complications

It includes electrolyte imbalances seen more commonly during massive transfusion.[1,3]

A. **Hypocalcaemia**: It is mainly due to excessive citrate infusion during massive blood transfusion and apheresis procedures. Citrate binds calcium and hence leads to hypocalcaemia. Inability to metabolize citrate predisposes liver failure patients to hypocalcaemia. Hypocalcaemia presents with perioral or peripheral paraesthesia, tingling, cramps, nausea, vomiting, arrhythmias, bradycardia and hypotension. QT prolongation on ECG is the hallmark of hypocalcaemia. It is managed by oral or iv calcium depending on the severity of the reaction. Prophylactic calcium may also be given to those who are at risk of developing hypocalcaemia. Magnesium should be given to patients unresponsive to calcium therapy.

B. **Hyperkalemia:** Any abnormally high potassium level (> 5 mmol/l) within an hour of transfusion can be classified as transfusion-associated hyperkalemia. It occurs due to the accumulation of potassium in the extracellular fluid in blood components with increasing blood centre storage times. Improper storage and handling of blood components increase potassium leakage from red cells due to haemolysis. ECG changes include peaked T waves, prolonged PR interval and ventricular arrhythmia. Patient with acidosis, renal failure and neonates are at risk of developing hyperkalemia. In these patients, fresh blood units (<7 days old) or washed PRBC may be given to prevent hyperkalemia.

C. **Hypokalemia:** A decrease in potassium levels may be seen in patients receiving massive transfusion with plasma containing components. When citrate is metabolized to bicarbonate, it leads to alkalosis in the patient and precipitates hypokalemia. It is self-limiting, but a potassium supplement may be required in severe cases.

D. Haemosiderosis (Iron overload): Transfusion-associated haemosiderosis is defined as a blood ferritin level of ≥ 1000 micrograms/L, with or without organ dysfunction in the setting of repeated RBC transfusion. It is seen in almost all transfusion-dependent thalassemia patients on chronic transfusion therapy. In these patients, there is downregulation of hepcidin (iron absorption regulatory protein) expression, which leads to enhanced gastrointestinal absorption of iron, leading to iron toxicity. Early initiation and regular transfusion therapy can help in decreasing ineffective erythropoiesis and hence hepcidin mediated iron toxicity. Iron chelation therapy is effective in removing the excess iron.

10. Transfusion-associated graft-versus-host disease (TA-GvHD)

It is a clinical syndrome characterised by symptoms of fever, rash, liver dysfunction, diarrhoea, pancytopenia and characteristic histological findings on biopsy occurring 1-6 weeks following transfusion with no other apparent cause.[1]

Mechanism:

The major risk factors for TA-GvHD are the degree of recipient immunodeficiency, the number of viable T-lymphocytes in the transfused blood component and the degree of genetic diversity in the population.[8] In an immunocompetent recipient, when there is a recognition of 'nonself' HLA of the transfused donor lymphocytes by the host immune system, it usually leads to attack and death of the donor lymphocytes contained in the transfused blood component, thus preventing engraftment and proliferation.[9] In circumstances when the blood donor is homozygous for the HLA antigens for which the recipient is heterozygous (one-way haplotype match),[3] the recipient's immune system does not recognize the HLA-homozygous transfused donor lymphocytes as 'non-self'(Figure 1). In contrast, the transfused lymphocytes are able to recognize the host cells as 'non-self'and are able to mount an immunologic attack on the host.[8] In a large compilation of TA-GvHD cases by Kopolovic et al,[10] nearly all cases were attributed to cellular components stored for ≤10 days.

Signs and symptoms:

These include fever, rash (erythematous), pancytopenia, bone marrow aplasia, diarrhoea, and deranged liver function tests. The clinical manifestations occur 1-6 weeks following transfusion.[1] All the cellular blood products including whole blood, packed red blood cells (PRBCs), platelets and granulocytes have been implicated but not the plasma products, or frozen-deglycerolised RBCs due to insufficient number of viable donor lymphocytes in these products.[3]

Differential diagnosis and investigations:

Other clinical conditions like drug reactions and viral illness may have similar presentation.[11] Diagnosis is usually made by biopsy of skin, gut or liver supported by evidence of persistence of donor lymphocytes which can be differentiated from host lymphocytes by measuring restriction fragment length polymorphisms or short tandem repeat analysis.[12] The skin biopsy reveals a superficial perivascular lymphocytic infiltrate, necrotic keratinocytes, compact orthokeratosis, and bullae formation.[11]

Treatment and prevention:

Despite immunosuppressive therapy, TA-GvHD is almost uniformly fatal (90% to 100%).[11] Irradiation of cellular blood components is the only reliable way to prevent TA-GvHD.[8] The American Association of Blood Banks (AABB) standards require a minimum dose of 25 Gray (Gy) delivered to the central portion of the container and 15 Gy elsewhere.[8] The British Society of Haematology (BSH) guidelines recommend a minimum of 25 Gy, but not exceeding 50 Gy.[12] Most blood centres use Cs-137, Co-60 or X-rays as a source of ionising radiation. PRBCs may be irradiated at any time up to 14 days of storage and subsequently stored for a further 14 days. Platelets can be irradiated at any stage during storage. All granulocytes should be irradiated before issue.[12] The major indications for irradiation of blood components include intrauterine transfusion (IUT), prematurity, low birthweight, exchange transfusion in neonates who received IUT, congenital immunodeficiencies, haematologic malignancies or solid tumours, haematopoietic stem cell transplantation, directed donations from first- or second-degree relatives, patients on purine analogues.[8,12]

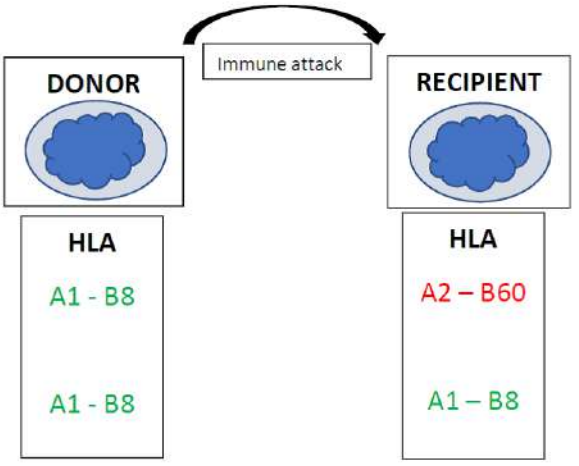


Figure 1: One of the HLA haplotypes of the recipient for which the donor is homozygous is treated as 'self' (*HLA-A1,B8*) by the recipient, but the donor considers the recipient's *HLA-A2,B60* as 'foreign' and mount an immune response against it, leading to transfusion-associated graft-versus-host disease (TA-GVHD)

11. Post transfusion purpura (PTP):

It is characterized by thrombocytopenia arising 5-12 days following transfusion of cellular blood components with findings of antibodies in the patient-directed against the Human Platelet Antigen (HPA) system.[1]

Mechanism:

Antibodies to HPA-1a and HPA-1b (and sometimes HPA-1b, -2b, -3a, -3b, -4b, -5a, -5b, and -15b) result in destruction of both transfused and host platelets (PLTs), leading to severe thrombocytopenia (<10000/ μL). These antibodies result from recipients'prior exposure(s) to PLT antigens through pregnancy (85-95% of cases) or transfusion. PTP can be triggered by transfusion of any blood components- PRBCs, PLTs, plasma-containing PLT antigens or secondary to passive transfer of antibodies.[13] The mechanism of destruction of the patient's own HPA-1a-negative platelets remains uncertain.[3]

Signs and symptoms:

Mucocutaneous bleeding (wet purpura, petechiae, epistaxis, gastrointestinal, and urinary tract) is common. Mortality rates could be up to 16%, due to intracranial haemorrhage.[8,3] Although, thrombocytopenia usually resolves spontaneously within a few weeks, it may be delayed by up to four months or more.[9]

Differential diagnosis and investigations:

Other causes of thrombocytopenia, such as autoimmune thrombocytopenia, thrombotic thrombocytopenic purpura, heparin-induced thrombocytopenia (HIT), disseminated intravascular coagulation (DIC) and drug-induced thrombocytopenia, should be ruled out. However, in PTP, the patients usually have normal platelet counts previously and are asymptomatic.[8]

Treatment and prevention:

Intravenous immunoglobulin (IVIG) is the current treatment of choice (0.4g/kg for five days).[8,3] Platelet transfusions are usually ineffective. Pulse methylprednisolone (1g/day) is also effective.[3] HPA-1a-negative patients should be transfused with HPA-1a-negative blood products, although the efficacy is uncertain.[8,3]

12. Transfusion transmitted infections

A. Transfusion transmitted bacterial infections (TTBI): It is the bacterial infection following transfusion, with evidence of blood product contamination or infection in the donor and in the absence of infection in the recipient before transfusion. However, an international consensus is not yet established.[14]

Mechanism:

It is frequent with platelet components as they are stored at room temperature (20-24^{0}C), serving as an excellent growth medium for many aerobic and microaerophilic bacteria.[3] Psychrophilic bacteria (e.g., *Yersinia enterocolitica*) are frequently implicated in PRBCs. It uses citrate as an energy source and grows better in the presence of iron, thus RBCs are an ideal culture medium. Organisms associated with plasma and cryoprecipitate are *Pseudomonas cepacia* and *Pseudomonas aeruginosa.* Bacteria most commonly enter the container at the time of phlebotomy (skin commensals like *Staphylococcus epidermidis, Propionibacterium acnes*), or at times through asymptomatic donor bacteraemia. Very rarely, contamination of the collection bag, tubing, or anticoagulant may involve environmental contaminants (*Serratia* spp., *Bacillus* spp., and *Pseudomonas* spp.). Contaminants in water baths are usually *Burkholderia cepacia* and *P. aeruginosa*.[3]

Signs and symptoms:

TTBI is suspected when signs and symptoms shortly after transfusion include fever (>38.5^{0}C or 101^{0}F), tachycardia, chills and rigours and/or hypotension. Gram-negative bacteria produce more severe symptoms of shock, renal failure and DIC.[4]

Investigations:

In case of suspected TTBI, any blood remaining in the container/blood bag should be sent for culture. A simultaneous follow-up of the corresponding other blood components should also be undertaken. Specific identification of the contaminating organism becomes an important clue for the donor follow-up as well.[3,15,16]

Treatment and prevention:

The treatment of TTBI should be prompt with antibiotics without waiting for the laboratory results. Careful donor screening and disinfecting the donor skin prior to phlebotomy by using iodophors, 2% chlorhexidine, or alcohol, are effective methods. Diversion of the first 10 to 40 mL of donor blood is the most effective way to decrease contamination. Tests for detection of bacteria in platelets (BacT/ALERT system, the Pall eBDS test, etc.) are also available but are of limited value. Leucoreduction during manufacture may decrease the risk of bacterial contamination.[3] Visual inspection of PRBCs for any evidence of haemolysis may help identify possibly contaminated units. Pathogen inactivation methods may also help to reduce the risk.

B. Transfusion transmitted viral infections:

Viral TTIs of global concern are hepatitis B, hepatitis C and HIV. For details on viral TTIs, refer to section 5.

C. Transfusion transmitted parasitic infections:

The incidence of transfusion-transmitted parasitic infections is low as compared to bacterial and viral infections. For details on parasitic TTIs, refer to section 5.

13. Transfusion-related immunomodulation (TRIM):

The constellation of all allogeneic blood transfusion-associated laboratory immune aberrations and their related clinical findings is known as transfusion-related immunomodulation (TRIM).[3] The TRIM effects may involve multiple biologic mechanisms. The support for the theory that TRIM is due to the allogeneic WBCs has come mainly from data from animal models. The overall evidence from randomised controlled trials (RCTs) seems to be inadequate to advocate universal white blood cell (WBC) reduction specifically for the prevention of this effect.[3] The association between leucocyte-containing allogeneic blood and increased mortality may be limited to cardiac surgery and should not be extended to other clinical settings.[15] The immunosuppressive effect of transfusion may be HLA dependent and directed against adaptive immunity and an additional non-specific effect directed against innate immunity due to apoptotic cells as a result of refrigerated storage. This immunosuppression may be linked to transforming growth factor-beta (TGF-β). Transfusion has also been shown to have a striking effect in improving the survival of subsequent renal grafts. This beneficial effect may be due to the induction of increased suppressor cell activity, decreased natural killer cell activity, specific

unresponsiveness due to idiotype antibodies, which inactivate T-cell clones and production of non-cytotoxic, Fc receptor-blocking antibodies. The relationship between blood transfusion, cancer growth and cancer-free survival has been quite controversial, as reported in many of the studies.[3]

14. Identification of adverse transfusion reaction in unconscious/anaesthetised patients:

a. In unconscious patients, the risk of identification errors is high; these patients should be identified very carefully.
b. During and after transfusion, periodically objective signs of a transfusion reaction should be checked (Table 5).
c. Anaesthesia can mask the symptoms of both haemolytic and non-haemolytic transfusion reactions, and certain side effects of anaesthesia may be falsely attributed to transfusion. Hence, care should be taken while deciding the imputability of a transfusion reaction.

Table 5: Identification of transfusion reaction in an unconscious patient

Type of reaction		Objective clinical signs
Non-haemolytic Reactions	FNHTR and Allergic reaction	Fever (an increase of body temperature > 1°C), skin changes (e.g., flushing, oedema, or pallor), tachycardia, hypotension
	Pulmonary reactions	Increased peak airway pressure, decrease in oxygen saturation, hyper/ hypotension, tachycardia
	TAH	Hypotension
Haemolytic Reactions	Acute HTR	Hypotension, microvascular bleeding (increased bleeding at the surgical site), urine colour change (transparent reddish colour in DIC or cola coloured due to haemoglobinuria), decrease in urine output.

15. Work-up of a transfusion reaction:

Transfusion reactions can be investigated by correlating clinical sign and symptoms with laboratory test (Figure 2).[19]

Identification:

a) Identify the patient to rule out any mismatched transfusion
b) Take immediate note and inform the blood centre
c) Record the vitals: temperature, pulse rate, respiratory rate, blood pressure and oxygen saturation.
d) If the patient is on a ventilator, record the ventilator setting pre- and post-transfusion.
e) Complete the transfusion reaction form and appropriately record the following:
   i. Clinical signs and symptoms
   ii. Time after the start of transfusion to the occurrence of reaction
   iii. Unit number and volume of component transfused

Immuno-haematological testing:

1. Both clotted and EDTA samples along with BT set (if available) are required for immune-haematological work up to rule out any immune cause of haemolysis.
2. Perform blood group of the patient, pre- and post-transfusion sample, along with blood group of the bag to rule out any ABO mismatched transfusion.

3. Direct Antiglobulin Test: To check for immune sensitization of red cells. Patient with a positive DAT on the post-transfusion sample and with a negative DAT on the pre-transfusion sample may be classified as having an immune haemolytic transfusion reaction
4. Repeat compatibility testing of the blood bag with both pre- and post-transfusion samples to rule out incompatible transfusion.
5. Antibody screen and identification (ID): If there is evidence of immune haemolysis due to non-ABO antibodies, an antibody screen and ID should be performed to identify the red cell antibody.

Other biochemical and haematological tests:

A. Haematological and biochemical tests:
   a. Complete blood count
   b. Plasma and urine haemoglobin
   c. Peripheral blood smear
   d. Coagulation screen
   e. Renal function test
   f. Liver function test
B. Blood culture of recipient and blood bag
C. Chest X-ray in cases of transfusion reaction with cardio-respiratory involvement.

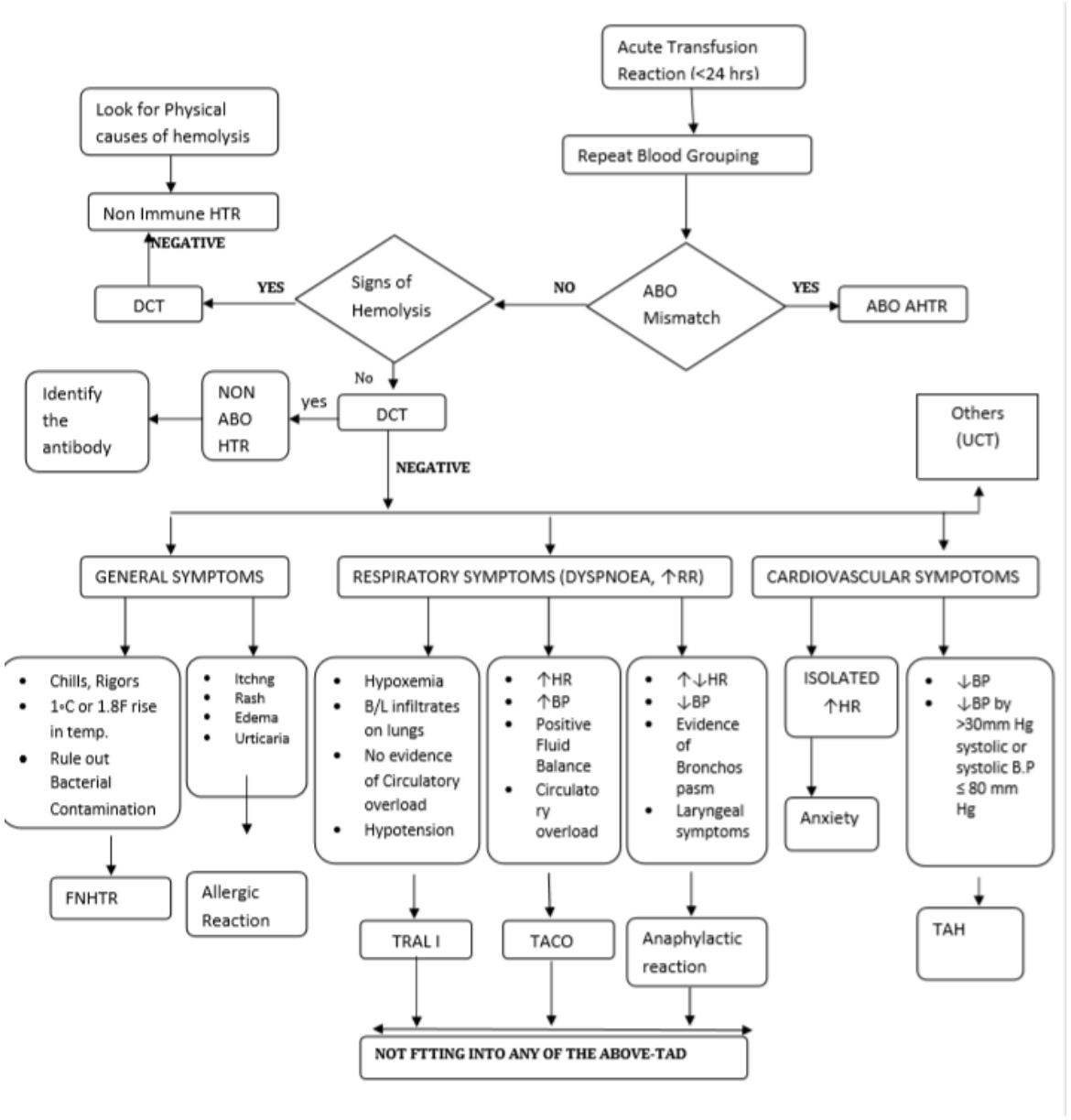


Figure 2: Flowchart for differential diagnosis of adverse transfusion reactions

16. Wrong blood in the tube (WBIT)

Definition:

The United Kingdom Serious Hazards of Transfusion (SHOT) scheme defines 'wrong blood in the tube'(WBIT) (SHOT, 2012) as events where:

a. Blood is taken from the wrong patient and is labelled with the intended patient's details.
b. Blood is taken from the intended patient but labelled with another patient's details.[20]

WBIT results from misidentification of the patient at the time of pretransfusion sample collection or mislabelling of the sample collection tube. This may result in inappropriate transfusion, i.e., exposing patients to the hazards of transfusions that they did not need.

It may be problematic if the patient has no previous ABO and Rh results with which to compare the current test results, and the blood centre might not detect it.

Prevention:

a. One approach to detect such specimen errors is to require that a second specimen be collected at a different time or phlebotomy than the original specimen that was used to determine ABO/Rh status for patients with no historical ABO grouping and Rh typing results. Such an approach permits identification of WBIT specimen errors and may prevent ABO-incompatible transfusions.[4]

b. Similar results on two independently collected samples before the release of non-group-O red cell products.[3]

17. Incorrect blood component transfused

These events result from avoidable system failures throughout the transfusion chain. Experience from the SHOT scheme over a period of 8 years has indicated that the observed rate of such events is 7:100,000 blood components issued, with a fatal outcome in 0.7: 100,000 blood components issued. Detailed analysis of these incidents reveals that in approximately 50% of events, there are multiple errors in the transfusion process, that approximately 70% of errors occur in clinical areas (the most frequent error being failure to ensure at the bedside that the right blood is being given to the right patient), and that 30% happens in hospital laboratories.[3]

Prevention:

a. Barcodes and radio-frequency identification (RFID) technologies serve to improve the accuracy of sample labelling and the bedside checking process. RFIDs are also capable of constantly monitoring blood product location and storage temperature, thereby improving compliance with good manufacturing practice regulations.

b. Computerized order entry

c. Locking devices that require allotment of a code to any potential transfusion candidate. This code is printed on the patient's identification bracelet and transcribed on the sample tube and requisition at the time of sample collection. Units of blood are placed into plastic bags, each of which is closed with a combination lock set to the patient's assigned code. At the bedside, the transfusion nurse can open the lock only by using the patient's code.[3]

18. Haemovigilance

a. Definition:

Haemovigilance is an important tool for blood safety. It is defined as "a set of surveillance procedures covering the whole transfusion chain from the collection of blood and its components to the follow-up of its recipients, intended to collect and assess information on unexpected or undesirable effects resulting from the therapeutic use of labile blood products, and to prevent their occurrence and recurrence" (http://www.ihn-org.net).[21]

b. Haemovigilance system and its constituents:

The establishment of a haemovigilance system is a complex process. The following requirements are to be considered before setting up a haemovigilance system

- The organizational set-up
- Mandatory through legislation or voluntary reporting
- The scope of haemovigilance can be comprehensive or restricted. In a comprehensive system, the transfusion chain is covered and includes complications in donors during blood collection, errors in blood processing, testing and release, adverse events in transfusion recipients, near misses and rapid alerts. It could begin in a restricted manner with a focus on one aspect of the transfusion chain.
- Uniform reporting formats as haemovigilance capture a large amount of data that needs analysis.
- Awareness and training of all stakeholders.

- Data management which includes report validation by experts, analysis, report publication and specific recommendations for blood safety.

c. Hemovigilance system in India:

The haemovigilance programme[22] was initiated under the overall ambit of the Pharmacovigilance Programme of India (PvPI). The PvPI was set up under the aegis of the Indian Pharmacopoeia Commission (IPC) and Central Drugs Standards Control Organization (CDSCO) in July 2010 with IPC as the coordinating centre, and resources were allocated for this programme by the government. The successful establishment of PvPI paved the way for Haemovigilance Programme as a joint venture between IPC and the National Institute of Biologicals (NIB), the coordinating centre haemovigilance programme of India (HvPI; http://nib.gov.in). Now, HvPI is a mandate of NIB. The programme commenced for recipient haemovigilance, but in 2016 donor vigilance was also added.[23,24]

- **Objectives of the recipient haemovigilance programme:**
  i. Monitor transfusion reactions
  ii. Create awareness amongst health care professionals
  iii. Generate evidence-based recommendations
  iv. Advise CDSCO for safety-related regulatory decisions
  v. Communicate findings to all key stakeholders
  vi. Create national and international linkages
- **Objectives of the donor vigilance programme:**
  i. Improve donor safety and satisfaction through monitoring, analyzing and researching adverse events
  ii. Analyze risk factors, implement and evaluate preventive measures
  iii. Provide evidence-based support for blood donation process improvement
  iv. Reduce the frequency of adverse events
  v. Increase donation frequency

The HvPI is based on WHO guidelines for Adverse Event Reporting and Learning Systems.[25] The key principle is to focus on systems improvements and not blame individuals. It should be non-punitive, the confidentiality of the reporting blood centre/nodal officer should be maintained, and the programme should be independent of punishing authority.

The assigned roles and responsibilities of the functional units of haemovigilance are as follows:

- **Reporting units (Blood centres/Departments of Transfusion Medicine)**
  - Generate transfusion reaction reports
  - Perform imputability assessment
  - Submit reports in Haemo-Vigil software
- **National Co-ordinating Centre – HvPI**
  - Review quality and completeness of data
  - Collation and analysis of data
  - Preparation of guidance documents
  - Training and awareness programmes
  - Feedback to reporting units
  - Recommendations for blood safety
  - Forward recommendations to CDSCO
- **CDSCO–DCGI (Drug Controller General India)**
  - Formulate safety-related regulatory decisions
  - Communication of blood and blood products transfusion safety-related decisions to stakeholders
  - To monitor compliance

d. International scenario of haemovigilance:

Several countries in the world have set up national haemovigilance systems. Governance mechanisms for haemovigilance systems are diverse and depend upon the organizations overseeing blood safety within a country. Exchange of information, harmonization of definitions and assistance in establishing haemovigilance systems in countries have been facilitated through the ISBT working party on haemovigilance, the International Haemovigilance Network (IHN) and AABB. In association with IHN and ISBT, the World Health Organization has published an Aide-Memoire to help countries intending to set up national haemovigilance systems.[26]

Key Points

- Blood transfusions are lifesaving but may also be associated with adverse effects.
- Reactions occurring within 24 hours of transfusion are termed acute, rest are delayed.
- A haemolytic transfusion reaction is when symptoms and clinical or laboratory signs of increased red cell destruction is produced by transfusion. It may occur due to immune (alloantibody mediated) and non-immune (handling and storage errors) mechanisms.
- IgM class of red cell alloantibodies causes intravascular haemolysis leading to haemoglobinemia, haemoglobinuria and its consequences. The IgG class of red cell antibodies leads primarily to extravascular destruction and unconjugated hyperbilirubinemia.
- TACO and TRALI are adverse events characterized by respiratory distress and pulmonary oedema but with different pathogenetic mechanisms.
- Plasma rich products from multigravida female donors should be avoided for the prevention of TRALI.
- Hypotension as a predominant feature occurring very rapidly after the start of the transfusion (within minutes) should draw attention to an anaphylactic or hypotensive transfusion reaction; the former is associated with allergic manifestations in addition.
- TTBIs are more frequent with platelet components as they are stored at room temperature (20-24^{0}C), serving as an excellent growth medium for many aerobic and microaerophilic bacteria. Psychrophilic bacteria are frequently implicated in PRBCs.
- Bacteria most commonly enter the container at the time of phlebotomy or at times through asymptomatic donor bacteraemia. Contaminants in water baths may be causal factors at the time of thawing of plasma components.
- In an anaesthetised or unconscious patient, hypotension, red coloured urine in catheter tubing and decreased oxygen saturation should raise an alert for transfusion reactions.
- Acute transfusion reactions have some common signs and symptoms and are to be investigated as per a standard laboratory workup protocol. Specific tests are performed as indicated.
- Near-miss events are errors or deviation from SOPs or policies that are discovered before the start of the transfusion and could have led to a wrongful transfusion or to a reaction in a recipient.
- Haemovigilance is an important tool for blood safety. It is a set of surveillance procedures covering the whole transfusion chain, from collecting blood and its components to the follow-up of its recipients to collect and assess information on unexpected or undesirable effects and prevent their occurrence and recurrence. The Haemovigilance Programme of India commenced for recipient haemovigilance in 2012, and in 2016 donor vigilance was also added. The National Institute of Biologicals (NIB) is the coordinating centre.

References:

1. International Society of Blood Transfusion Working Party on Haemovigilance. Proposed standard definitions for surveillance of non-infectious adverse transfusion reactions. July 2011, incorporating TRALI definition 2013 and TACO definition 2018. http://www.isbtweb.org/Haemovigilance/ISBT_definitons_final_2011_TRALIcorrection adopted_IHNlogo.doc (Accessed on October 15,2019).

2. Simon TL, Snyder EL, Solheim BG, Stowen CP, Strauss RG, Petrides M, editors. Rossi's Principles of Transfusion Medicine. 4th ed. West Sussex, United Kingdom. Wiley Blackwell; 2009.
3. International Society of Blood Transfusion Working Party on Haemovigilance and IHN. Proposed standard definitions for surveillance of sentinel types of errors and incidents. Adopted2015. https://www.isbtweb.org/ISBT_definitions_sentinel_events_adopted2015.pdf (Accessed on October 15, 2019)
4. Mazzei CA, Mark A, Kopko PM. Non-infectious complications of blood transfusion. In: Fung MK, editor. Technical Manual. 18th ed. Bethesda, MD (USA). American Association of Blood Banks (AABB) Press; 2014. p. 665-92.
5. Alexander P.J. vlaar et.al. A consensus definition of transfusion related acute lung injury. Transfusion 2019; 59; 2465-76.
6. Semple J W, Rebetz Johan, Kapur R. Transfusion associated circulatory overload and transfusion related acute lung Injury. Blood 2019; 133:1840-53.
7. Metcalf RA, Bakhtary S, Goodnough LT, Andrews J. Clinical Pattern in Hypotensive Transfusion Reactions. Anesth Analg 2016; 123:268-73.
8. Savage WJ, Hod EA. Non-infectious complications of blood transfusion. In: Fung MK, editor. Technical Manual. 19th ed. Bethesda, MD (USA). American Association of Blood Banks (AABB) Press; 2017. p. 569-98.
9. Bahar B, Tormey CA, Prevention of transfusion-associated graft-versus-host disease. Arch Pathol Lab Med 2018; 142:662-7.
10. Kopolovic I, Ostro J, Tsubota H, Lin Y, Christine M. Cserti-Gazdewich CM, Hans A. Messner HA, Keir AK, Hollander ND, Dzik WS, Callum J. A systematic review of transfusion-associated graft-versus-host disease. Blood 2015; 126:406-14.
11. Ruhl H, Bein G, Sachs UJ. Transfusion associated graft-versus-host disease. Transfus Med Rev 2009; 23:62-71.
12. Treleaven J, Gennery A, Marsh J, Norfolk D, Page L, Parker A, Saran F, Thurston J, Webb D. Guidelines on the use of irradiated blood components prepared by the British Committee for Standards in Haematology blood transfusion task force. Br J Haematol 2010; 152:35–51.
13. Menis M, Forshee RA, Anderson SA, McKean S, Gondalia R, Warnock R, Johnson C, Mintz PD, Worrall CM, Kelman JA, Izurieta HS. Posttransfusion purpura occurrence and potential risk factors among the inpatient US elderly, as recorded in large Medicare databases during 2011 through 2012. Transfusion 2015; 55:284–95.
14. Chew E, Benjamin RJ, McDonald CP, Wiersum-Osselton JC, Wood EM. International survey on definitions and current practices in prevention, diagnosis, management and reporting of transfusion-transmitted bacterial infections. ISBT Science Series 2015; 10:31–40.
15. Klein HG, Anstee DJ. Mollison's Blood Transfusion in Clinical Medicine. 12th ed. West Sussex, United Kingdom. Wiley Blackwell; 2014.
16. Bloch EM. Residual risk of bacterial contamination: what are the options? Transfusion 2017; 57; 2289-92.
17. WHO clinical use of blood. Available from: https://www.who.int/bloodsafety/clinical_use/en/Handbook_EN.pdf.
18. Bolton-Maggs PHB, Wood EM, Wiersum-Osselton JC. Wrong blood in tube–potential for serious outcomes: can it be prevented? Br J Haematol 2015;168:3–13.
19. International Haemovigilance Network http://www.ihn-org.net.
20. Haemovigilance Programme of India. National Institute of Biologicals.www.nib.gov.in.
21. Marwaha N, Singh S, Bisht A. Setting up haemovigilance from the very first step. The Indian perspective. ISBT Science series 2014; 9:178-83.
22. Bisht A, Singh S, Marwaha N. National Blood Donor Vigilance Programme of India. Asian J TransfSci 2016; 10:1-2.
23. World Health Organization WHO-EU Advancing, Reporting and Learning systems 2012, http://www.who.int/patient safety/implementation/reportingandlearning/en

24. World Health Organization. Aide-Memoire, National Haemovigilance Systems, 2015. www.who.int/blood safety/haemovigilance.
25. Bisht A, Marwaha N, Kaur R, Gupta D, Singh S. Haemovigilance Programme of India: Analysis of transfusion reactions reported from January 2013 to April 2016 and key recommendations for blood safety. Asian J Transfus Sci 2018; 12:1–7.

Section 11

Hospital Transfusion Policy, Audit and Patient Blood Management

1. Introduction

For the best patient outcome, blood transfusion services must ensure safe and adequate vein-to-vein transfusions. The transfusion safety chain is complex, and the outcome of transfusion depends on multiple denominators, i.e., multiple processes and people etc. For example, the personnel involved in completing the transfusion process have different qualifications, different levels of experience, and different background practices due to their previous experiences and may be following different standards. This could lead to an unstructured methodology with unreliable outcomes and compromise transfusion safety. To achieve transfusion safety and appropriate use of blood, it is important that all stakeholders, i.e., blood centre personnel and multi-disciplinary clinical staff making a decision about transfusion and executing transfusion across the hospital follow standardized procedures commensurate with the updated standard and national guidelines.

Therefore, it is recommended that every hospital should have a hospital transfusion policy (HTP) detailing the standard procedures and guidelines for different processes to be followed in different situations. To ensure that HTP is followed in the true spirit, every hospital should have a Hospital Transfusion Committee (HTC), which is a bigger force and creates a sense of ownership of responsibility towards transfusion safety. On the one hand, it helps improve communication with key players involved in clinical transfusion to understand, and it becomes their goal too; on the other hand, administrators are also required to be on board, leading to greater administrative accountability and allocation of much-needed resources.

2. Hospital transfusion committee

Hospital transfusion committees play an important central role as strategic, operational, and reporting channels for transfusion safety, i.e. dissemination of guidelines, monitoring, and implementation of new programs for improving transfusion outcomes in hospitals.[1] Lack of knowledge regarding transfusion medicine among clinicians is possibly the major obstacle in making bedside transfusion safety more consistent.[2] The Serious Hazards of Transfusion (SHOT) initiative has also consistently shown, since its inception in 1996, that bedside errors are the main causes of transfusion-related morbidity and mortality.[2] A functional HTC, fulfilling its mandate, can be a powerful mechanism to ensure patients receive the safest and most appropriate blood transfusion therapy possible. The success of the HTC in improving blood management, reducing inappropriate use of blood, and reduction in red cell transfusions is well known.[3,4,5]

2.1 HTC requirements worldwide and in India:

HTCs have been created in different countries to oversee transfusion activity within their scope, and though the approaches used to achieve their goals have varied historically and between nations, their principles are the same. It is also one of the regulatory and accreditation requirements in the United States of America (USA). The USA Code of Federal Regulations (CFR) requires hospitals to implement a quality assessment and improvement plan for overseeing the practice. The American Association of Blood Bank (AABB), College of American Pathologists, and the Joint Commission also mandate quality oversight

of this process at the hospital level.[6] In Europe, The "Guide to Preparation, Use and Quality Assurance of Blood Components" produced by the Council of Europe encourages the establishment of HTC and suggests that it should include representatives of the hospital transfusion service, the blood establishment, and the main clinical units with significant transfusion activity.[7] World Health Organization (WHO) also recommends "a transfusion committee should be established in each hospital to implement the national policy and guidelines and monitor the use of blood and blood products at the local level'.[8] National Blood Policy of India, which came out in 2002, has one of its mandates," To encourage appropriate clinical use of blood and blood products", and suggests that every institution/ hospital having a blood centre/blood transfusion service should have an active HTC with a defined role.[9]

1.1. Constitution:

HTC should include key stakeholders both from multidisciplinary clinical specialities and management who are directly or indirectly involved with blood usage and safety. International Society of Blood Transfusion (ISBT)[10] and World Health Organization (WHO)[11] have suggested typical membership, adding that additional roles may be required in different countries.

WHO[11] recommended that the constitution of HTC can be with minimum members as follows:

- A senior representative of clinical specialities of blood and blood components users like anaesthesiologists, surgeons, physicians, paediatricians, haematologists, oncologists, gynaecologists etc.
- Hospital administrators or medical superintendents
- Representative/in-charge of blood centre or blood transfusion services
- Clinical nursing representative
- The hospital staff member is responsible for the supply of intravenous replacement fluids, pharmaceuticals, medical devices etc.

Chairperson:

The chairperson of HTC ideally should be a blood user department or hospital administrator. The chairperson cannot be permanent; they may rotate from two to four years. The role of a chairperson is to ensure that terms of reference (TOR) or agendas are developed, meetings are regularly planned, agendas are discussed and approved by all members, encourage all members to participate actively, and follow-up on the decisions taken.

Secretary:

The secretary of HTC should be in-charge/representative of a blood centre or blood transfusion services. The role of the secretary is to assist the chairperson to prepare the agenda before the meeting, scheduling the meeting, present the agenda to members in the meeting, record minutes of the meeting and distribute it to all members, and ensure that action items and decisions are properly documented, distributed and implemented.

The HTCs should work in tandem with patient safety, clinical governance, and senior hospital management to ensure any risks identified are accounted for; and to ensure blood transfusion remains on the hospital agenda.

1.2. Roles and responsibilities:

A work plan for the activity should be made to ensure the committee's work is focused on agreed TORs.

The roles and responsibilities of HTC include:

- Governance of administrative issues related to transfusion, i.e., to be the link with regulatory authorities and agree on any submissions and inspection outcomes.
- Defining blood transfusion policies for the hospital commensurate with national guidelines and developing systems for their implementation.
- Sharing transfusion-related information, including changes to national guidance, audit results, and examples of good practice with users.
- To monitor the implementation of national guidelines in the hospital and to take appropriate action to

overcome any hindrance in their effective implementation.

- To ensure appropriate blood utilization and best practice standards.
- To review and minimize blood component loss due to time expiry and wastage.
- To develop a mechanism for safe collection and disposal of used empty blood/component bags and transfusion sets from clinical areas.
- To implement patient blood management (PBM) initiatives, i.e., reviewing transfusion alternatives and making recommendations for their use.
- To reduce the number of inappropriate dose transfusion incidents.
- To introduce hospital-wide transfusion safety initiatives, i.e., use of wrist bands with barcodes etc.
- To empanel blood centres for supplying blood/ components in hospitals without their own blood centres and in times of shortages in hospitals with their own blood centres.
- To maintain adequate communication with blood/component providers to ensure the availability of required blood and blood components.
- Training and proficiency evaluation for all healthcare personnel of the hospital involved in the blood transfusion process.
- To monitor, report, and investigate adverse events and near misses related to transfusion and use these examples to improve learning.
- To ensure a cycle of clinical audits to assess transfusion practice, safety, and compliance with national requirements.
- To assess if recall and other quality manual processes are working as they are intended to be.
- To review quality indicators and transfusion service performance metrics, ensuring that all-important indicators of relevance are included to improve the systems.
- To review the TORs of HTC and membership at set intervals.

1.3. Frequency of meetings:

The reported frequency of HTC meetings varies from 6 weekly to once a year in different countries.[12] The organization must choose the effective frequency that works well for them.

1.4. Challenges for the HTC:

It is important to realize that there could be many challenges to making the HTCs function optimally so that they can be taken in stride and efforts made to remove them.

Most HTCs struggle to ensure adequate and consistent attendance. The factors responsible could be voluntary attendance/ participation requirements, meeting timings, clinical commitments, other priority engagements, etc. As poor attendance impacts collective decisions, every effort should be made to ensure enthusiastic participation. The chairperson of HTC should encourage members to attend the meeting.

Another significant challenge of HTC is the implementation of decisions or recommendations in routine practices. This is because of insufficient knowledge of clinicians about the importance of blood transfusion practices. The continuous medical education of clinicians about blood transfusion practices can overcome this.

The Transfusion Medicine expert has to spearhead to overcome the challenge of unawareness among committee members and chairperson by providing resources and tailored educational support, key performance indicators, completion and sharing of results of audits with the chair and members who are not transfusion medicine subject experts. Linking with other regional/associated HTCs or other hospitals to share practice also helps.

2. Hospital transfusion policy

Policies communicate the highest-level goals, objectives, and intent of the organization. All hospitals need to have HTP meeting the standards required by national blood policy, regulations, and guidelines for transfusion

practice. The aim is to ensure compliance for rationale, effective and safe use of blood and components. The scope of HTP extends from ensuring a safe and adequate blood/component supply to every step of the clinical transfusion process.

The policy is formulated by HTC and endorsed by the hospital executive or board. For its successful implementation, management commitment and support are imperative. To ensure a hospital quality system for transfusion, all involved staff must understand the importance of quality and the consequences of failure in the quality system for patients. A hospital transfusion policy coming through HTC becomes binding for all individuals involved in a safe transfusion chain to follow.

Format for writing hospital transfusion policy:

HTP should include the following parts:

1. Policy introduction i.e., policy number, version and version history, date of review, date of next review, valid from date, valid till date, approval date, reference to related documents, distribution, authors, etc.
2. Scope: Area and stakeholders to whom it applies.
3. Purpose: Should elaborate on why this policy is needed and what it strives to achieve.
4. Goals and aims.
5. Core accountabilities/responsibilities are forming review, ratification, dissemination, and compliance.
6. The policy statement, i.e., policy about:
    a. Operational systems based on written instructions/standard operating procedures (SOPs)
    b. Standard procedures for all stages of clinical transfusion include:
        i. The basis for transfusion needs to be evidence-based and justified.
        ii. Responsibility for transfusion rests with the prescriber.
        iii. Informed consent for transfusion and protocol for refusals.
        iv. Ordering for blood in routine and emergencies. Mode i.e., electronic/ hard copies.
        v. Patient identification for specimen collection and blood product administration including deviations or special requirements in special circumstances.
        vi. Policy for using validated means for delivering blood samples to and collecting blood components from blood centres i.e., pneumatic tube systems or porters.
        vii. Selection of components and compatibility procedures. Receive back of blood components into inventory.
        viii. Maintenance of cold chains at all levels of storage and transportation.
        ix. Guidelines for administration and monitoring of transfusion in adults / neonates /paediatric patients for different indications.
        x. Recording of all transfusions in patient records.
        xi. Massive transfusion protocol.
        xii. Introduction of new blood components/products.
        xiii. Blood shortage management.
        xiv. Availability and use of maximum surgical blood order schedule (MSBOS) and PBM.
    c. Availability of standard approved transfusion related documents across the hospital i.e., blood component request form, compatibility form, transfusion reaction reporting form, and transfusion consent form etc.
    d. Reporting incidents.
        i. Transfusion adverse event identification, intervention, reporting, and monitoring
        ii. Non-conformance/error reporting, complaints, corrective and preventive measures.
    e. Risk, audit, and assessment: Management of the performance of audits, lookback/trace back for reported transfusion-transmitted infections.

f. Training: Staff training and ongoing competency evaluation.
g. Source of blood supply for the hospital.
h. Supplies and management
i. Monitoring compliance.

3. Audits in transfusion medicine

In general, audits are the means of monitoring to determine whether actual activities comply with planned activities, are implemented effectively, and achieve desired objectives. Audits in transfusion medicine should cover all the processes and procedures found in the blood centre and transfusion therapy. The purpose of conducting audits is to improve patient care and outcomes through the systematic review, monitor adherence to the transfusion guidelines and policy, and identify the critical areas for improvement and create a culture of delivering quality service to patients whereby transfusion care will be improved on a continuous basis.

3.1. Types of audits:

According to the temporal relationship**,** transfusion audit data may be gathered retrospectively, prospectively, or concurrently.

3.1.1. Retrospective audit:

In this type of audit, information regarding transfusions given to patients is gathered and reviewed sometime after the patient's transfusion episode and subsequent discharge. Therefore, the review is retrospective by necessity, and the information is used in an attempt to alter clinicians' behaviour in the future.[13]

Retrospective audits do have limitations, mostly owing to the fact that appropriate recording of all the necessary information is very often lacking, and obviously, it does not have any impact on the treatment of the patients that were included in the audit.

The advantage of this type of audit is that it does not need many resources to be implemented and maintained. It can also be managed on a manual system. Therefore, this method is often used in areas with limited resources. This provides some information on what current practices consist of and will guide clinicians' education. It also serves as a tool to establish whether current transfusion practices are acceptable in terms of the agreed guidelines and whether the guidelines are of an acceptable standard.

3.1.2. Prospective audit:

This involves reviewing and validating the decision to transfuse at the time when it is made against the agreed clinical guidelines. It, therefore, implies the review of orders for blood before the transfusion episode and thus stands a very high success rate for improving transfusion practice. This approach, however, needs considerably more resources than a retrospective audit approach. This can be done manually by reviewing each requisition by a transfusion medicine physician who must also have access to laboratory results continuously or by integrated hospital information system and algorithms to assist and critique blood requisitioning according to transfusion guidelines and laboratory parameters of the patient. This approach has been shown to give more accurate information and improvement in transfusion practice in the form of decreasing the inappropriate use.[14]

3.1.3. Concurrent audit:

It involves gathering information on a transfusion episode and giving feedback within the time scale of a patient's stay in the hospital. The advantage of this approach is that the data are likely to be accurate, and the outcome can be provided to the clinicians making the decisions regarding the transfusion episode while the specific incident can still be recalled. The disadvantage is the same as retrospective audits that this type of audit will not influence the transfusion decision for the current patient.

Other classification

- Depending on the agency conducting the audit: external or third-party audit / internal audit.
- Single institution/ multi-institutional audits: multi-institutional audits can be very useful benchmarking tools.
- Depending on the monitoring focus, audits can monitor
  a. Process outputs, i.e., quality indicator data
  b. A single process (targeted audit)
  c. Set of inter-related processes (system audits)

3.2. Areas for Audit:

Audits may be comprehensive audits or targeted audits.

- Audits in the blood donation area
- Audits in component preparation laboratory
- Audit of bedside activities related to transfusion, i.e., blood sample collection and labelling, completeness of blood requisition, the turnaround time for processing requisition, time to start transfusion, pre -medications, transfusion time, any other activities which may affect transfusion safety, i.e., warming or dilution with any fluid, etc.
- Audit to blood transfusion.
  - The hospital's or department's transfusion rate (i.e., the number of units transfused per hospital bed per year).
  - The percentage transfused per user or user department for each indication.
  - Crossmatch/transfusion ratio per user or user department for the type of surgery and/or indication.
  - Transfusion index or transfusion proportion.
- The transfusion failure rate, i.e., the percentage of transfusions that did not achieve the expected outcome or result.
- The incidence of non-compliance with transfusion guidelines. Transfusion triggers are measures that can be used for initial audits. Transfusion triggers that might be useful are the following:
  - For red cells:
    - Haemoglobin or haematocrit levels.
    - Clinical parameters such as pulse rate and blood pressure (baseline and post-transfusion).
  - For platelets:
    - Platelet count.
    - Presence or absence of clinical bleeding.
  - For fresh frozen plasma:
    - Results of coagulation tests (prothrombin time or international normalized ratio and activated partial thromboplastin time).
  - For all blood components: The reason/s for the transfusion and operation (if applicable).

3.3. How to conduct an audit:

Auditing transfusion practices is a cyclical process. Within the cycle, some stages follow a systematic process of establishing best practices, taking into account local circumstances and limitations, measuring care against established criteria, taking action to improve the care given, and monitoring to sustain improvement. (Figure 1)

Depending on the criteria used, a rather extensive amount of information may be needed about each transfusion to determine whether it met the criteria specified for the transfused blood component. Furthermore, the number of transfusion episodes that are reviewed to determine whether the use of the blood component was

consistent with the guidelines will also influence the extent of the audit (sample size). In countries with limited resources, an audit must be planned carefully to keep it manageable and gain the maximum benefit from it.

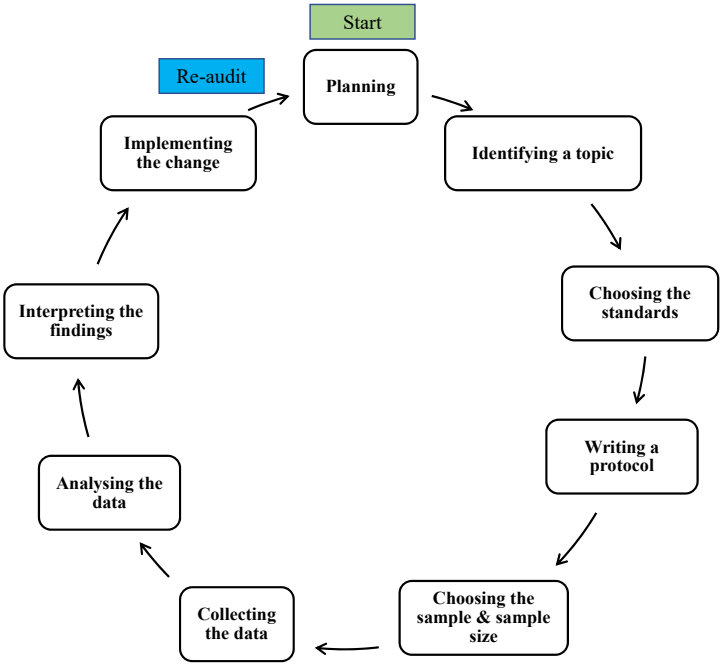


Figure 1: Audit Cycle

A very elementary approach can be used for the first audit cycle. As confidence is gained, the audit process can be extended to a higher level. Once results are obtained from the initial audits, they can also serve as tools to motivate increased resources to be allocated to the process. These measures do have shortcomings that should be recognized in that limited clinical information is included, but the measures will indicate how well the clinicians adhere to the guidelines in well-controlled circumstances. It also serves as a simple way to start off with auditing in settings with limited resources and start to create a culture of improving the level of care continuously. An additional advantage is that it does not put a huge demand on existing resources within a resource-poor setting.

When starting an audit system within a hospital, it might be easier to begin with one of the hospital departments. It is, however, beneficial to add more departments as this will allow for comparisons. It is also beneficial to compare audit results with published results from the literature. Keep in mind, though, that most of these types of publications originate from developed countries. Hence, there are differences in the patient population, type of surgery done, diseases treated, facilities and expertise available, etc. This may make comparisons difficult.

3.4. Follow-up of an audit:

Feedback on the audit should take the format of an audit report, and this should be presented and discussed in the hospital transfusion committee to implement recommendations to overcome lacunae as reflected in the audit. This may be in the form of physician and paramedical staff education and training, usage of computer software for intelligent blood requisitioning, recommendations for electronic patient identification systems, or specialist transfusion nurses.

In summary, to improve hospital transfusion services for best patient outcomes, a robust hospital transfusion policy, well thought PBM program, and diligent audits to cover all the processes in transfusion are indispensable, and the hospital transfusion committee is an essential instrument for having a vision and their successful implementation.

4. Patient blood management

The Society for Advancement of Blood Management (SABM) has defined patient blood management (PBM) as

"the timely application of evidence-based medical and surgical concepts designed to maintain haemoglobin concentration, optimize haemostasis and minimize blood loss to improve patient outcome" .[15]

The concept of PBM goes beyond the quality assurance and optimal utilization of blood components for transfusion in a patient to minimizing/mitigating the need for blood components. It requires a multipronged 'Patient Centric' approach for any patient who is being considered for blood component transfusion. PBM applies to a full spectrum of medical care, be it outpatient/inpatient settings; long before transfusion or even hospital admission is contemplated, as a part of discharge planning, and from very young to very old patient populations.

4.1. Principles of the PBM program:

It is reported that 94% of transfusions in surgical patients can be attributed to low preoperative haemoglobin levels, excessive surgical blood loss, and inappropriate transfusion practices**,** all of which are modifiable risks**.**[16] Additional evidence has largely come from literature on treating anaemia in patients who refused transfusions, and the four core principles of any successful PBM program have been identified as follows:[17,18,19]

4.1.1. Anaemia management:

This principle focuses on identifying the treatable causes of anaemia, optimizing the red cell mass, and determining patient-specific physiological anaemia reserve to mitigate transfusion requirements, thereby reserving transfusion of blood products essentially for situations when it is clinically indicated. Every effort should be made to generate awareness of the prevalence of pre-surgical anaemia. A system should be developed to perform an audit of preadmission haemoglobin levels, advocate for early pre-surgical laboratory testing, and collaboration with clinical specialists to develop guidelines and standardized protocols for anaemia evaluation and management of pre-surgical patients with regard to the mitigation of anaemia.

4.1.2. Multidisciplinary and multimodal blood conservation strategies:

It aims to reduce the need for a blood transfusion by minimizing blood loss by performing minimally invasive procedures, minimizing iatrogenic blood loss (e.g., repeated laboratory draws), using surgical techniques that limit blood loss, detecting and halting blood loss as quickly as possible, and employing transfusion techniques that minimize allogeneic transfusion (e.g., autologous transfusion, intraoperative red cell salvage, normovolemic haemodilution). Iatrogenic blood loss due to the drawing of blood samples is one of the major causes of hospital-acquired anaemia in patients. Therefore, every effort should be made to develop strategies to minimize it. Some of the suggested means are identifying the key stakeholders, limiting the ordering of tests, launching educational efforts directed at changing physician practice, redesigning the computer-displayed test order screens, using newer technology for small volume blood sample requirements, and limiting the volume of the samples collected.

4.1.3. Optimization of haemostasis and/or coagulopathy:

It requires appropriately evaluating haemostasis for the presence of coagulopathy, determining its aetiology and treating it with targeted therapies, thereby reserving transfusion of blood product components for situations when it is clinically indicated and unavoidable.

4.1.4. Patient-centred care:

This signifies incorporating the patient's needs and concerns in the decision-making process, informing the patient on PBM-based treatments, alerting the patient to potential risks/benefits/alternatives of different treatment options, and working with the patient to come up with the final treatment plan.

In elective surgery patients, the PBM principles can be applied in three integrated phases, i.e., preoperatively, intraoperatively, and postoperatively, whereas in the emergency or trauma setting, when there is little time for patient optimization, aggressive application of the intra- and postoperative principles are used. Therefore, PBM strategies are most effective when used in combination and tailored to the individual patient's specific

needs. Furthermore, the application of PBM to other areas of medicine needs to be explored to expand evidence-based guidelines.

Over the years, PBM programs have demonstrated improvements in transfusion and patient outcome-based metrics across specialities. In 2010, World Health Assembly endorsed the implementation of PBM, and a concept paper was published by WHO in 2011 that supported and encouraged the implementation of PBM programs.[20, 21]

4.2. Implementation of PBM:

Successful implementation of the PBM program requires

- A commitment of time and staff
- The development of new standard operating procedures
- Appropriate motivation and training of staff to adopt new guidelines
- Appropriate systems to monitor outcomes based on changes implemented as part of the PBM.

The Institute of Healthcare Improvement first described the term bundle as "a straightforward set of evidence-based interventions for a defined patient population that, when implemented together, will result in significantly better, more penetrating and sustainable outcomes than when implemented individually." Meybohm et al. proposed using six PBM bundles to help the implementation of PBM (Figure 2).In this model bundles, the second, third, fourth, and fifth bundles comprise four PBM strategies. In contrast, the first bundle deals with PBM project management and the sixth bundle deals with the evaluation of PBM related metrics, i.e. patient outcomes and benchmarks.[22] Bundles proposed by Meybohm et al. are the following:[22]

4.2.1. Bundle 1: PBM project management:

The first bundle is the preparatory phase for the successful launch and execution of the PBM program. Engaging HTC is one of the primary steps for obtaining a mandate for PBM from hospital management. It also involves recruiting the key stakeholders in the PBM program, as is required for the successful management of any other project. The key players include the PBM coordinator, who plays a central role in developing and implementing the program. Other stakeholders include physicians, nurses, the transfusion committee, quality personnel, and administrative officers such as the chief medical officer and chief executive officer. Additionally, the involvement of the laboratory and information technology department is essential to successful PBM implementation. Looking at the constitution, it can very well be a special interest group in the HTC. This bundle also involves educating the key stakeholders and the remaining hospital staff on the program and developing standard operating procedures and protocols. Specific educational objectives are 1) provide background on the current evidence for transfusion benefits and risks; 2) present the institutional best practice guidelines for transfusion; 3) provide comparisons between the institution's historical use of blood products with utilization under the newly adopted transfusion guidelines, and 4) strategize on how best to adhere to institutional guidelines. Assessment of case mix and clinical services should be done to determine the main targets for PBM.

4.2.2. Bundle second to five:

Already elaborated above in points 4.1.1 to 4.1.4.

4.2.3. Bundle 6: PBM-related metrics, patient's outcome, benchmark:

It requires the establishment of auditing criteria and mechanisms to evaluate the change and monitor the effectiveness of the implementation of the PBM program. A PBM scorecard system can be adopted to monitor adherence to guidelines for blood avoidance and the use of blood, including the use of benchmarking to identify clinicians/clinical teams who are consistently well outside of average blood use for a specific procedure.

Institutions can take advantage of already available systems and mould them to the needs of their patients and institution.

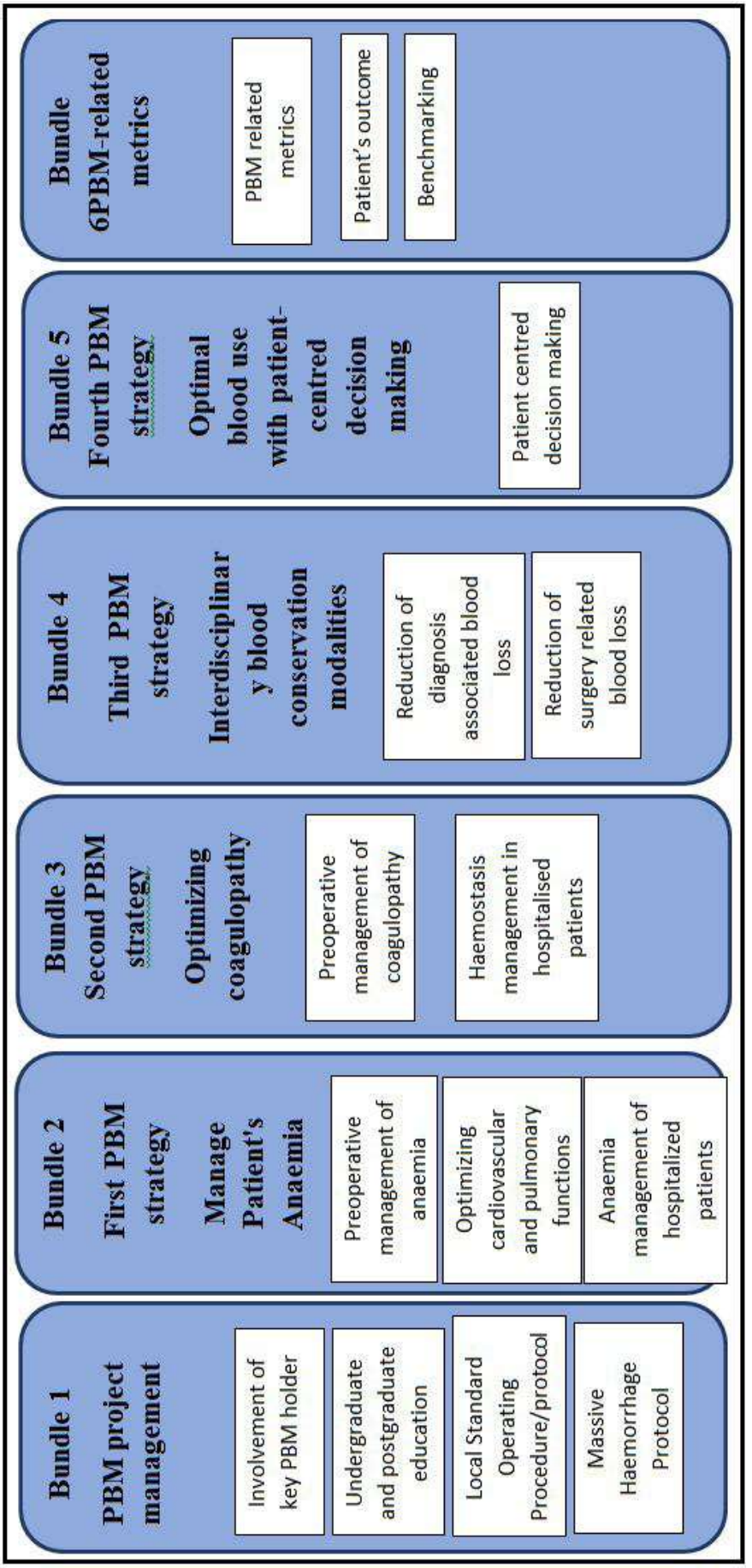


Figure 2: Six bundles of patient blood management

Key points

- To achieve transfusion safety and appropriate use of blood in a hospital, it is pertinent that all stakeholders in the transfusion chain follow standardized procedures consistent with national guidelines and standards.
- A functional HTC is a powerful mechanism to ensure rational, effective, and safe transfusion use and outcome for the patients.
- HTP should be revised as and when required according to the latest guidelines and should be made available to key stakeholders for implementation across the hospital.
- The four core principles of PBM, i.e., anaemia management, optimization of haemostasis, multidisciplinary blood conservation strategies, and a patient-centric approach, should be applied in every patient with potential blood component transfusion requirements to improve patient outcomes.
- For successful implementation of the PBM program, it is essential to build a solid foundation, i.e., having a PBM project management plan that includes identifying the champion coordinators, key clinical areas or patient groups, and continual education.
- Audits in transfusion medicine are effective tools to determine if actual activities comply with the planned activities, are implemented effectively, and achieve desired objectives. They also bring to notice the critical areas which need improvement.

References

1. Haynes SL, Torella F.The role of hospital transfusion committees in blood product conservation. Transfus Med Rev 2004; 18: 93-104.
2. Friedman MT. Blood transfusion practices: A little consistency please. Blood Transfus 2011; 9:362-5.
3. Serious Hazards of Transfusion, Recommendations for Hospital Transfusion Committees, SHOT 2015.
4. Sanquin Blood Supply, National Users' Board Sanquin Blood Supply, Blood Transfusion Guideline Sanquin 2011
5. Kaur G, Kaur P. Hospital transfusion committee: Role and responsibilities. Indian J PatholMicrobiol2014; 57:352-4
6. Sullivan HC, Roback JD. The pillars of patient blood management: key to successful implementation. Transfusion. 2019; 59:2763-67.
7. European Directorate for the Quality of Medicines & Healthcare. Guide to the preparation, use and quality assurance of blood components. 18th ed. Strasbourg: Council of Europe; 2015.
8. Developing a national policy and guidelines on clinical use of blood. WHO 2001https://www.who.int/bloodsafety/clinical_use/en/who_bct_bts_01_3.pdf?ua=1,assesed on 10th July 2019.
9. National Blood Policy, NACO, MoH & FW, India 2007.
10. www.isbtweb.org/working-parties/clinical-transfusion/11-the-role-of-transfusion-committees/, assessed on 10th July 2019.
11. Developing a national policy and guidelines on the clinical use of blood, World Health Organization (publication 2001, accessed date; October 10th, 2020, https://apps.who.int/iris/bitstream/handle/10665/330085/WHO-BCT-BTS-01.3-eng.pdf
12. PBM: a central focus for patient safety and quality care. http://www.sabm.org/ [accessed July 7, 2019
13. SABM. Administrative and clinical standards for patient blood management programs. 4th ed. Society for the Advancement of Blood Management; 2018. Available from: http://www.sabm. org/wp-content/uploads/2018/11/SABM-Standards-2018.pdf [accessed July 1, 2019
14. Shander A, Javidroozi M, Lobel G. Patient blood management in the intensive care unit. Transfus Med Rev 2017; 31:264-71.
15. Althoff FC, Neb H, Herrmann E, et al. Multimodal patient blood management program based on a three-pillar strategy: A systematic review and meta-analysis. Ann Surg 2019;269: 794-804.

16. Leahy MF, Hofmann A, Towler S, et al. Improved outcomes, and reduced costs associated with a health-system-wide patient blood management program: a retrospective observational study in four major adult tertiary-care hospitals. Transfusion 2017; 57:1347-58.
17. WHA63.12—availability, safety, and quality of blood products. WHA resolution; sixty-third world health assembly. World Health Organization; 2010.
18. Global forum for blood safety: patient blood management— concept paper. World Health Organization; 2011
19. Resar R, Pronovost P, Haraden C, et al. Using a bundle approach to improve ventilator care processes and reduce ventilator associated pneumonia. Jt Comm J Qual Patient Saf 2005; 31:243-8
20. Meybohm P, Richards T, Isbister J, et al. Patient blood management bundles to facilitate implementation. Transfus Med Rev 2017; 31:62-71.

Section 12

Autologous Blood Transfusion

1. Introduction

Blood collected from a patient for re-transfusion later into the same individual is called “autologous blood”. Minimum or no allogeneic transfusion certainly leads to fewer adverse reactions, fewer donor exposure, and reduced treatment cost. Autologous blood is possibly the safest blood because the patient receives his or her own blood and is not exposed to any foreign antigens or any infectious diseases other than those that the patient may already have. The use of autologous blood for transfusion is one of the strategies of patient blood management to decrease the use of allogeneic blood in the perioperative period.

2. Advantages and disadvantages of autologous transfusions

2.1. Advantages:

- Prevention of transmission of transfusion transmissible infections as well as emerging pathogens through transfusion.
- Prevention of alloimmunization to red cells, leucocytes, platelets, and plasma proteins.
- Prevention of adverse transfusion reactions especially allergic and febrile reactions.
- Blood management in patients with rare blood groups or with antibodies against high-frequency antigens.
- Some types of autologous transfusions are acceptable to patients who refuse allogeneic blood transfusions due to certain religious beliefs (Jehovah’s Witness) and can thus help in their management.
- It is an essential part of the patient blood management program.

2.2. Disadvantages

- Preoperative autologous blood donation subjects the patient to anaemia and hypovolemia and increases the likelihood of transfusion.
- The risk of administrative errors leading to ABO incompatibility is not eliminated.
- The risk of bacterial contamination or volume overload is not eliminated
- Additional development of policies, processes, and procedures related to its labelling, storage, and reinfusion is required.
- Results in wastage of blood if the procedure is postponed or transfusion is not needed.
- It costs more than allogeneic blood donation.

3. Types of autologous blood donation

- Preoperative autologous blood donation (PABD)
- Perioperative autologous blood donation
  - Acute normovolemic haemodilution (ANH)
  - Intraoperative blood salvage

- Postoperative blood recovery.

3.1 Pre-operative autologous blood donation (PABD)

Preoperative autologous blood donations are most practical for patients likely to require transfusion during elective surgery scheduled to take place within 35 - 42 days (the shelf life of blood stored in a liquid state). As preoperative anaemia is common in surgical patients, correction, and management of anaemia is an important step in preoperative blood collection.

Each patient must be carefully evaluated by his or her physician and the transfusion medicine expert for preoperative autologous donation. Written advice is required from the patient's physician for autologous donation. A consent form needs to be signed by the patient and his relatives, acknowledging that they have been well informed and understand the risks and advantages of autologous donation. Figure 1 shows the sequential steps of a PABD program.

Indications for preoperative autologous blood donation (PABD):

Preoperative autologous donation is utilized in elective surgical procedures with:

- Reasonable probability for transfusion
- Likelihood of blood loss over 500 to 1000 mL
- Sufficient time to obtain one or more units of blood with minimum risk and without creating significant haemoglobin deficit in patient-donor

Some of the indications of preoperative autologous blood deposit may include:

- Orthopaedic surgery (joint replacement)
- Plastic and reconstructive surgery
- Cardiovascular surgery
- Major abdominal surgery (elective splenectomy)
- Individuals with rare blood group/ with multiple alloantibodies/antibodies to high-frequency antigens

Contraindications for preoperative autologous blood donation*:*

- Bacteraemia and/or acute localized infection
- Myocardial infarction in the past 6 months
- Unstable angina / angina at rest
- Aortic stenosis
- Congestive heart failure
- Significant ventricular arrhythmia
- Marked uncontrolled hypertension
- Cerebrovascular accident within 6 months
- Transient ischemic attack
- It may also be prudent to discourage PABD in patients over the age of 80 years. These patients are at minimal risk from conventional transfusion of allogeneic blood.

Eligibility criteria of preoperative autologous blood donation:

Patient-donor, for autologous donation, does not always require meeting all the criteria of homologous blood donation.

Major points to consider eligibility are as below:

- **Haemoglobin**: Should be at least 11 gm/dl or 33% (0.33) haematocrit or higher. Below this level, phlebotomy should not be done. Under special circumstances, with the approval of the patient's physician, it may be done with haemoglobin (Hb) between 10-11g/dl but never if Hb is less than 10g/dl

- **Age**: There is no upper or lower limit of age. Age less than 10 years and above 80 years should not be considered for PABD.
- **Weight and volume of blood withdrawn**: Similar to the allogeneic blood collection, 450ml and 350 ml of autologous blood should be collected from donors weighing ≥55kg and 45-55kg, respectively. For donors weighing less than 45 kg, a proportionately smaller volume in the range of 7-8ml/kg body weight could be collected. PABD is not recommended for children younger than 10 years, mainly because of technical difficulties (large-bore needle in veins of limited size), and it can be difficult to gain sufficient co-operation.[1] Therefore, a full collection of 10.5 mL of blood per kilogram (kg) of the donor's weight for each donation is permitted.[2] If a lesser volume of blood is collected, the anticoagulant volume should be adjusted according to the volume collected.

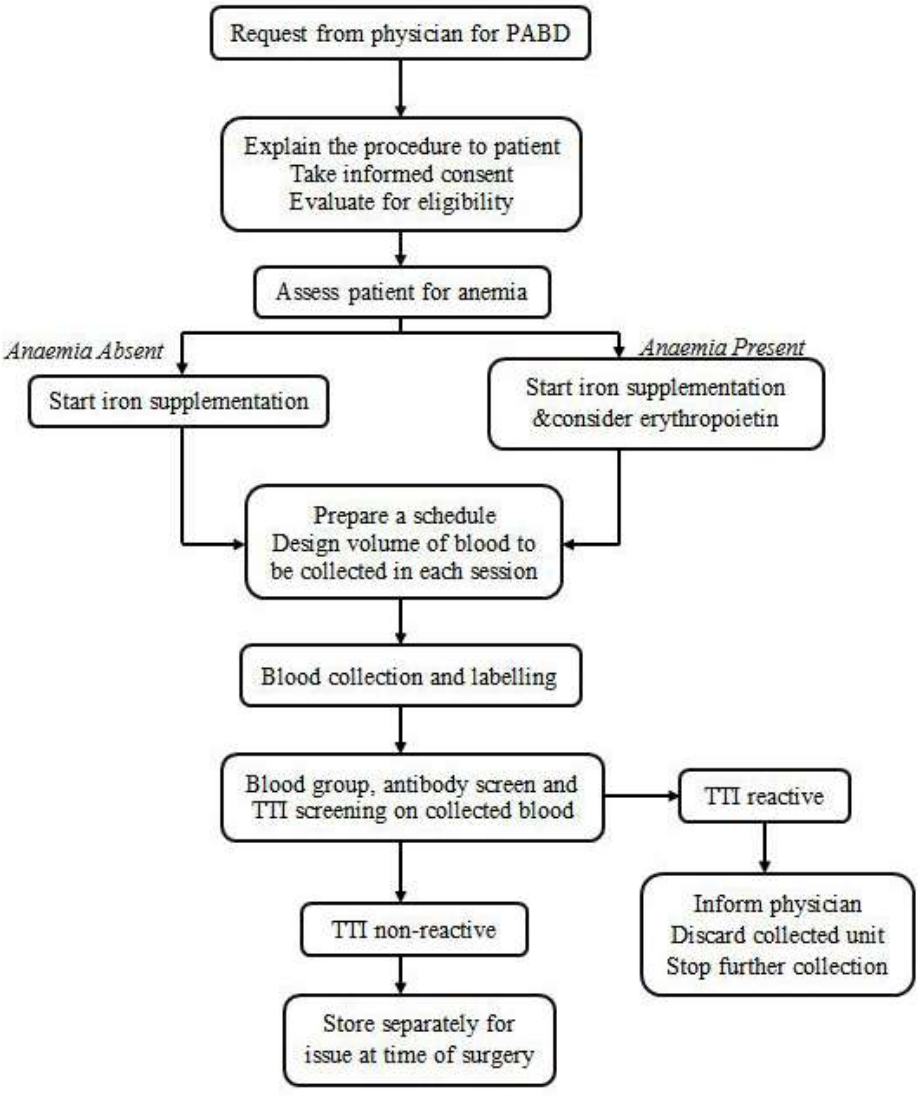


Figure 1: Sequential steps in a PABD program *TTI: Transfusion Transmitted Infections

- **Frequency of donation:** Donations can be taken weekly or even at 4 days intervals, with the last phlebotomy performed 72 hours or more before the operation. This helps the patient's plasma volume to return to normal before surgery.
- **Iron supplementation and use of erythropoietin:** Oral iron (300 mg of ferrous sulphate three times a day) is prescribed to enhance the restoration of haemoglobin to pre-donation levels as soon as the surgery is scheduled. Erythropoietin is primarily indicated in those patients in a PABD program who are anaemic

before the first donation or who have small blood volumes and would otherwise be unable to meet their anticipated needs. The dose has ranged from 12,000 to 40,000 units per week (given subcutaneously), and iron replacement is given concurrently.

- **Labelling:** Each autologous blood unit collected should be assigned a unique identification number, and a record of the same should be maintained. This will help the unit to be tracked to its final disposition. A label stating "For Autologous Use Only" must be placed on the bag. Machine-readable labels may be produced in black and white;otherwise, blood labels for autologous use have green colour traditionally (Figure 12.2).[3, 4] Autologous blood units should be stored on a separate shelf of the blood bank refrigerator.
- **Use of autologous donation for homologous use:** The policy of allowing autologous blood to cross over for allogeneic use is controversial, and as the patient does not fulfil allogeneic blood donation criteria in most of the autologous donations; its use for other patients is not permissible.
- **Laboratory testing:** Minimum pre-transfusion laboratory testing is required for the autologous unit, such as ABO grouping, Rh typing, and transfusion-transmitted infection (TTI) disease markers. The reason for testing TTI markers of diseases is to protect the hospital staff rather than the recipient himself or herself. However, some literature suggests that only the first unit of autologous blood unit collected should be tested for the markers of the transfusion-transmitted diseases. If any infection disease test is reactive, it should be labelled as biohazard and discarded as per the biomedical waste management (BMW) guidelines, the same should be informed to the patient's physician for counselling and further treatment.

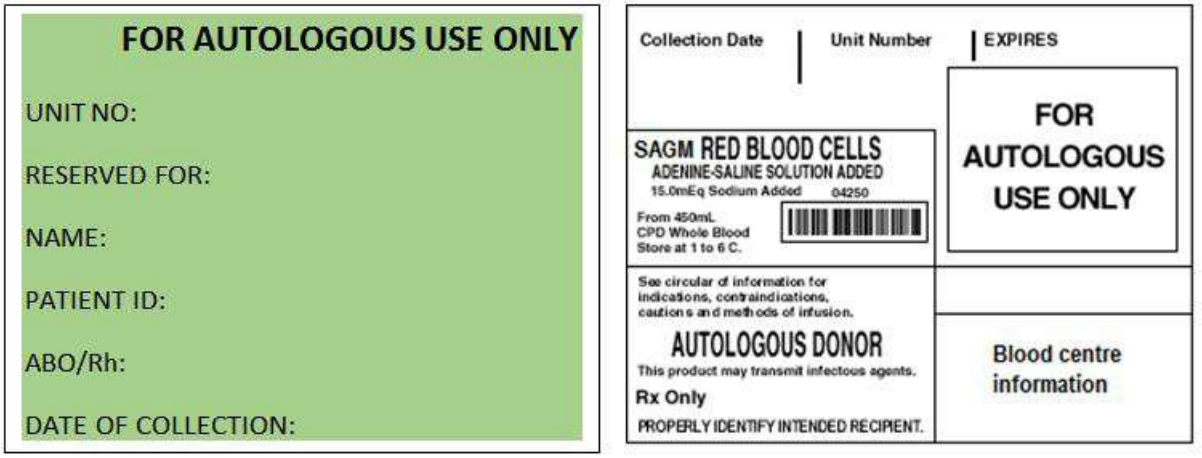


Figure 2: Example of the traditional and machine-readable label for autologous blood

With the advancement of better blood screening and improvement of blood supply, the scope of PABD is limited. It may be a reasonable approach in certain conditions such as patients with rare blood group, multiple alloantibodies/antibodies to high-frequency antigens and patients who refuse allogeneic blood. Proper planning and evaluation before the onset of the PABD program are essential to prevent anaemia induced by autologous blood donation.

3.2 Perioperative autologous blood donation

3.2.1 Acute normovolemic haemodilution:

Acute normovolemic (ANH) or isovolumic haemodilution is a blood conservation technique. It involves removing whole blood from the patient into a standard blood bag containing anticoagulant, either immediately before or shortly after the induction of anaesthesia in the operation theatre with the maintenance of normovolemia using crystalloid and colloid replacement. As a result, any blood loss from the patient during the surgery is diluted, resulting in reduced red cell loss.

Advantages:

- Surgical bleeding occurs at the lower haematocrit (Hct); therefore, the loss of red cells is less.
- The blood flow through microcirculation is improved because of the reduced haematocrit.
- The donated blood that can be used during or immediately after surgery contains viable platelets, adequate-protein levels and good levels of all plasma clotting factors.
- ANH is a cost-effective option since testing, storage and crossmatching cost are not incurred.

Disadvantages:

- ANH requires anaesthesia personnel trained in this area and the required intensive monitoring of aggressively haemodiluted patients.
- Chances of dilution of coagulation factors may increase blood loss.
- The efficacy of ANH regarding the reduction of bleeding is questionable because bleeding and allogeneic blood requirements have not been well established.

Contraindications to ANH:

- Impaired cardiac function
- Impaired renal function with oliguria
- Baseline haemoglobin<11 g/dl
- Abnormalities of coagulation and platelets
- Non-availability of point of care testing for complete blood count and coagulation factors
- Severe obstructive and/or restrictive pulmonary disease as near-normal oxygen transport is essential.
- Poor vascular access
- Bacteraemia
- Pregnancy with anaemia

Selection of patient:

ANH may be considered for patients with normal haemoglobin levels (12-16 g/dl) who are expected to undergo procedures or surgery with high blood loss [1-1.5L] and have a relatively high haematocrit to allow maximum blood units to be removed. ANH is a good option for Jehovah's Witnesses.

Blood volume that can be withdrawn:

$$\text{The volume of blood that can be removed} = \text{EBV} \times \frac{\text{Hi–Hd}}{\text{Average (Hi \& Hd)}}$$

EBV = Estimated Blood volume = Body Weight x 70ml (80ml for children)

Hi = Initial haematocrit

Hd = Desired haematocrit

Example: Weight=70kg, Initial Hct=45%, Desired Hct=30%,

BV= 70 x 70 = 4900ml

Therefore, volume to be removed = 4900 x ([0.45-0.30]) / 0.375 = 1960ml

The desired haematocrit should be decided in such a way that the blood volume to be withdrawn should never exceed 40 % of the patient's estimated blood volume.

Vascular access:

Adequate vascular access is essential for performing the procedure. The desired amount of blood is collected by following the aseptic venepuncture technique in a standard blood bag with anticoagulants (350ml/450 ml) if specialized blood bags are not available.

Volume replacement:

Either crystalloids or colloid solutions may be used as replacement fluids. As crystalloids are distributed across the extracellular compartment (intravascular + interstitial), the volume to be replaced usually exceeds the volume of blood withdrawn. On the contrary, colloids are restricted within the vascular space, so the volume replaced is the same as the blood volume withdrawn. The volume replaced is usually colloid 1 ml or crystalloid 3 ml for every 1 ml of blood collected.

Labelling and storage:

Each unit of blood should be labelled with the patient's name, registration number, withdrawal time, sequence number (if more than one unit), and blood group. Blood units collected by ANH may be stored in the operation theatre at room temperature (22°C) for up to 8 hours. If it is not transfused within 8 hours of collection, it can be stored at 2°C - 6°C for up to 24 hours labelled "FOR AUTOLOGOUS USE ONLY".

Reinfusion:

Patients of ANH should be monitored regularly during surgery, and the decision of reinfusion depends on the anaesthesiologist's and surgeon's assessment of blood loss. Usually, blood is reinfused when haemoglobin level falls in the range of 7-8 g/dl (Hct 21-24% approximately). The units of blood are reinfused to the patient in the reverse order of collection (i.e., the unit collected first is reinfused at last). This allows the most concentrated unit to be transfused at the time of least bleeding. Hence, the first unit, containing the highest haemoglobin, the maximum number of platelets and undiluted coagulation factors, is administered last. However, in certain situations, it may be wise to change the sequence to ensure that the first unit(s) is not wasted and is administered within eight hours of collection.

3.2.2 Intraoperative blood salvage

The process of collecting shed autologous blood and its processing and re-administration has been termed as cell salvage, auto transfusion, intraoperative blood recovery, and cell saving. Cell salvage can happen either in the intraoperative period or in the post-operative period. Salvage involves washing of the collected blood, or it may be simply re-administered with micro aggregate filtration.

Blood salvage in surgical procedures is recommended if there is the expectation of a significant blood loss (greater than 1 litre).

Indications:

- Cardiovascular surgery
- Vascular surgery
- Orthopaedic procedures (especially total hip replacement and spinal surgery)
- Liver transplant
- Ruptured ectopic pregnancy
- Trauma

Contraindications:

- Relative contraindications:
  - Infection - reinfusion of contaminated (even if washed) blood may lead to bacteraemia
  - Malignancy (malignant cells) - reinfusion of malignant cells may lead to metastatic spread
  - Haemoglobinopathies (thalassemia and sickle cell disease)
  - Cold agglutinin disease
- Absolute contraindications:
  - Contamination with antiseptics or disinfectants or any other solution may cause RBC haemolysis.
  - Admixture of haemostatic products or bone cement, heavy metals

- o Faecal contamination

Traditionally cell salvage is avoided in obstetrics (contaminated amniotic fluid), and malignant cell contamination.

The procedure of intraoperative cell salvage:

The intraoperative cell salvage machine (commonly known as a cell saver) separates, washes, and concentrates salvaged red blood cells. The procedure of intraoperative cell salvage is as below[5]

- Aspiration of blood from the surgical field: The surgeon starts aspirating the blood from the surgical field through a suction wand (-80 to -120 mmHg). The blood is mixed with an anticoagulant (heparin or citrate) to prevent coagulation.

  Heparin may cause heparin-induced thrombocytopenia (HIT), so a citrate-based anticoagulant is preferred in some patients. In addition, citrate is metabolized in the liver, so in patients with impaired liver function, calcium level has to be monitored carefully.
- Processing of aspirated blood: The aspirated blood is collected in a sterile filtered reservoir up to the volume of 375-750 ml.
- Centrifugation of collected blood: The salvaged blood is subsequently pumped into the centrifuge bowl, where RBCs are separated and concentrated. The supernatant plasma and platelets are sent to the waste bag.
- Washing of salvaged blood: The salvaged blood may have a high concentration of free haemoglobin, inflammatory mediators, and other cellular debris. It is washed with isotonic solution (0.9% NS or PlasmaLyte-A). In typical cell salvage, the entire procedure can be performed within a few minutes.
- Reinfusion: Finally, the washed red cell suspension is pumped into the cell salvage bag and is ready for reinfusion. The use of a 40-micron microaggregate filter or leucocyte depletion filters may be required for the reinfusion of salvaged blood.

Storage and handling*:*

Intraoperative salvaged blood should be used within 6 hours of collection at room temperature. It may be kept up to 24 hours at 1-6 degrees if collected under aseptic conditions. It must be labelled with the name and registration number of the patient, ***"FOR AUTOLOGOUS USE ONLY",*** and the time of expiry.

It should never be transfused to any other patient.

Complications of intraoperative salvaged blood are:

- Haemolysis
- Coagulation defects especially dilutional coagulopathy since blood loss is replaced only by reinfusion of salvaged blood that does not contain plasma or platelets.
- Sepsis
- Air embolism
- Fat may be present in the unwashed product, which increases the risk of fat embolism.
- Thrombogenic substances (e.g., inflammatory mediators and microaggregates consisting of white cells and platelet debris) may be introduced.

Newer devices (e.g., Sorenson Auto transfusion Systems, Hemonetics Cell Saver) that transfuse salvaged whole blood or washed red cells have proved to be safer and no major complications reported.

3.2 Postoperative blood salvage

Postoperative blood salvage refers to collecting blood from surgical drains and subsequent reinfusion through a 40-micron microaggregate filter, with or without a washing process.

These techniques available for collecting the postoperative drainage are usually of worth if blood collection can be done within 24 to 48 hours after surgery in patients actively bleeding into a closed site (e.g., after

cardiopulmonary bypass, blood from the chest following traumatic haemothorax, joint cavity drainage). However, if there is evidence of infection or any evidence of malignant cells, or when the rate of blood loss is less than 50 ml per hour, this procedure is contraindicated. In addition, the blood collected from postoperative drainage should be sterile and defibrinated without any clotting properties.

The same devices are utilized for collecting and processing blood that is used in intraoperative autotransfusion. Blood must be filtered (washing is optional) before it is returned to the patient and must be reinfused within six hours from the start of collection in order to minimize infection and proliferation of bacteria. The blood bag should be labelled having the patient's name and an identifying number.

Complications:

Similar complications as discussed in the section on intraoperative blood salvage are associated with postoperative blood salvage.

Establishing a blood salvage program:

All hospitals or institutions with intraoperative blood salvage programs and blood centres providing the service should establish written policies and procedures for adequately collecting, labelling, and storing the collected blood.

All policies and procedures should be regularly reviewed by a clinician responsible for the program. Periodic quality control testing of collected blood is recommended to ensure that collection equipment and techniques result in an aseptic blood product.

Conclusion:

Minimizing allogeneic blood transfusion may lead to a decrease in the rate of transfusion reactions, blood donor exposure as well as transfusion-transmitted infections. Autologous transfusion has limited utility in certain areas like Jehovah's Witness, patients with rare blood group or with alloantibody(s) against high incidence antigens. Proper selection of patients who may be benefited from autologous transfusion is the key to a successful autologous transfusion program. However, its utility in other patient groups has been questioned from time to time in view of the increased wastage, higher cost, and the increased safety of allogeneic blood by the introduction of nucleic acid testing.

Key points

- Blood collected from a patient for re-transfusion into the same individual is called "Autologous blood."
- Autologous blood transfusion aims at improving patient safety and clinical outcomes by appropriately managing the patient's blood.
- It is a reasonable approach in patients with rare blood group, multiple alloantibodies, antibodies to high-frequency antigens and patients who refuse allogeneic transfusion (Jehovah's Witness). It also prevents some adverse transfusion reactions and the transmission of transfusion-transmitted infections.
- Autologous transfusion can be broadly categorized as:
  - Preoperative autologous blood donation
  - Intraoperative blood collection
  - Post-operative blood salvage
- Preoperative autologous blood donation: Two or more units are drawn from the patient and stored before surgery. The units are available for replacement if operative loss necessitates transfusion.
- Intraoperative blood collection can be classified as acute normovolemic haemodilution (ANH) and intraoperative blood salvage.
- In acute normovolemic haemodilution, a predetermined volume of the patient's blood is removed before the commencement of surgery and simultaneously replaced with colloid/ crystalloid solution to maintain

circulatory blood volume. The units are then reinfused in reverse order of their collection on assessment of blood loss.

- Intraoperative blood salvage involves collecting shed blood from a body cavity or wound during surgery and its subsequent reinfusion to the patient following centrifugation and washing of salvaged blood.
- Post-operative blood collection is the recovery of blood from surgical drains followed by reinfusion with or without processing.
- All autologous units should be tested for ABO & Rh grouping and TTI markers (HIV, hepatitis B, Hepatitis C, malaria, and syphilis)
- Each autologous unit must be labelled "***FOR AUTOLOGOUS USE ONLY***" along with the patient's name, registration number, withdrawal time, sequence number (if more than one unit), blood group, and date of expiry.

References

1. British Committee for Standards in Haematology, Transfusion Task Force, Boulton FE, James V. Guidelines for policies on alternatives to allogeneic blood transfusion. 1. Predeposit autologous blood donation and transfusion. Transfus Med 2007 Oct;17(5):354-65.
2. Kakaiya R, Aronson CA, Julleis J. Whole blood collection and component processing at Blood collection centers. In: Roback JD,Grossman BJ, Harris T, Hillyer CD, editors.Technical Manual of AABB 17th edition. AABB: Bathesda; 2011.p.187-226.
3. Food and Drug Administration, Centre for drugs and Biologics. Guideline for the uniform labelling of blood and blood components. 1985; p- II-6, II-17. Available at http://academy.gmp-compliance.org/guidemgr/files/unilabel.pdfaccessed on 05/10/2020
4. JPAC, Guidelines for the Blood Transfusion Services/23.5: Blood group labels. Available at https://www.transfusionguidelines.org/red-book/chapter-23-specification-for-the-uniform-labelling-of-blood-blood-components-and-blood-donor-samples/23-5-blood-group-labels accessed on 05/10/2020.
5. Triulzi DJ, Yazer MH, Waters JH. Patient blood management In: Simon TL, McCullough J, Snyder EL, Solheim BG, Strauss RG, editors. Rossi's principles of Transfusion Medicine.5th edition. Wiley Blackwell: West Sussex;2016. p.11-22.

Section 13

Documentation in Blood Transfusion Services

1. Introduction

Documentation is a vital and integral part of all aspects of blood transfusion services. It forms a framework for understanding and communication throughout the organization. In addition, good documentation practices can provide a detailed and logical sequence of steps for the implementation of any process.

Document management should include the following:

- It should be verified and approved before the issuance
- It should be reviewed, modified, and reapproved periodically
- It should be legible, identifiable, and readily available in the location in which it will be used
- It should be appropriately numbered for easy traceability
- Previous versions should be retained and easily retrievable for the required retention period
- It should be safe from the unintended use of significantly outdated or obsolete documents
- It should be protected from alteration, damage, or unintended destruction

2. Objectives of documentation:

The main objectives of the documentation are to provide:

- Consistency in procedures
- Reproducibility of all test results
- Information regarding the service
- Statistics
- Traceability of blood units/donors and patients
- Auditing
- Decision making
- Training programs and generating knowledge
- Improve the overall efficiency of the services

3. Types of documents

The documentation system has a hierarchy containing four levels (figure 1) includes[1]:

Level 1- Policies “what to do”
Level 2- Processes “how it happens”
Level 3- Procedures “how to do it”
Level 4- Forms, records, worksheets, templates etc.

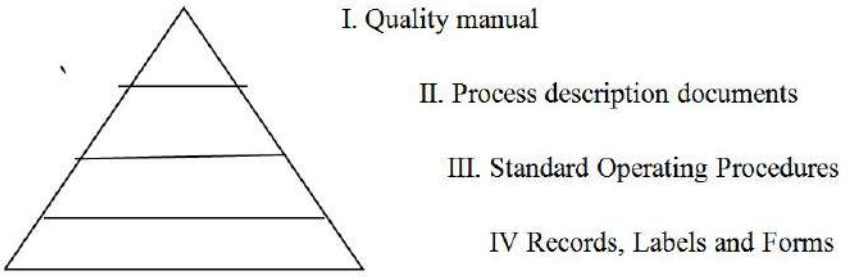


Figure 1: Hierarchy pyramid of documentation system

Type of documents required in blood centre:

- Quality manuals
- Policies
- Process documents
- Standard Operating Procedures
- Records/Registers
- Forms
- Work instructions
- Labels

3.1. Quality manual:

It is the main, top-level document that establishes an organization's quality policies and objectives. It provides evidence of what specific controls are to be implemented to ensure product/service quality. It includes quality policy which is the statement of the blood centre's standard of service, i.e. the objectives of the quality management system. It includes an organogram of the blood centre along with the job responsibilities of all the staff.

3.2. Policies:

Policies that are documented in the quality manual must reflect actual laboratory practice. Clear cut policies about the various aspects of blood transfusion services maintain consistency and confidence in the blood centre staff. Policies can be mandatory by regulatory authorities, or they can be optional on an institutional basis. Policies are supplemented by a set of other documents like procedure manuals, work instructions, forms, etc.

3.3. Process document:

Process can be defined as a set of interrelated resources and activities that transforms inputs into output. It indicates the strategies that are used to implement and perform the stated policies in the blood transfusion service, i.e. who should perform the specific tasks, when the task should be done and how to record the task when it is done. It can also present as a flow chart for better understanding.

3.4. Standard Operating Procedures:

The standard operating procedures (SOPs) are the vital written procedure document in any blood transfusion service. SOPs are mandatory for licensing of the blood centre. The development of SOPs is a multi-step process that involves preparation/writing, validation, review, approval, and training for its use. It should be written by a responsible person who is well trained in undertaking the procedure and authorized by the In-charge of the blood centre. The person working in the area (user) should validate the SOPs.

It should be written in first-person voice or active voice with clear, simple, and understandable information with one idea per sentence. Finally, it should be authorized with the signatures of the appropriately qualified

person. A valid copy of SOPs should be at the point of use and easily available for staff performing the job. One copy of all the SOPs should be maintained in the master file with the blood centre incharge. The review period of all SOPs should be defined, preferably annual but not more than two years. The review is targeted to improve the quality and incorporate the changes in the responsible personnel, operational processes, equipment, reagents, premises, and methodology. Training and retraining of all the personnel are the only mechanisms to ensure that the SOPs secure good manufacturing practices in the blood centre.

SOPs should follow strict regulatory guidelines and standards to control the actuality and precision of their content.

3.4.1. Components of SOP:

The first page should have information regarding the SOP followed by its description

The information part of the SOP should have the following components:

- Name of the blood centre
- Subject
- Location
- Function
- Distribution
- Unique number
- Version and revision
- Effective date
- Review period
- Number of pages and number of copies
- Name and signature of the person who has been authorized to approve
- Name and signature of the person who is responsible for authorizing the use of SOP from the effective date

As per the Drugs and Cosmetic Act, 1940[2], SOPs of a blood centre shall include:

a) Criteria used to determine donor suitability
b) Methods of performing donor qualifying tests
c) Procedure to prepare the site of phlebotomy
d) Method of accuracy relating to the product(s) to the donor
e) Blood collection procedure, including in-process precautions taken to measure accurately the quantity of blood drawn from the donor
f) Methods of component preparation
g) All tests performed on blood and blood components during processing
h) Storage temperatures and methods of controlling storage temperatures for blood and its components and reagents
i) Length of expiry dates, assigned for all final products
j) Pre-transfusion testing, including precautions to be taken to identify the recipient blood components accurately during processing
k) Procedures for managing adverse reactions in donor and recipient
l) Criteria for determining whether returned blood is suitable for re-issue
m) Procedures used for relating to a unit of blood or blood components from the donor to its final disposal
n) Quality control procedures for supplies and reagents employed in blood collection, processing and pre-transfusion testing
o) Schedules and procedures for equipment maintenance and calibration

p) Labelling procedures

q) Procedures of plasmapheresis, plateletpheresis and leucapheresis if performed

r) Procedures for preparing recovered (salvaged) plasma if performed, including details of the separation, pooling, labelling, storage, and distribution

s) All records pertaining to the lot or unit maintained according to these regulations shall be reviewed before the release or distribution of a lot or unit of the final product. The review or portions of the review may be performed at appropriate periods during or after blood collection, processing, testing, and storage. A thorough investigation, including the conclusions and follow-up, of any unexplained discrepancy or the failure of a lot or unit to meet any of its specifications, shall be made and recorded.

3.5. Records:

Records are the documents that provide evidence of the activities performed and results achieved. Records should be created concurrently with the performance of each significant step and should clearly indicate the identity of the individuals who performed each step and when each step was completed. Data should be recorded in a format that is clear and consistent. When forms are used for capturing or recording data, steps or test results, the forms become records. Records must be protected from accidental and unauthorized modification/destruction. The integrity of the records (electronic or hard) should be ensured. They should be stored properly and can be retrieved at the time of need. Confidentiality of the donor and the patient/ recipient records has to be maintained. Records should be legible, and corrections should be initialled.

3.5.1. Type of Records

As per the Drugs and Cosmetic act. 1940[2] various records required to be maintained in blood centres are:-

1) **Blood donor record:** It shall indicate the serial number, date of bleeding, name, and signature of the donor with other particulars of age, weight, haemoglobin, blood grouping, blood pressure, medical examination, bag number, and patient's detail for whom donated in case of replacement donation, category of donation (voluntary/replacement) and deferral records and signature of medical officer-in-charge.

2) **Master records register:** It shall indicate bag serial number, date of collection, date of expiry, quantity in millilitres, ABO/Rh "D" group, results for testing of HIV I and HIV II antibodies, Malaria, Syphilis, Hepatitis-B surface antigen, and Hepatitis-C virus antibody and irregular antibodies (if any), name and address of the donor with particulars, utilization issue number, components prepared or discarded and signature of the medical officer-in-charge.

3) **Issue register:** It shall indicate serial number, date and time of issue, bag serial number, ABO/Rh "D" group, total quantity in millilitres, name, and address of the recipient, group of recipients, unit/institution, details of cross-matching report and indication for transfusion.

4) **Records of components supplied:** Quantity supplied, compatibility report, details of recipient and signature of issuing person.

5) **Records of blood bags:** Details of the manufacturer, batch number, date of supply, and testing results.

6) **Register for diagnostic kits and reagents used:** Name of the kits/reagents, details of batch number, date of expiry, and date of use.

7) **Crossmatch register:** The blood centre must issue the blood's cross-matching report to the patient and the blood unit.

8) **Transfusion adverse reaction records**.

9) **Purchase record register:** Records of purchase, use, and stock in hand of disposable needles, syringes, and blood bags shall be maintained.

3.5.2. Retention of the records:

The blood centres shall maintain all the records for a period of five years. Some of the records like notification of donors of permanent deferral, the record of rare blood groups donors or patients, and patients with multiple

clinically significant alloantibodies may be kept for an indefinite period.

3.5.3. Disposal of records:

Records should be destroyed after a specified period of retaining by shredding by an approved document disposal committee of the hospital. Maintain the records of the disposal.

3.6. Labels:

Labels on every bag having blood and/or blood components shall contain the following particulars:

I. The proper name of the product in a prominent place, and bold letters on the bag
II. Name and address of the blood centre
III. License number
IV. Serial number/donor unit number
V. Pharmacopeia followed to prepare the component such as Indian Pharmacopeia (IP), British Pharmacopeia (BP), United State Pharmacopeia (USP)
VI. The date of collection of blood and the date of expiry
VII. The coloured label shall be put on every bag containing blood

The following colour scheme for the said labels shall be used for different groups of blood

Blood Group	Colour of the label
O	Blue
A	Yellow
B	Pink
AB	White

VIII. The results of the test for HIV I and II antibodies, Hepatitis B surface antigen, Hepatitis C virus antibodies, syphilis, and malarial parasite.
IX. ABO and Rh “D” group
X. Total volume
XI. Instruction to use:
- Keep continuously at the recommended temperature for blood and blood components.
- Disposable transfusion sets with filters shall be used for administration equipment.
- Appropriate compatible cross-matched blood without atypical antibodies in the recipient shall be used.
- The contents of the bags will not be used if there is any visible evidence of deterioration like haemolysis, clotting or discolouration.

XII. The label shall indicate the appropriate donor classification like a voluntary donor or replacement donor in no less prominence than the proper name

4. Computerization:

In an era of digitization, it is important that blood transfusion services should adopt the use of information and communication technology. In blood centres, a large amount of repetitive data needs to be documented every day in different registers, which is labour intensive and sometimes error-prone. Many computerized blood centre management systems have been developed for managing blood transfusion services. The management system should comply with the regulatory requirements of the Drug and Cosmetic Act, 1940[2] and, in addition, allows the customization to incorporate the specific requirements of the stakeholders, for example, regional transfusion centres, district hospitals and storage centres.

Advantages of computerization in blood transfusion services:

- Help to streamline the blood transfusion processes
- Help to maintain uniformity and standardization of documentation in blood centres as well as in hospitals
- Improve the efficiency and effectiveness of the staff for documentation and record-keeping
- Help in planning of blood donation camps
- Help in management for motivation, recruitment, and retention of blood donors
- Help to maintain an accurate and easily accessible donor registry, including rare blood donors
- Help in the correct identification of blood and blood products throughout the process and easy traceability of them from donor to the patient or vice versa
- Validation of all laboratory test results is possible
- Label blood products, transcription, and labelling errors can be reduced
- Help to manage the blood and blood component inventory
- Easy preparation of monthly or yearly statistics, indicators, and other reports for planning, monitoring, and evaluation

4.1. Infrastructure required for computerization

- Hardware- desktop, keyboard, printers, barcode printer, barcode reader, webcams, fingerprint scanner.
- Power supply and UPS (uninterrupted power supply),
- Software- mainly operating system software, application software and interface software (allows the systems to communicate with other computer systems) are used by the computers.
- Server
- Data storage facility and backup storage
- Internet/Intranet connection
- Manpower for entry of commands and data into the system

All the staff should be trained to use the software application and should have his/her own confidential user ID and password to protect the donor/patient confidentiality and also for the protection of data from accidental and unauthorized destruction and modification. Only authorized users must have access to the information in the computer system.

4.2. Blood centre software applications

Basic applications/ modules that should be incorporated into the blood centre management/information system are: -

A. Donor management
B. Blood component preparation and storage management
C. Immunohaematology laboratory test results: blood grouping, antibody screening, antibody identification, phenotyping etc.
D. Transfusion transmitted infections screening
E. Validation of all test results
F. Labelling and storage
G. Blood stock inventory management
H. Traceability of blood and blood components
I. Requisition and issue of blood and blood components
J. Patient management

4.3. System Management

Once a computerized blood centre management system is installed and put into regular use, it needs regular maintenance and updating to ensure uninterrupted operations.

There should be clear cut instructions or written standard operating procedures on the following subjects

- Archiving data- Periodic assessment of the storage space and when required data can be archived safely in an orderly fashion. The procedure for archiving the data should adhere to regulatory guidelines.
- Backup of software program and data in case of failure of hardware, software, loss of internet connectivity, disasters like fire or floods, etc.
- Hardware maintenance- regular maintenance contracts with the vendors, cleaning of the equipment, etc., must be in place.
- Maintenance of security- procedure for adding and deleting the users must be established. Sensitive information like test results of transfusion-transmitted diseases marker should be available only to the selective personnel.
- Personnel training- staff members should be adequately trained to use the system efficiently.

Validation of the software must be performed before it is implemented for consistent functioning.

5. e- Raktkosh

Ministry of Health and Family Welfare, Government of India, has developed a centralized blood centre information system "e-Raktkosh".[3] It is a comprehensive IT solution attempting to connect, digitize, and streamline blood centres' workflow and modernize blood centres in India by providing critical inputs under the blood services program. It incorporates the acquisition, validation, storage, and circulation of various live data and information electronically regarding blood donation and transfusion services. This system can assemble the heterogeneous data into legible reports to support decision making from effective donor screening to optimal blood dissemination in the field. These electronic processes will help the public easily access the blood availability status and make a requisition of particular blood group-specific components in nearby blood centres. The e-Raktkosh application facilitates compliance with the regulatory requirement of the Drug and Cosmetic Act and rules, 1945[2]. It has been developed with a modular and scalable approach and allows customization to incorporate specific information from nationwide stakeholders.

It has a citizen-centric portal that the citizens can use to get information about their nearest blood centre, stock, and blood donation camp schedule. There is also an e-Raktkosh mobile app available for Windows, Android, and iOS platforms. Various SMS are also sent from this system to the donors with thank you messages for registering and donating blood.

To accommodate the workflow of blood centres and blood storage centres, e-Raktkosh has the following modules:

- Donor management
- Camp management
- Blood stock management
- Blood grouping
- Investigation
- Requisition and issue
- Billing
- Enquiry
- Quality control
- Inventory
- Biomedical waste
- Equipment management
- Reports/ dashboard
- Web portal
- Mobile Apps

The e-Raktkosh application can be accessed on www.eraktkosh.in

Key Points

- The document consists of all the written procedures, instructions, records, forms, labels, and logbooks involved in processing blood and its components.
- Documentation in a quality management system has four levels of hierarchy with a quality manual at the apex, process description at the second level, standard operating procedures at the third level and records, forms, labels etc., at the bottom.
- SOP should be available for all processes and procedures, written in simple and understandable language, authorized by an in-charge medical officer, and validated.
- SOPs should be available in the respective working area.
- Records represent that activities have been performed and results obtained.
- All the records should be retained for a period of five years.
- Computerization of blood transfusion services improves blood transfusion services' efficiency and effectiveness, benefiting the donors and patients.
- Computerization should meet the standards as per national regulatory guidelines, incorporating all the aspects of blood transfusion services.
- Staff shall be adequately trained to utilize the services of computerization.

References

1. Tiehen A, Volny M. Information system in Blood Bank. vol 7th. Modern Blood Banking and Transfusion Practices. F.A. Davis Company; 2019.
2. Drug and Cosmetic Act 1940. Accessed 11/10/2020, https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf,documents/acts_rules/2016DrugsandCosmeticsAct1940Rules1945.pdf
3. eRaktkosh blood cell, National health Mission, Ministry of Health and Family Welfare. (Accessed 11/10/2020), https://www.eraktkosh.in/BLDAHIMS/bloodbank/transactions/bbpublicindex.html

Section 14

Human Leucocyte Antigen System

1. Introduction

Human Leucocyte Antigens (HLA) is the human version of the major histocompatibility complex (MHC), initially identified in mice as responsible for graft rejection between genetically unrelated strains (transplantation antigens). In 1958, Dausset, Snell, and Rappaport found HLA antigens in human leucocytes for the first time. In the same year, van Rood et al. reported the production of antibodies against leucocytes in the sera of multi-transfused individuals and multiparous women.[1] Based on these discoveries; these antigenic structures were named as HLAs because they were first identified on leucocytes. Later, it was observed that these antigens were present not only on leucocytes but also on tissue cells.[2] HLA contains loci of graft rejection, hence also termed as "transplant antigens", but the primary biological role of HLA molecules is in regulating the immune response. Under their extreme polymorphism, the HLA loci ensure that only very few individuals are identical, and thus the population at large would recognize anything that is "foreign". Since few HLA antigens have been recognized on all of the tissues of the body (rather than just blood cells), the recognition of HLA antigens is more appropriately described as "Tissue Typing" ("HLA Typing" is used interchangeably with tissue typing).

2. Genomic organization of the HLA system

The HLA refers to a cluster of highly conserved polymorphic genes on the sixth chromosome that spans approximately 4000-kilo bases of deoxyribonucleic acid (DNA). HLA includes two major isoforms, termed class I and class II.

The HLA is classified into three regions:

- **The class I region**: It is mostly telomeric, which includes the classical HLA-A, HLA-B and HLA-C genes that encode the heavy chains of class I molecules and the non-classical HLA-E, HLA-F, and HLA-G loci; and several pseudo-genes.
- **The class II region**: It consists of a series of sub-regions, each containing A and B genes encoding α (alpha) and β (beta) chains, respectively. In the centromeric to telomeric direction, the first is the HLA class II region comprising α-and β-chains of classical HLA-DR, HLA-DQ, HLA-DP, and non-classical HLA-DM, and HLA-DO.
- **The class III region:** It is sandwiched between the class II and class I region, does not encode HLA molecules, but contains genes for functionally unrelated genes such as complement components, heat shock proteins, and tumour necrosis factor (Figure 1).

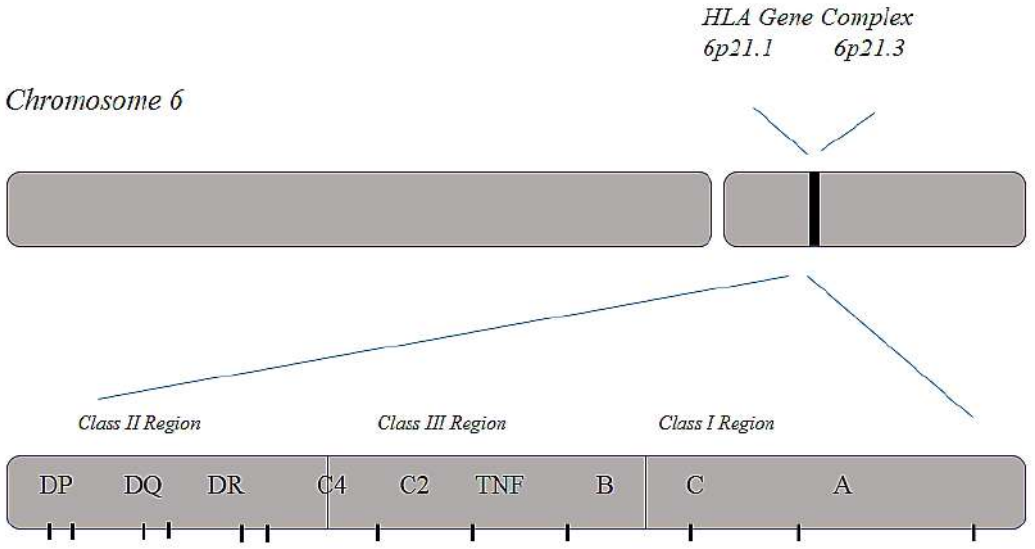


Figure 1. Gene map of HLA region on chromosome number 6.

The common definitions related to HLA genetics are illustrated in Table 1.

Table 1: Common definitions in Human Leucocyte Antigen genetics

Term	Definition	Example
Allele	A unique sequence of an HLA gene defined by molecular methods	DRB1*04:01 allele is a unique sequence defined as DR4 by serologic methods
Antigen	Antibody-defined protein	DR4 antigen is a serologically defined protein product of an HLA gene
Haplotype	HLA genes inherited as a chromosomal unit	HLA-A1, HLA-B8, and HLA-DR3 are common haplotypes among the white population
Genotype	Molecularly defined HLA allele or sequence	Genotypically-matched donor and recipient are identical for the HLA alleles at a given HLA gene (e.g., HLA-DRB1*04:01
Phenotype	Serologically defined HLA protein or antigen	Phenotypically matched donor and recipient share the same HLA antigen (e.g., HLA-DR4)

3 Structure of HLA molecules

3.1 Structure of HLA class I molecules:

HLA class I molecules contain two different non-covalently bound polypeptide chains (α-chain and β2-microglobulin). The α-polypeptide chain is 44 Kilo-Dalton (kDa) and consists of three loops containing 338 amino acids bound to each other by di-sulphide bonds. The α-polypeptide molecule is divided into three parts: extracellular hydrophilic region (1–281 residues), trans-membrane hydrophobic region (282–306 residue), and intracellular hydrophilic region (307–338 residues). Extracellular parts of the heavy chain (α1 and α2) are located on the distal membrane and constitute the peptide binding region of the molecule. These α1 and α2 regions are encoded by second and third exons and determine the variability of the molecules. The other part (α3) on the proximal membrane carries the interaction region for the CD8 molecule on T-cells.[3] β2-micro-globulin, which is encoded by a gene on the 15th chromosome, is a 12-kDa subunit. Non-polymorphic β2-microglobulin is not membrane-bound and is required for heavy chain stability and antigenic peptide association. [Figure 2(A)]

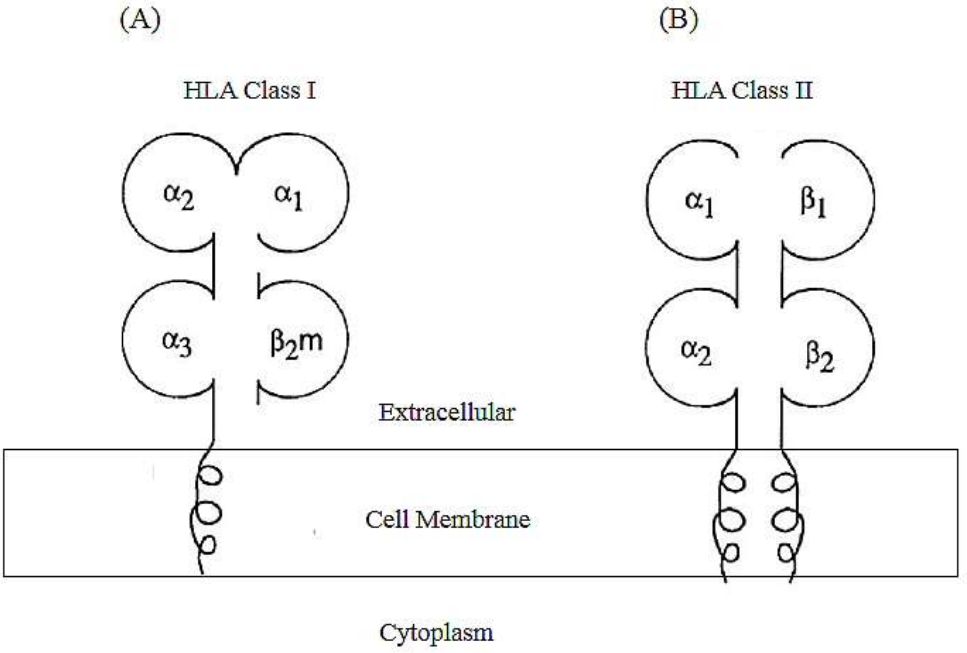


Figure 2: (A) The structure of class I HLA molecule (B) The structure of class II HLA molecule

3.2 Structure of HLA class II molecules:

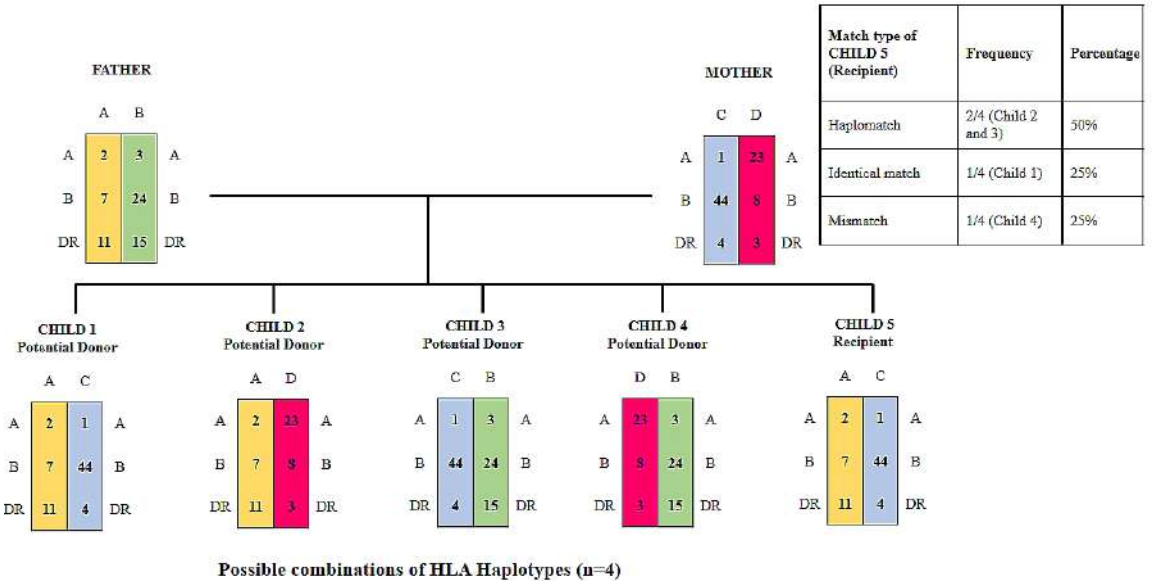
HLA class II molecules consist of six different loci (HLA-DM, DN, DO, DP, DQ, and DR). DM, DN, and DO loci are pseudo-genes and do not encode proteins. They are type I integral membrane proteins with heterodimer structure. HLA class II molecules have two polypeptide chains (α, β), which are non-covalently bound to each other. The α-chain is 24–32 kDa, and the β-chain is 29–32 kDa. The second exons of α and β chains (α1 and β1) encode the variable peptide-binding region.[3] This variable region can bind to peptides more than 11 amino acids in length and present them to CD4+ T helper cells [Figure 2(B)].

4. Inheritance

4.1 HLA haplotypes:

The entire set of A, B, C, DR, DQ, and DP genes located on one chromosome is known as a haplotype. HLA genes are closely linked, and the entire MHC is inherited as an HLA haplotype in a Mendelian fashion from each parent. Genetic crossovers and recombination in the HLA region are uncommon (less than 1%). Thus a complete set of alleles located on a chromosome is usually inherited by children as a unit (haplotype). Figure 3 illustrates the segregation of HLA haplotypes in a family. The two haplotypes of the father are labelled as A and B, and the mother's haplotypes are C and D. Each child inherits two haplotypes, one from each parent. Thus, two siblings have a 25% chance of being genotypically HLA identical, a 50% chance of being HLA haploidentical (sharing one haplotype), and a 25% chance to share no HLA haplotypes.

An important inference is that a parent and child can share only one haplotype, making an identical match between the two unlikely. It should also be apparent that uncles, grandparents and cousins are unlikely to have identical haplotypes with any given child. These are important factors when looking for a well-matched organ or blood donor.



Match type of CHILD 5 (Recipient)	Frequency	Percentage
Haplomatch	2/4 (Child 2 and 3)	50%
Identical match	1/4 (Child 1)	25%
Mismatch	1/4 (Child 4)	25%

Figure 3: Inheritance of HLA haplotype demonstrated in a family study. Possible combinations of HLA haplotype are four. If the child 5 is recipient for kidney, then the chances of match with four siblings (potential donors) are depicted in figure 3.

An important characteristic of HLA antigens is the existence of linkage disequilibrium between the alleles of the loci. Possible random combinations of antigens from different HLA loci on an HLA haplotype are enormous, but certain HLA haplotypes are found more frequently in some populations than expected by chance. This phenomenon is known as linkage disequilibrium.(e.g., HLA-A1, B8, DR17 is the most common HLA haplotype among Caucasians, with a frequency of 5%; in the north Indian population, the three most haplotypes are HLA-A33-B44-DR7, HLA-A33-B58-DR3, and HLA-A2-B50-DR3.[4]

4.2 Cross-reactivity:

Cross-reactivity is the phenomenon whereby one antibody reacts with several different antigens, usually at one locus. This is not a surprising event as it has been demonstrated that different HLA antigens share exactly the same amino acid sequence for most of their molecular structure. Antibodies bind to specific sites on these molecules, and it would be expected that many different antigens would share a site (or epitope for which a specific antibody will bind). Thus, cross-reactivity is the sharing of epitopes between antigens, and the term **CREG** is often used to describe the "**C**ross **Re**acting **G**roups" of antigens.

5. HLA nomenclature

The history of the HLA system nomenclature was summarized by Sir Walter Bodmer, who, together with Ruggero Ceppellini, was primarily involved in its development.[5] It began as HL-A, for 'human leucocyte locus A'.With the recognition that HLA molecules are encoded by more than one locus, the A came to stand for 'antigen', and a locus designation was added after HLA (i.e., HLA-A, HLA-B, HLA-C, HLA-D, etc.). At present, two systems are used; one is immunologically defined nomenclature, and the other one is sequence-defined allelic nomenclature.

5.1 Immunologically defined HLA nomenclature:

Immunologically defined nomenclature is based on the identification of HLA antigens on the surface of leucocytes. Therefore, HLA phenotypes described by immunologic methods are conventionally called HLA antigens. The rule that follows in this is that HLA is separated by a hyphen from a capital letter identifying the locus encoding distinct HLA class I (-A, -B, -C) or class II (-DR, -DQ, -DP) antigens. The letter is followed by a number that identifies a serologic family of alleles sharing epitopes recognized by alloantibodies or

alloreactive cytotoxic T-cells. For e.g., HLA-DR3; where HLA stands for "Human Leucocyte Antigen" - a name that has been kept more as a tribute to history than actual function; DR: the name of the specific locus; 3: the number refers to the actual antigen at the locus. HLA-DR3 is the broadest description of the antigen. It is the name of a specific group of antigens. The DR3 group can be divided into HLA-DR17 and HLA-DR18 by using antibodies (serology).

5.2. Sequence-defined allelic nomenclature:

The 10th International Histocompatibility Workshop recommended in 1987 a sequence-based nomenclature to describe alleles not distinguishable by immunologic methods. This is based on the molecular identification of nucleotide sequences in genomic DNA, and the results are conventionally referred to as HLA alleles. Molecular typing has gained popularity because of its higher resolution. As a result, the number of HLA alleles has rapidly increased. As of October 2019, a total of 23,907 alleles for HLA exist. HLA designates molecules belonging to the human MHC followed by the locus (-A, -B, etc.). Alleles are then identified after an asterisk (*). Each HLA allele name has a unique number corresponding up to four sets of digits separated by colons. The first two digits indicate the allele family, corresponding on most occasions to the serological equivalent; the next two to three digits represent the allele number according to its nucleotide sequence, within that allele family, and each allele consists of an HLA protein differing at least in 1 to 2 amino acids; the next two digits indicate silent synonymous nucleotide differences, and the last two digits indicate differences in the introns or the 3' or 5' non-coding regions. After the numerical designation, further letters are used as follows to designate other characteristics of the HLA alleles: (N) to indicate non-coding sequences or null alleles; (L) low expression; (S) secreted, soluble but not a surface expression; (C) cytoplasmic expression only; (Q) questionable expression; (A) aberrant or doubtful expression. For example, a given allele could be denoted as HLA-A*01:01:111:01A.

HLA:	prefix for an HLA gene
HLA-A:	a particular HLA locus i.e. A
HLA-A*01:	a group of alleles that encode the A01 antigen
HLA-A*01:01:	a specific HLA allele
HLA-A*01:01:111:	an allele that differs by a synonymous mutation from HLA-A*01:01:110
HLA-A*01:01:111:01	an allele that contains a mutation outside the coding region from HLA-A*01:01:111:02
HLA-A*01:01:111:01A	an allele encoding a protein with an aberrant or doubtful expression, where the mutation is found outside the coding region.

New sequences are submitted to

- European Molecular Biology Laboratory (EMBL; www.ebi.ac.uk/Submissions/index.html),
- GenBank(www.ncbi.nlm.nih.gov/Genbank/index.html), or
- DNA Data Bank of Japan (DDBJ; http://www.ddbj.nig.ac.jp/submission-e.html).

6. HLA polymorphism

The HLA system is known to be the most polymorphic in humans. The HLA polymorphism is not evenly spread throughout the molecule but is clustered in the antigen-binding groove. Amino acid variations in several regions change the fine shape of the groove and thus alter the peptide-binding specificity of HLA molecules. The distribution and frequency of HLA antigens vary greatly among different ethnic groups. It has been postulated that this diversity of HLA polymorphism has evolved under unique selective pressure in different geographic areas. This could be related to the role of the HLA molecules in the presentation of prevalent infectious agents in different areas of the world.

7. Expression of HLA

HLA class I molecules are expressed on the surface of almost all nucleated cells and platelets, whereas class II molecules have a much more restricted distribution. Class II molecules are expressed only on B lymphocytes, antigen-presenting cells (monocytes, macrophages, and dendritic cells), and activated T lymphocytes.

8. The function of HLA molecules

The fundamental role of Class I and II molecules is to bind to self and non-self-peptides and transport them to the plasma membrane of cells for recognition by the T cell antigen receptor. Autoimmunity is avoided by several mechanisms to delete or suppress T-cells which bind with high affinity to self-peptides in the context of an individual's Class I and II molecules. However, viral and bacterial peptides displayed by the Class I and II molecules to T cell antigen receptors generally result in an immune response.[6] Class I molecules bind 8–10 amino acid peptides primarily resulting from proteasomal degradation of cytoplasmic proteins and present these peptides to CD8+ cytotoxic T-cells. Thus, Class I molecules are a primary means of alerting T-cells to virally infected cells. Class II molecules bind 13–25 amino acid peptides largely resulting from endosomal degradation of exogenous and endogenous proteins and present these peptides to $CD4^+$ T helper cells. Class II molecules play an important role in eliciting immune responses to organisms such as pyogenic bacteria. $CD8^+$ and $CD4^+$ T cell activation by these two routes results in cell division and differentiation, resulting in cellular and antibody-mediated immune responses.[7]

9. Clinical laboratory testing for HLA

a. **HLA antigen typing techniques**

i. **Serologic HLA typing technique:** In the lymphocytotoxicity (serological) test, lymphocytes are added to sera which may or may not have antibodies directed to HLA antigens. If the serum contains an antibody specific to an HLA (Class I or Class II) antigen on the lymphocytes, the antibody will bind to this HLA antigen. When complement is added, it binds to positive cells (i.e., where the antibody has bound) only and, in doing so, causes membrane damage. The damaged cells are not completely lysed but suffer sufficient membrane damage to uptake vital stains such as eosin or fluorescent stains such as ethidium bromide. Microscopic identification of the stained cells indicates the presence of a specific HLA antibody. The cells used for the test are lymphocytes because of their excellent expression of HLA antigens and ease of isolation compared to most other tissue. The most important use of this test is to detect specific donor-reactive antibodies present in a potential recipient prior to transplantation. Historically, this test has long been used to type for HLA Class I and Class II antigens, using antisera of known specificity. However, the problems of cross-reactivity and non-availability of certain antibodies have led to DNA-based methods. Currently, many laboratories have moved to molecular genetics methods for HLA typing.

ii. **Molecular HLA typing techniques:** The polymerase chain reaction (PCR) has been developed for investigating the DNA nucleotide sequence of a particular region of interest in any individual. Very small amounts of DNA can be used as a starting point. The first step in this technique is to obtain DNA from the nuclei of an individual. The double-stranded DNA is then denatured by heat into single-stranded DNA. Oligonucleotide primer sequences are then chosen to flank a region of interest. The oligonucleotide primer is a short segment of complementary DNA, which will associate with the single-stranded DNA to act as a starting point for reconstructing double-stranded DNA at that site. If the oligonucleotide is chosen to be close to a region of special interest like a hyper-variable region of HLA-DRB then the part of the DNA, and only that part, will become double-stranded DNA when DNA polymerase and deoxy-ribonucleotide triphosphates are added. From one copy of DNA, it is thus possible to make two. Those two copies can then, in turn, be denatured, re-associate with primers and produce four copies. This cycle can then be repeated until there are sufficient copies of the selected DNA to isolate on a gel and then sequence or type.

There are several PCR based methods in use. These are:

A. **Sequence-specific priming (SSP) typing:** Genomic DNA (gDNA) is isolated from the sample, and HLA regions of interest are amplified using PCR technology. PCR utilizes in-vitro prepared small oligonucleotide sequences (primers or oligos). According to complementarity rules these 'oligos' bind to an exact location of a DNA molecule, acting as starting points for the production of multiple complementary copies of the intermediate regions between a pair of them (amplification). More primers per reaction or more pairs of primers may be included in a single PCR, depending on the purpose of each protocol.[8]

   The amplification primers are polymorphic-specific, meaning that they only extend and form a product if the targeted polymorphism exists. The primers are designed so that their 3'end nucleotide is complementary to the investigated genomic alteration. Thus, products of specific lengths are produced depending on the polymorphism and the primer design. Afterwards, these are visualized using gel electrophoresis. PCR/SSP is a time and labour intensive technique. This technique is also prone to false-positive bands and false-negative results, especially for old (degraded) samples.

B. **Sequence-specific oligonucleotide (SSO) typing:** The procedure relies on the locus-specific amplification of the genomic DNA segment comprising the polymorphic sites of HLA alleles. Amplified DNA is then immobilized on a solid support, usually a nylon membrane, and then hybridized with a battery of sequence-specific oligonucleotide probes (SSOP) (direct hybridization). Fluorochromes are linked with the probes to allow their detection by the chemiluminescence technique. Alternatively, SSO probes can be immobilized on a solid support, for example, colour-coded microspheres, and hybridized with labelled PCR product (reverse hybridization). The higher the number of probes better is the resolution level. Usually, 50-100 probes per locus are used for intermediate/high-resolution typing, however, only the probe completely matched with the target sequence amplified will hybridize and give a positive signal.

C. **Sequence-based typing (SBT):** Another approach to high-resolution HLA typing is the PCR amplification and subsequent, direct sequencing of previously described class I and II exons. Di-deoxy-based Sanger sequencing, using capillary electrophoresis, provides increased reliability, especially after SSO or SSP. The two alleles of heterozygous samples, which represent a substantial source of ambiguities, are usually sequenced separately following SSO or SSP typing, thus increasing the resolution of possible genotypes.[8]

Although more automated, easier to implement, and less prone to technical and interpretation errors, this technique is less sensitive than others, like SSO, which, when optimized, provides more accurate and less ambiguous results.[9] The summary, advantages, and disadvantages of the three techniques, SSP, SSO, and SBT are detailed in Table 2.

Table 2: Molecular techniques for HLA typing

Method	Summary	Advantages	Disadvantages
PCR-SSP	PCR amplified DNA using Sequence-specific primers (SSP). Primers are designed with specificity dependent nucleotides on the 3' end. PCR product visualized directly on gels	• Cheaper than other HLA techniques. • Easily performed • Does not require expensive equipment. • Simple, and easy to interpret	• Subjective in interpretation. • Labour-intensive • Time-consuming
PCR-SSO	Labelled sequence-specific probes are hybridized to PCR amplified DNA and then detected.	• Suitable for a higher number of samples.	• This may lead to false-positive results. • Hybridization temperature is critical, and could lead to false-negative hybridization. • Lacks accuracy in precise allelic typing

PCR-SBT	DNA amplified by PCR using primers specific for the site of interest. PCR products are purified and then sequenced	• More reliable and specific than other methods. • Used for perfect matching • New alleles can be detected quite easily	• High-end equipment is needed.

HLA antibody screening assays (for HLA sensitization)

Anti-HLA antibodies are generally defined as panel reactive antibodies (PRA). They are one of the immunological factors affecting graft survival. In recent studies, researchers have assessed the pre-transplant incidence of anti-HLA antibodies and their clinical significance. Numerous studies reported that post-transplant anti-HLA antibody detection was associated with a high graft rejection ratio.[10] Anti-HLA antibodies can directly recognize undamaged foreign HLA molecules on the cell surface. Thus, these antibodies play a critical role in solid organ transplantation and also in haematopoietic stem cell transplantation (HSCT). The humoral response directed against foreign HLA molecules can be encountered during pregnancies, blood transfusions, and/or previous transplantations.

Transplant candidates are tested for these anti-HLA antibodies by different techniques, such as the PRA test. HLA laboratories perform PRA tests routinely in patients waiting for solid organ transplantation. Physicians can measure the probability of a negative cross-matched donor.[11] Earliest studies determined class I and II anti-HLA antibodies with complement-dependent lymphocytotoxicity (CDC) techniques. However, antibodies at low levels cannot be detected using this technique. In addition, only complement-dependent antibodies (IgG1, IgG3, and partly IgG2) can be detected by this method. Over the years, other more sensitive analytical methods have become available such as flow cytometer based and solid-phase assays. All IgG subtypes (IgG1, IgG2, IgG3, and IgG4) can be detected by flow cytometry.[12]

i. **Lymphocytotoxicity assay:** Preformed HLA antibodies can be detected by testing the patient's serum against a panel of lymphocytes with known HLA types. The complement-mediated micro-lymphocytotoxicity technique has been the standard, and the anti-human globulin (AHG) enhancement method provides higher sensitivity. This is known as HLA antibody screening, and the results are expressed as the percentage of the reactive panel cells; this is called the percent panel reactive antibody (% PRA) e.g., if 10 of 40-panel cells are reactive with a serum, the PRA is 25%. With a panel of well-selected cells representing various HLA antigens, antibody specificities can sometimes be assigned. This information is particularly important for the prospective organ transplant recipient to predict the chance of finding a compatible or cross-match-negative deceased donor and to avoid specific mismatched HLA antigens in the donor. When a potential donor becomes available, a final crossmatch is performed between the recipient's serum and the donor's lymphocytes to determine the compatibility.

ii. **Panel reactive antibody screening testing:** Affinity-purified HLA class I and class II antigens of known HLA type, either pooled or specific, are bound directly to wells of micro-titre plates. Antibody reactivity is detected by the addition of a secondary antibody. This enzyme-conjugated antihuman immunoglobulin induces a colour change upon the addition of the enzyme-substrate, detected by measuring optical density. This is a quick, objective, semi-quantitative assay that detects both complement and non-complement-fixing HLA antibodies with a reported greater sensitivity than CDC tests.[13]

PRA detection using methods based on enzyme-linked immunosorbent assay (ELISA) and fluorescence-based flow cytometry or single antigen bead (SAB) technologies offer higher sensitivity and specificity. PRA screening, identification, and SAB assays have similar test principles consisting of two main incubation steps. Patient and control sera are primarily incubated with appropriate beads, while fluorescence-conjugated anti-human secondary antibodies are added during the second incubation step. At the end of the assay, a fluorescence peak is generated, which indicates a positive result (binding of the antibody to HLA antigens on the beads).[14]

c. **Compatibility testing assays (between donor and recipient)**

Tissue typing of the recipient and donor determines their HLA match. The MHC class I HLA-A and HLA-B and class II HLA-DR antigens are routinely determined because rejection responses most commonly

occur from mismatches at these alleles. There is an increasing awareness of the importance of immune responses to other HLA antigens, and many centres now also look for the presence of antibodies to HLA-C, HLA-DQ, and HLA-DP. A six-antigen (HLA-A, HLA-B, and HLA-DR) match confers a graft survival advantage compared with zero antigen matches for both deceased and living donor transplantation of 10% at 10 years.[15] In addition to the determination of the HLA compatibility, screening for anti-HLA antibodies and cross-matching are performed to assess the risk for rejection.

i. **Lymphocytotoxicity crossmatch or complement-dependent cytotoxicity crossmatch (CDCXM):** The long-established complement-dependent cytotoxicity crossmatches (CDCXM) assay is over 30 years old and is still widely used. The technique involved isolating donor lymphocytes (cadaveric/living). The B and T-cells are separately tested against serum from the recipient. The humoral immunological response is mediated through are activation of the complement system by the classical pathway. To demonstrate the effects of this cascade, complement is added to the mixed recipient serum, and donor lymphocytes and cell lysis of the lymphocytes is observed. The addition of anti-human immunoglobulin (AHG) helps increase the sensitivity of CDC as multiple AHG's binds to a single donor-specific antibody, amplifying its complement activation response at smaller titres.

   CDC is a robust method for detecting and defining complement-fixing IgG (IgG1 and IgG3) and IgM antibodies directed against HLA and/or non-HLA targets (including autologous reactive antibodies). Although criticized for its low sensitivity [16], the pattern of CDC panel reactivity as a measure of anti-HLA sensitization (in vivo) is a useful indicator of an increased risk of rejection and potential hyper-acute rejection. Its inherent disadvantages include requiring a large panel of viable lymphocytes to cover the most commonly occurring HLA specificities. It is subjective. It requires manual reading of the test and expert analysis, and it is unable to detect non-complement-fixing antibodies.

ii. **Flow cytometry crossmatch (FCXM):** A FC-XM test is an antibody-binding assay following incubation of donor lymphocyte targets with recipient serum; a fluorescent-labelled detecting antibody is used to tag any bound alloantibody. Following the passage of the target cells through a flow cytometer, a semi-quantitative assessment can be made of the degree of binding of alloantibody to donor lymphocytes, leading to an assessment of XM positivity.

   FCXM is a more sensitive method compared to the CDCXM test. Thus, donor-specific antibodies (DSAs) that CDCXM cannot detect can be detected by the FCXM technique. In this situation, the antibody concentration may be insufficient to generate hyper-acute rejection. However, positive T FCXM (and negative T CDCXM) results indicate a high risk of graft failure. Positive FCXM may not affect graft survival in non-sensitized patients who get transplanted for the first time. Hypersensitive patients with previous transplantations and patients with positive FCXM would have shorter graft survival compared to patients with negative FCXM. Positive FCXM results obtained after previous transplantations can be due to memory cells' production, differentiation, and antibody production. If these antibodies recognize new HLA antigens during the new transplantation, they will lead to rejection. Low levels of antibodies may indicate memory cell activation.[17]

iii. **Donor specific antibody crossmatch (DSA-XM):** DSA-XM tests consisting of beads coated with antihuman Class I and Class II antibodies are available to isolate HLA molecules from a specific donor to be used in donor specific crossmatches. DSA-XM allows performing a real crossmatch using donor lysates which are easier to keep than living cells for post-transplantation studies.[18]

   Donor lysate is prepared using beads provided by the manufacturer. The lysate is preserved at −80°C for 5 years. The kit includes a single blend of beads. Two of the beads in the blend are conjugated with monoclonal antibodies specific for Class I HLA or Class II HLA. When mixed with a lysate, these two beads will capture the solubilized HLA, making a donor-specific HLA target for antibodies in a serum sample. The bead blend also includes control beads to monitor the amount of background in the assay and ensure that the appropriate conjugate has been used in the assay. The patient serum, diluted in the specimen diluent, is then added and incubated with the beads for 30 minutes. Following another wash, the diluted antihuman IgG phycoerythrin (PE) conjugate is added to the beads. After a final 30-minute incubation, wash buffer is added to the wells, the plate is placed in the instrument, and data is collected for analysis.

iv. **Virtual crossmatch:** Virtual cross-matching refers to comparing the anti-HLA antibodies of the recipient, as defined by SAB, with the HLA of the donor.[19] If there is a DSA present, this would represent a positive virtual

crossmatch. Antibodies are defined against HLA class I and II antigens. Synthetic microspheres (beads) coated with HLA antigens are commercially available for this testing. Beads may be coated with multiple HLA antigens for screening purposes or a single HLA antigen for defining the specificity of antibodies more precisely. For the virtual crossmatch, multiple beads, each coated with a single HLA antigen, are mixed with the recipient serum. Anti-HLA antibodies present bind to the beads and are detected by an isotype-specific (e.g., IgG) detection antibody via flow cytometry. Unique fluorochromes within the beads mark the HLA antigen specificity of each bead. This technique is as sensitive as flow cross-matching and provides the specificity of the antibody.

10. Clinical relevance of the HLA system

The most important function of the HLA molecule is in the induction and regulation of immune responses. T-lymphocytes recognize foreign antigens in combination with HLA molecules. In an immune response, the foreign antigen is processed by and presented on the surface of a cell (e.g., macrophage). The presentation is made by way of an HLA molecule. Antigens bind to the antigen-binding cleft of the HLA molecule. T-lymphocytes interact with the foreign antigen/HLA complex and are activated. Upon activation, T-cells multiply and set up an immune response by releasing cytokines that will recognize and destroy cells with this same foreign antigen/HLA complex when next encountered. The exact mode of action of HLA Class I and HLA Class II antigens is different in this process. HLA Class I molecules, by virtue of their presence on all nucleated cells, present antigens that are peptides produced by invading viruses. These are specifically presented to cytotoxic T-cells (CD8), which will then act directly to kill the virally infected cell.

HLA Class II molecules have an intracellular chaperone network that prevents endogenous peptides from being inserted into its antigen-binding cleft. They bind antigens (peptides) derived from outside of the cell (and have been engulfed). Such peptides would be from a bacterial infection. The HLA Class II molecule presents this “exogenous” peptide to helper T-cells (CD4) which then set up a generalized immune response to this bacterial invasion. Thus, it is apparent that HLA products are an integral part of immunological health. Therefore it is no surprise to see a wide variety of areas of clinical and genetic implications.

10.1. HLA and transfusion:

The HLA system can cause adverse immunologic effects in transfusion therapy. These effects are primarily mediated by HLA antibodies developed against the donor leucocytes contained in the cellular blood components. These HLA antibodies induced from previous allo-sensitization (pregnancy, transfusion, or transplant) can cause platelet transfusion immune refractoriness, febrile transfusion reaction, and transfusion-related acute lung injury.

10.1.1. HLA alloimmunization:

Anti-HLA antibodies are most commonly induced by multiple pregnancies and blood transfusions (caused by the leucocytes normally contained in the blood products). The incidence of HLA alloimmunization following transfusions can vary with the patient’s diagnosis and therapy.[20] HLA antibodies can be detected in 25% to 30% of transfused leukemic patients and can be present in as high as 80% of aplastic anaemia patients. Leukemic patients are usually transfused while receiving intensive chemotherapy, which induces immunosuppression and reduces the incidence of transfusion-induced alloimmunization. Severe aplastic anaemia patients who had developed HLA alloimmunization have a higher incidence of graft rejection following stem cell transplantations.

To reduce HLA alloimmunization, the leucocyte content must be reduced to less than 5×10^6, which can be achieved using one of several leucocyte-reduction filters.[21] Leucoreduction can be achieved for platelet and red cell components using third or fourth-generation leucocyte reduction filters. The wider use of leucocyte–reduced blood products is likely to reduce the number of newly HLA allo-immunized patients from blood transfusions. However, the incidence of HLA antibody development is not decreased or delayed by leucocyte reduction in patients with previous pregnancies. Mostly multiparous women appear to develop HLA antibodies by a secondary immune response during transfusion therapy.

10.1.2. Platelet refractoriness:

Haematopoietic stem cell transplantation and the more aggressive use of chemotherapy in the treatment of malignancies have led to a dramatic increase in platelet transfusions in the past few decades. HLA class I antigens are expressed variably on platelets.[22] The development of antibodies to these antigens can cause immune destruction of transfused incompatible platelets, resulting in immune refractoriness to random donor platelet transfusions. Refractoriness is manifested by the failure to achieve a rise in the circulating platelet count one hour after infusion of adequate numbers of platelets and repeated failure to achieve satisfactory responses to platelet transfusions from random donors. Platelet refractoriness is associated with a number of adverse outcomes, including longer hospital stays, increased risk of bleeding, and decreased survival.[23] The refractory state is often associated with lymphocytotoxic HLA antibodies. Considering the highly polymorphic nature of the HLA system, it is nearly impossible to obtain sufficient numbers of HLA typed donors to provide HLA-matched platelets for all allo-immunized patients. However, some blood centres maintain large pools (several thousand or more) of HLA-A and HLA-B typed voluntary apheresis platelet donors. If the specificity of the patient's antibodies can be determined, donors who are negative for corresponding HLA antigens can be selected. Donors who are not perfectly matched with the patients but homozygous for a given locus can also be used (e.g., patient HLA-A2, 3, and donor HLA-A2 only). HLA-matched siblings or HLA-haploidentical family members can donate platelets by apheresis. However, these blood-related donors should not support patients' transfusions before a stem cell transplant to prevent alloimmunization to minor histocompatibility antigens. Good platelet survival and HLA matching are not absolute. For example, poor transfusion results are sometimes obtained despite a perfect HLA match. Poor recovery may be a result of sensitization to non-HLA antigens, such as platelet-specific antigens. In contrast, excellent transfusion results are at other times obtained in the presence of a complete HLA mismatch. Good recovery may be a function of:

- A restricted pattern of alloimmunization—private versus public antibodies
- Variable expression of HLA antigens on the platelet

Numerous techniques have been tried to determine platelet compatibility.[24] Platelet cross-matching using a solid-phase red cell adherence technique has been developed. This technique detects antibodies against HLA class I and platelet-specific antigens. Apheresis platelet units are cross-matched with the patient's serum, and cross-match compatible units are identified. Surface antigens on leucocytes are more immunogenic than antigens on the surface of platelets, and therefore, refractoriness is probably initiated by HLA antigens on the surface of contaminating leucocytes. The transfusion response can be improved by removing the leucocytes.

10.1.3. Febrile non-haemolytic transfusion reaction (FNHTR):

Refer to section 10 for details

10.1.4. Transfusion-associated graft-versus-host disease (TA-GvHD):

Refer to section 10 for details

10.1.5. Transfusion-related acute lung injury (TRALI):

Refer to section 10 for details

10.1.6.Neonatal alloimmune thrombocytopenia (NAIT):

A rare condition occurs when a mother becomes sensitized to a foreign antigen of paternal origin present on fetal thrombocytes (platelets). These platelet antigens provoke the production of maternal antiplatelet IgG antibodies that cross the placenta and destroy fetal platelets and cause neonatal immune thrombocytopenia. The most commonly involved platelet-specific antigen is HPA-1a. Platelet-specific antigens are generally weak immunogens, and additional genetic factors may influence whether HPA-1a-negative women will develop anti-HPA-1a antibodies. Individuals with certain HLA haplotypes with HLA-DRB3*0101 alleles are more likely to develop antibodies against the HPA-1a antigen. Traditionally, it has been thought that only antibodies against platelet-specific antigens cause NAIT. Several case reports suggest that HLA class I antibodies may occasionally be involved.[25]

10.2. HLA and transplantation

10.2.1. Renal transplantation:

HLA typing was applied to kidney transplantation very soon after the first HLA determinants were characterized. The best graft survival rates occur when kidneys are obtained from HLA-identical, ABO-compatible siblings, but such donors are available for relatively few patients. It is apparent that the effect of HLA matching is significant, even with the highly efficient immunosuppression used today. There are two major priorities in renal transplantation that reduce the (already low) chance of obtaining good HLA matching. These are the need for ABO compatibility and the need for a negative T-lymphocyte crossmatch (using cytotoxicity). ABO compatibility of solid organs is the same as that of Red Blood Cells (RBC); 'A' recipient can receive from 'A' and 'AB', 'B' recipient can receive from 'B' and 'AB', 'AB' recipient can receive from 'AB', 'A' and 'B', and 'O' recipient can receive from 'O' donor only. Anti-HLA Class I antibodies present (i.e., when the T cell crossmatch is positive) at the time of transplant will cause "hyper-acute rejection" of the graft, i.e., within a few hours of transplant.

10.2.2. Liver transplantation:

Patients awaiting liver transplantation can seldom afford to wait for a well-matched graft. Therefore, liver transplantation is more involved with problems such as physical size rather than HLA. With the effects of drug tacrolimus (or cyclosporin-A) and the action of the liver itself as a form of "immunological sponge" (to mop up immune complexes), the effect of HLA matching is difficult to determine. The lymphocytotoxic T-cell crossmatch is an important factor in liver transplantation. Transplants, which are, through urgency, carried out despite a positive T-cell crossmatch, have a significantly lower success rate.

10.2.3. Bone marrow transplantation (BMT)/Haematopoietic stem cell transplantation (HSCT):

Haematopoietic stem cell transplantation (HSCT) is used to treat haematologic malignancy, severe aplastic anaemia, severe congenital immune-deficiencies and selected inherited metabolic diseases. Complete HLA matching (10 of 10 alleles of HLA-A, -B, -C, -DR -DQ) of bone marrow donor and recipient is crucial to the success of allogeneic BMT. Matching for the HLA-A, -B, -C, -DRB1, and –DQB1 loci are referred to as a 10/10 match; when HLA-DPB1 is included, it becomes a 12/12 match. Matching for HLA-A, -B, -C, and –DRB1 loci is an 8/8 match. There is still no international standard for reporting DRB3/4/5 as well as DQA1 and DPA1 mismatches. A mismatch between a recipient and donor may not only lead to rejection but also to the greater problem of graft-versus-host-disease (GVHD). Most bone marrow transplants involve HLA-identical siblings with the HLA identity confirmed by family studies and highly definitive molecular genetics techniques. Failing an HLA identical sibling being available, a close relative with very similar (e.g., one HLA antigen mismatch) may be considered. However, since 60% to 70% of potential candidates do not have a suitable family member to act as a donor, there has been interest in developing lists of tissue typed volunteers prepared to donate bone marrow or peripheral blood stem cells. Several such registries (depicted in Table 3) are established to serve as a source of HLA-matched unrelated donors. The success rates of these transplants are getting better results.

Table 3: List of International and Indian registries (arranged in alphabetical order)

Prominent International Registries	All Indian Registries
• Anthony Nolan, UK • Australian Bone Marrow donor registry (ABMDR), Australia • Bone Marrow Donors Worldwide (BMDW), • Bone Marrow Registry (BMRN), Nigeria • Chinese Marrow Donor Program (CMDP), China • Croatian Bone Marrow Donor Registry, Croatia • Cyprus Bone Marrow Donor Registry, Cyprus • Czech National Marrow Donor Registry, Czechoslovakia	• Arjanveer Foundation, New Delhi • Bangalore Medical Services Trust (BMST), Bangalore • Datri, Chennai • Genebandhu, New Delhi

Prominent International Registries	All Indian Registries
• European Marrow Donor Information System (EMDIS), Europe • National Marrow Donor Program (NMDP), US, • NHS Blood and Transplant, UK • Zentrales Knochenmarkspender-Register (ZKRD), Germany	• Jeevan Stem Cell Registry, Chennai • Marrow Donor Registry (MDRI), Mumbai

10.2.4. Heart, lung and corneal transplantation:

There has only recently been a sufficient accumulated experience to show the effect of HLA antigens in heart and lung transplantation. In heart and lung transplantation, HLA match at the DR locus is important, but there are some difficulties like ischemic times, availability of donors, and clinical need of recipients. Corneal grafts are not usually influenced by HLA matching unless being transplanted into a vascularised bed.[26]

Key points

- HLA (Human Leucocyte Antigen) refers to a cluster of highly conserved polymorphic genes on the human chromosome number six (6). HLA represents the Major Histocompatibility Complex (MHC) in humans.
- The primary biological role of HLA molecules is to regulate the immune response. It also plays a role in graft rejection and is hence termed as “transplant antigen”.
- HLA genes are closely linked, and the entire MHC is inherited as an HLA haplotype in a Mendelian fashion from each parent.
- HLA class I molecules are expressed on the surface of almost all nucleated cells and platelets, Class II molecules are expressed only on B lymphocytes, antigen-presenting cells (monocytes, macrophages, and dendritic cells), and activated T lymphocytes.
- Anti-HLA antibody is generally defined as panel reactive antibody (PRA). PRA is one of the crucial immunological factors affecting “graft survival”.
- Anti-HLA antibodies can directly recognize undamaged foreign HLA molecules on the cell surface. Therefore, Anti-HLA antibodies play a critical role in solid organ transplantation as well as in haematopoietic progenitor cell transplantation (HPCT).
- Tissue typing of the recipient and donor determines their HLA match. The MHC class I HLA-A and HLA-B and class II HLA-DR antigens are routinely determined because rejection responses most commonly occur from mismatches at these alleles.
- Preformed HLA antibodies can be detected by testing the patient’s serum against a panel of lymphocytes with “known HLA” types. The complement-mediated micro-lymphocytotoxicity (CDC) technique has been the standard for several decades, and the anti-human globulin (AHG) enhancement of the CDC method provides higher sensitivity.
- The HLA system can cause adverse immunologic effects in transfusion therapy. These effects are primarily mediated by HLA antibodies developed against the donor leucocytes contained in the cellular blood components.
- HLA antibodies induced from previous allo-sensitization (pregnancy, transfusion, or transplant) can cause febrile non-haemolytic transfusion reaction, HLA alloimmunization, and platelet refractoriness. Leucodepleted blood components reduce the chances of these adverse events.

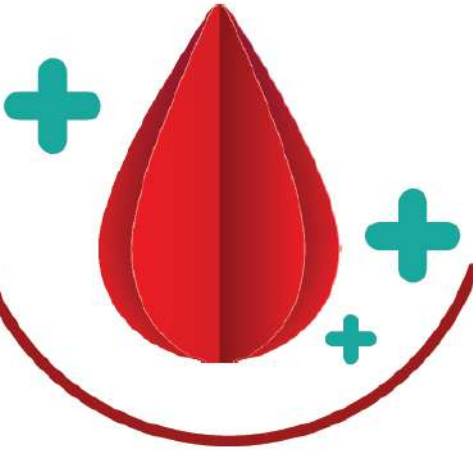
References:

1. Abbas AK, Lichtman AH, Pober JS. Cellular and Molecular Immunology. 2nd ed., Elsevier, Dunfermline, UK; 1994
2. Kurihara K, Kitada H, Miura Y, et al. Impact of flow cytometry crossmatch B-cell positivity on living renal transplantation. Transplant Proc. 2013; 45:2903-6.

3. Gebel HM, Bray RA. HLA antibody detection with solid phase assays: great expectations or expectations too great? Am J Transplant. 2014;14:1964-75.
4. Mehra NK. Defining genetic architecture of the populations in the Indian subcontinent: Impact of human leucocyte antigen diversity studies. Indian J Hum Genet. 2010;16:105-7.
5. Bodmer WF. HLA: what's in a name? A commentary on HLA nomenclature development over the years. Tissue Antigens. 1997; 49:293-6.
6. Williams TM. Human leucocyte antigen gene polymorphism and the histocompatibility laboratory. J Mol Diagn. 2001; 3:98-104.
7. Janeway CA Jr, Travers P, Walport M, et al. Immunobiology: The immune system in health and disease. 2nd edition. New York: Garland Science; 1997. Antigen recognition by T-cells.
8. Erlich H. HLA DNA typing: past, present, and future. Tissue Antigens. 2012; 80:1-11.
9. Spínola H, Bruges-Armas J, Brehm A. Discrepancies in HLA typing by PCR-SSOP and SBT techniques: a case study. Hum Biol. 2007; 79:537-43.
10. Amico P, Hönger G, Steiger J, Schaub S. Utility of the virtual crossmatch in solid organ transplantation. CurrOpin Organ Transplant. 2009; 14:656-61.
11. Ayna TK, Ciftci HS, Isitmangil G, Gurtekin M, Carin M. Flow cytometric crossmatching and panel-reactive antibodies in chronic renal failure patients. Transplant Proc. 2011; 43:805-8.
12. Kuby J, Editor. Major histocompatibility complex. Immunology. 3rd ed. Freeman, Houston; 1997. pp. 224–46.
13. Kerman RH, Susskind B, Buelow R, et al. Correlation of ELISA-detected IgG and IgA anti-HLA antibodies in pretransplant sera with renal allograft rejection. Transplantation. 1996; 62:201-5.
14. Lobashevsky AL. Methodological aspects of anti-human leucocyte antigen antibody analysis in solid organ transplantation. World J Transplant. 2014; 4:153-67.
15. Opelz G, Wujciak T, Döhler B, Scherer S, Mytilineos J. HLA compatibility and organ transplant survival. Collaborative Transplant Study. Rev Immunogenet. 1999; 1:334-42.
16. Süsal C, Pelzl S, Simon T, Opelz G. Advances in pre- and posttransplant immunologic testing in kidney transplantation. Transplant Proc. 2004; 36:29-34.
17. Holgersson J, Elsheikh E, Grufman P, Sumitran-Holgersson S, Tydén G. A case of acute vascular rejection caused by endothelial-reactive non-HLA antibodies. ClinTranspl. 2006;535-8.
18. Caro-Oleas JL, González-Escribano MF, Toro-Llamas S, et al. Donor-specific antibody detection: comparison of single antigen assay and Luminex crossmatches. Tissue Antigens. 2010; 76:398-403.
19. Bielmann D, Hönger G, Lutz D, Mihatsch MJ, Steiger J, Schaub S. Pretransplant risk assessment in renal allograft recipients using virtual crossmatching. Am J Transplant. 2007; 7:626-32.
20. Weinstock C, Schnaidt M. Human Leucocyte Antigen sensitisation and its impact on transfusion practice. Transfus Med Hemother. 2019; 46:356-69.
21. Chapman CE, Stainsby D, Jones H, et al. Ten years of haemovigilance reports of transfusion-related acute lung injury in the United Kingdom and the impact of preferential use of male donor plasma [published correction appears in Transfusion. 2011 Dec;51(12):276]. Transfusion. 2009; 49:440-52.
22. Duquesnoy RJ, Testin J, Aster RH. Variable expression of w4 and w6 on platelets: possible relevance to platelet transfusion therapy of alloimmunised thrombocytopenic patients. Transplant Proc. 1977; 9:1829-31.
23. Kerkhoffs JL, Eikenboom JC, van de Watering LM, van Wordragen-Vlaswinkel RJ, Wijermans PW, Brand A. The clinical impact of platelet refractoriness: correlation with bleeding and survival. Transfusion. 2008; 48:1959-1965.
24. Engelfriet C, Reesink H. Detection of platelet-reactive antibodies in patients who are refractory to platelet transfusions and the selection of compatible donors. Vox Sang. 2003; 84:73-88.
25. Saito S, Ota M, Komatsu Y, et al. Serologic analysis of three cases of neonatal alloimmune thrombocytopenia associated with HLA antibodies. Transfusion. 2003; 43:908-17.
26. Sheldon S, Poulton K. HLA typing and its influence on organ transplantation. Methods Mol Biol. 2006; 333:157-74.

Section 15

Biosafety



1. Introduction

Biosafety is an essential component of blood transfusion services (BTS) as the stakeholders involved are the laboratory workers, nurses, doctors, and the general public who are blood donors. The process of blood collection, processing, issue, and transfusion to recipients involves a lot of hazards and risks as well the use of blood bags, blood collected in blood bags, syringes, needles, sharps, use of kits, reagents in serology for testing blood units, and various other chemicals, thus, leading to generation of biological waste and the need for waste management and waste handling as per the statutory norms and national guidelines.

Knowledge about the methodologies and basics of biosafety is important for all waste handlers and personnel involved in BTS. Over the years, the government has taken a stand for the safety of both handlers and the public by laying down statuary rules. The act was passed by the Ministry of Environment and Forests in 1986 in India[1]. The Bio-Medical Waste (BMW) (Management and Handling) Rules were notified in July 1998,[2] making it mandatory for all occupiers (heads of institutions or controlling authorities) to treat the generated waste and dispose it without affecting the individuals involved in generation or public by at large. Thus, biosafety is an essential statutory component of BTS. Health care workers and society is exposed to risks, the probability of causing harm to humans and the environment, hazards capable and causing harm to humans and the environment and both can be controlled by measures to minimize exposure to hazards and understanding and minimizing risks.

2. Microorganism risk groups and biosafety levels

World Health Organization (WHO) has defined both microorganism risk groups and biosafety levels.[3] WHO microorganism risk groups 1,2,3,4 are decided as per relative hazard levels of infectious agents.[3] Other than risk groups, biosafety levels 1, 2, 3, and 4 have been related to the organism's risk groups and are based on operational procedures, requirements of laboratory designs, containment, practices, and procedures required to conduct work safely in the laboratory. Procedures done in the blood centre require biosafety level 2 precautions. (Table1)

Table 1. Classification of infective microorganisms by risk groups and relation with biosafety levels

Risk groups	Level of risk	Treatment and prevention	Biosafety levels	Laboratory type
Risk Group 1	No or low individual and community risk	Not required	Basic- Biosafety Level 1	Basic Teaching research
Risk Group 2	Moderate individual risk, low community risk	Effective treatment and preventive measures are available and the risk of spread of infection is limited	Basic- Biosafety Level 2	Primary Health Services; diagnostic services, research laboratories, the Blood centre

Risk Group 3	High individual risk, low community risk	Effective treatment and preventive measures are available	Containment – Biosafety Level 3	Special Diagnostic services, research
Risk Group 4	High individual and community risk	Effective treatment and preventive measures are not usually available.	Maximum containment – Biosafety Level 4	Dangerous Pathogen units

3. Blood centre: design, function and biosafety

A blood centre should have a suitable location, area, and construction to function optimally. The blood centre should be designed to ensure a safe operational environment for both the donors and the staff taking care of norms of biosafety with minimum risk to the personnel involved. In India, the design of the blood centre is controlled by the norms of the Drugs and Cosmetic act. 1940 and Rule 1945[4] and the licensing authorities have laid out certain minimum requirements that need to be followed.

The major areas of a blood centre are-

A. Blood donation complex includes
   i. Waiting area
   ii. Donor registration area
   iii. Counsellor room
   iv. Medical examination room
   v. Blood collection area
   vi. Refreshment- cum- restroom
B. Laboratory Areas
   i. Blood group serology laboratory
   ii. Infectious marker testing laboratory
C. Component preparation room
D. Washing/ Sterilization room
E. Office / Store/ Record areas

3.1 Blood donation complex

The blood donation complex is usually located in that part of the hospital or transfusion centre, which has easy access to the public without having to intermingle with patients and hospital staff. This is a sensitive area as haemoglobin testing of blood donors and blood collection is done here. This involves the use of needles which are a prime biosafety hazard. The general public has access to this area, and therefore it is all the more important to ensure the biological safety of both the staff and the blood donors. To this end, first and foremost, this area should be segregated from other areas of the blood centre except for the office area. A functional plan is advisable in which written signage and structural cues direct the donor along the "path of the donor", which refers to the sequence of rooms in the blood centre that the donor has to pass through to donate blood. Separate entry and exit for donors help smoothen the flow of donors. Overcrowding should be avoided at all costs, and unauthorized persons such as the donor's friends and relatives must not be allowed into the blood collection room. Whenever possible, the 'path of blood,' i.e., the route through which blood is taken from the blood collection room to the component preparation room and testing laboratories, should be separate from the "path of the donor' and there should be no intermingling. A sufficient area has to be provided for the staff movement. Each blood collection couch should have all the required equipment and consumables as a separate unit, and there should be no sharing between two adjacent donor couches.

The blood collection room is an area where gross quantities of blood are handled and thus at risk of spills; therefore, it is advisable to have washable floors and walls. All work surfaces in this area should be scratch, stain, moisture, and chemical resistant to be amenable to cleaning and chemical disinfection. An easily accessible area for handwashing is advisable. If space and finances permit, eyewash stations and emergency showers should also be provided. Air conditioning is essential as this reduces the incidence of adverse donor reactions and facilitates biosafety measures such as wearing protective gear like gloves and lab coats. Illumination should be adequate, and an uninterrupted power supply with a backup is essential to prevent accidents during phlebotomy and blood collection.

3.2 Component preparation and laboratory areas

The component preparation and laboratory area must be located in a more secluded part of the blood centre as access to this area is restricted to staff only. The office area should be located outside the lab area. Biosafety level II precautions apply to this area. This area should be separated from other areas by a door, and the main entry door and doors to individual areas should remain in a closed position when not being used and should be lockable. Demarcated areas with ample space for each function prevent overcrowding. The equipment required for each function must be within easy reach as this improves functioning. The laboratory rooms should have an inward flow and a negative air pressure differential. A controlled ventilation system with 100% outside air is preferable. The walls and floors of the labs should be impervious, and slip-resistant floors are desirable. The laboratory doors should have appropriate signage such as 'Restricted Entry' or 'Biohazard'. The doors, frames, casework, and benchtops should be non-absorptive (the use of organic material is to be avoided). The work surfaces must be scratch, stain, moisture, chemical, and heat resistant. They are to be continuous and compatible with adjacent and overlapping material and should not have open seams. The edges of the benchtops are to contain spills of liquids by having marine edges or drip stops. Benches, doors, door handles, drawers, furniture, etc., having rounded rims and corners are preferable. The drawers are to be one-piece construction and must be equipped with catches to prevent drawers from being pulled out of the cabinet. The doors of cabinets should not be self-closing as this increases the risk of spills. The ergonomics of the workbenches, cabinets, stools, and chairs should be carefully studied, and the plan should be such that the staff should not have to work in awkward postures such as reaching overhead, twisting, bending too often, kneeling, or squatting. A handwashing sink at the exit of the laboratory is essential, while eyewash and emergency showers are advisable where possible. There should be separate hanging areas for lab coats and street clothes.

3.3 Washing and sterilization room

The washing and decontamination of laboratory glassware and autoclaving of blood bags take place here. The walls, floors, and work surfaces should be impervious, resistant to chemicals, and washable. There should be two sinks, one for pre-cleaning, i.e., removing gross dirt, and the second one for cleaning. The sinks used for washing should be deep to avoid splattering of water; they should be made of ceramic as hypochlorite, one of the major disinfectants used in the blood centre, erodes metal. There should be heavy-duty exhausts in this area to remove the steam from washing and autoclaving. Fire and electrical safety are major concerns in this area and should be looked into. Clean glassware is very important to avoid any erroneous reactions in the laboratory, and the standard operating procedure must exist for the same. Glassware soaked in hypochlorite should be cleaned with mild soap/ cleaning powder and preferably rinsed with lukewarm water. Test tubes which are the major glassware used, can be dried in the oven overnight before use. Infection control standards need to be kept in mind in maintaining cleaning and sterilization in laboratories. Planning for environmental and biological safety should begin when the blood centre is envisioned. A sound design with inbuilt safety features is the key to a safe working environment.

4. Universal precautions for laboratory staff

- Wear gloves when handling infectious material.
- Do not touch eyes, nose, or skin with gloved hands.

- Do not leave the workplace or walk around wearing gloves; remove gloves after completion of work.
- Wash hands with soap and water immediately after any contamination.
- Wear a laboratory apron only when working in the laboratory.
- Entry to the laboratory should be restricted; doors should have a 'Biohazard' sign and 'Restricted Entry' labels.
- Keep the laboratory clean, neat, and free from extraneous materials and equipment.
- Disinfect work surfaces at the end of procedures and each working day with 1% hypochlorite solution.
- Avoid the usage of needles and other sharp instruments, and if in use, place them in a puncture-resistant container.
- Do not recap used needles and do not remove needles from syringes.
- Never pipette by mouth; use pipetting aid.
- Do not eat, drink, smoke, apply cosmetics or apply contact lenses in the laboratory.
- Remove gloves before attending the telephone while working in the laboratory.
- Use separate markers, pens, and other stationery for laboratory work.

5. Points of special reference for blood transfusion service and biosafety

5.1 Blood bags:

While procuring blood bags for the blood centre, specifications should include safety features such as needle protectors that can cover the needle on withdrawal of the needle from the donor's arm, thus avoiding the risk of accidental puncture to the staff handling the blood bag. The tubing of the blood bag should be at least 80 cm in length to allow for easy manoeuvring during phlebotomy. Vacuum tubes are optimal for sample collection, and it is even better if they are integrated with the blood bag system.

5.2 Tube sealers:

A tube sealer is a device that has dramatically improved biosafety in the blood collection room by doing away with the need for a sharp object like a blade or scissor to cut the tubing after blood collection while also eliminating the risk of blood oozing from the cut ends.

5.3 Sample collection:

The blood samples for grouping and infectious marker testing should be collected on the donor's couch so that the staff does not carry the bare needles around. Ideally, there should be a tube sealer and sharps disposal container beside each donor couch, but if this is not possible due to resource constraints, the staff must be instructed to carry the needle inserted in a vacutainer/ test tube to the disposal site.

5.4 Points to remember while collecting blood/blood samples

- Always use gloves
- Take care to avoid contamination of hands
- The tourniquet should be removed before the needle is withdrawn
- Place needles and syringes in a puncture-resistant container containing disinfectant (disinfection at source)
- Do not bend/break or recap used needles
- Wash hands following completion of sample collection
- Never pass used needles directly from one person to another
- Always dispose off your sharps

5.5 Laboratory work rules and precautions

- Bench-tops of impervious material should be decontaminated daily with hypochlorite.
- Laboratory rooms should be with closable doors and a 'one pass in flow' air system.
- Sinks, and waste decontamination facilities should be available within the work area.
- Gowns and gloves should be used routinely.
- Mouth pipetting should not be allowed.
- Eating, drinking, smoking, etc. should not be allowed in the work area.
- High-risk activities should be clearly separated from low-risk activities.
- Extreme caution in handling needles should be taken.
- Any accidental exposure should be immediately reported to the laboratory supervisor.

➢ **Safe use of pipette and pipetting aids**

- A pipetting aid should always be used.
- Pipetting by mouth should be prohibited.
- All pipettes should have cotton plugs to reduce contamination of pipetting devices.
- Air should not be blown through an infectious sample.
- The liquid should not be expelled forcibly from pipettes by vigorous shaking as aerosols are formed.
- An absorbent blotting paper should be used for soaking the last drop of liquid in a pipette. This paper should be soaked in a disinfectant before disposal.
- Contaminated pipettes should be soaked in a disinfectant overnight.
- Pipetting aids should be wiped with a disinfectant after use.

➢ **Safe use of laboratory centrifuges**

- Always ensure that loads are evenly balanced before a run.
- Never attempt to open the door while the rotor is spinning or stop the rotor by hand.
- Do not attempt to move the centrifuge while it is in operation.
- Do not fill a tube with more than ¾ of its capacity.
- When possible, samples should be aspirated rather than poured from centrifuge tubes.
- When washing small quantities of red cells, invert tubes to discard liquid by blotting on a blotting paper to remove the last drop.
- Do not use the centrifuge at speeds higher than the manufacturers' maximum speed limit.

➢ **Management of spills and leaks in the centrifuge**

- Close the centrifuge lid immediately with the samples remaining inside and turn the centrifuge off and allow it to stand undisturbed.
- Wait for 30 minutes.
- Wear protective gear. Wipe and disinfect the centrifuge with suitable disinfectant available.
- Wipe the centrifuge with a surface cleaning solution.

➢ **Disinfection and cleaning of work surfaces in the blood centre**

Hypochlorite($NaOCl^-$) (70.91 gm of available Cl^-/ mole) is the most commonly used disinfectant in the blood centre.

- In solution, $HOCl^-$ liberates nascent oxygen, which-
  - Combines with components of the microbe and causes oxidative damage.
  - Forms N-chloro compounds that interfere with cell metabolism.
  - It inactivates cell-free virus within 30 seconds of contact at 1% (10,000 ppm).
- Advantages-
  - Cheap and easy to handle.

- Powerful germicide against a wide range of micro-organisms including viruses.
- Non-poisonous to humans at used concentration.
- Colourless and non-staining.

 Disadvantages-

- Corrodes metal.
- The concentrated solution is toxic (pH – 9-10).
- Causes corrosion of mucous membranes.

 Method of use-

- It is usually available as a 40% solution. Working dilutions should be prepared on each day of use.
- 10,000 ppm of available chlorine should be present (1% solution).
- There should be a holding time of at least 30 minutes.
- Use plastic buckets only.
- Do not flush in metal washbasins.

➢ **Disinfection of work surfaces**

- All work surfaces, walls and floors of the laboratory should be made of impervious washable surfaces.
- Every morning and at the end of each duty, the work surfaces should be cleaned and wiped with a disposable wiping sheet dipped in 1% hypochlorite. It is essential to use thick gloves for cleaning work surfaces.
- It is the person's responsibility working at each workspace to ensure that it is clean and disinfected.
- Laboratories handling blood should be kept scrupulously clean and free of bloodstains.

➢ **Managing spills of blood and plasma**

- Clean the area taking all precautions (gloves, masks, and protective gown).
- Cover the infected material with a filter paper sheet or absorbent material.
- Pour 5% sodium hypochlorite over and around the absorbent material and leave for 10-15 minutes
- Clean the mixture of disinfectant and spilled material with absorbent material.
- Place it in a contaminated waste container.
- Wipe the surface again with disinfectant.
- Broken glass/plastic should be swept with a brush and dustpan (Do not use hands).
- All spills and accidents are to be reported to the laboratory supervisor.

6. Biomedical waste management in reference to BTS

6.1 General principles:

Any health care facility, blood centre, or laboratory generating biomedical waste shall be called as occupier. Collection, transport, treatment, and final disposal are done by the Common Biomedical Waste treatment facility (CBWTF). Current BMW Rules for blood safety are to be followed for different types of waste generated in a BTS. The amount of waste generated depends on the scale of activities the BTS carries out.

Waste can be classified based on the level of hazard as follows:

- **Hazardous waste:** Any waste poses a substantial or potential threat to human health or the environment. The threat may be due to the quantity or concentration of the waste or to its physical, chemical, radioactive, genotoxic, or infectious characteristics.
- **Non-hazardous waste:** It is generally material that is not contaminated with blood or other body fluids or chemicals and includes items such as packaging, boxes, and wrappings.

Handling the waste generated in a BTS poses a risk of infection. Therefore, a standard operating procedure (SOP) should exist so that all waste generated within the BTS should always follow an appropriate and well defined process from its point of generation until its final disposal. The institute's policy should be followed in institute-based blood centres. A contract should be made with biomedical waste management and disposal agencies approved by State Pollution Control Board. The waste should be handed over in colour coded and labelled / barcoded bags, and the records of the same should be maintained. Biohazard register should be maintained as per the requirement of the Drugs and Cosmetics Act, 1940 and rules 1945, and all other required records must be maintained.

5.2 Waste segregation:

The waste generated should be segregated at the point of generation both in the blood centre and at the camp site. Waste management is equally important, and the waste should be carried from the blood donation camp site back to the blood centre with special consideration for the transportation of sharps and infectious waste generated. Waste is segregated as biomedical and general waste. The biomedical waste as per the category Yellow, Red, White (Translucent), and Blue goes in the yellow coloured non-chlorinated plastic bags, red coloured non-chlorinated plastic bags or containers, cardboard boxes with blue coloured markings, and white translucent containers respectively as per the BMW rules 2016, Schedule I, part I.

5.3 Waste storage

- The waste should be stored in a biomedical waste holding area for not more than 24 hours and should be given for disposal to CBWTF.
- Hazardous and Non – hazardous waste should be stored separately.

5.4 Waste treatment

- Autoclaving at a temperature of 121^0C with a pressure of 15psi for a minimum of 60 minutes (residence time) is the preferred method for infectious waste treatment. An autoclave of a capacity that is appropriate to the needs of the BTS should be used.
- Place the waste to be treated into an autoclavable polythene bag and open the mouth of the polythene bag before placing it in the autoclave chamber to facilitate the mixing of hot steam with waste. Non-hazardous waste does not require treatment. Chemical treatment using at least 10% sodium hypochlorite having 30% residual chlorine for twenty minutes or any other equivalent chemical reagent that should demonstrate 10^4 log reduction efficiency for microorganisms as given in Schedule- III of BMW 2016.
- All laboratory and highly infectious waste should be pre-treated onsite before sending to CBWTF.

5.5 Waste disposal

- Appropriately treated waste can be sent to an approved CBWTF approved by the pollution control board of the area for disposal.
- Non-hazardous general waste can be disposed of together with domestic waste or municipal waste.
- Incineration or plasma pyrolysis or deep burial for category yellow, pre-treatment/ disinfection of chemical liquid waste with pre-treatment onsite, autoclaving or microwaving or hydroclaving by shredding or mutilation or sterilization for the red and white category, and disinfection of washed glass waste after cleaning with detergent and sodium hypochlorite followed by autoclaving or microwaving or hydroclaving are to be done by CBWTF as methods of final disposal.
- Hazardous chemical/ inflammable waste should be disposed of according to the manufacturer's instructions.
- Disposal of radioactive products: Modern transfusion services have the facility of blood centre irradiators for irradiating cellular blood products. However, due to the self-contained nature of irradiators, the blood products which are irradiated are not radioactive. Thus, irradiated blood products are treated as infectious waste.

5.6 Health hazards associated with poor waste management

- Injuries from sharps – applicable to all categories of BTS staff.
- Risk of infection (e.g., HIV, HBV, and other TTIs) – applicable to waste handlers and the general public.
- Exposure to harmful toxins like dioxin and furans – applicable to BTS staff, waste handlers, and the public.

Table 2. Segregation, treatment, and disposal of biomedical waste in different areas of BTS.

S. No.	Area	Type of waste generated	Segregation in colour coded containers /Bags
1.	Donor Screening Area	Swabs	Yellow
		Lancets	White Translucent Puncture Proof Container
		Micropipette tip	Red
		Gloves	Red
		Adhesive tapes	Red
		Cuvettes	Red
		Copper sulphate solution	Yellow
2	Blood collection Room	Swabs	Yellow
		Bag needle	White translucent containers
		Blood bag tubing without needle	Yellow
		Gloves	Red
		Wrapper (all labels)	Black
		Adhesive tapes	Red
3	Component Laboratory	Blood bag tubing – with blood	Yellow
		Buffy coat bags	Yellow
		Inline filters	Yellow
		Under collected/ over collected/ red cell contaminated components/ lipemic blood bags	Yellow
		Ruptured blood bags	Yellow
		Gloves	Red
4	TTI Lab	Sample tubes (glass)	Blue
		Sample tubes (plastic)	Red
		Discarded plates	Red
		Tips	Red
		Washer waste	Treatment and Yellow
		Chemical reagents, kits, controls, Rapid cards	Yellow
		Reactive bags	Autoclave/ Yellow
		Reactive tubes	Blue
		Broken blood sample tubes (glass)	Blue
		Gloves	Red
5	Serology Lab	Sample vials (glass)	Blue
		Blood bag tubing	Red
		Test tubes	Red

S. No.	Area	Type of waste generated	Segregation in colour coded containers /Bags
		Reagent vials	Blue
		Gel cards	Yellow
		Microtips	Red
		Glass Pasteur pipettes	Blue
		Analyzer reagent vials and plates	Yellow
		Gloves	Red
		Blotting sheets/ swabs	Yellow
		Vacutainers	Blue
6	Apheresis Room	Discarded kits and tubing	Red
		Gloves	Red
		Swabs	Yellow
		Plasma collected after the therapeutic plasma exchange procedure	Yellow
		Reagents	Yellow

5.6 Salient features of Biomedical Waste Management (Amendment) Rules 2018

Bio-medical waste generators including hospitals, nursing homes, clinics, dispensaries, veterinary institutions, animal houses, pathological laboratories, blood centres, health care facilities, and clinical establishments will have to phase out chlorinated plastic bags (excluding blood bags) and gloves by March 27, 2019.[5]

I. All healthcare facilities shall make available the annual report on their website within a period of two years from the date of publication of the BMW (Amendment) Rules, 2018.[5]

II. Operators of common bio-medical waste treatment and disposal facilities shall establish a barcoding and global positioning system for handling bio-medical waste in accordance with guidelines issued by the Central Pollution Control Board by March 27, 2019.

III. The State, Pollution Control Boards/ Pollution Control Committees have to compile, review and analyze the information received and send this information to the Central Pollution Control Board in a new Form (Form IV A), which seeks detailed information regarding district-wise bio-medical waste generation, information on Health Care Facilities having captive treatment facilities, information on common bio-medical waste treatment and disposal facilities.

IV. Every occupier, i.e. a person having administrative control over the institution and the premises generating biomedical waste, shall pre-treat the laboratory waste, microbiological waste, blood samples, and blood bags through disinfection or sterilization on-site in the manner as prescribed by the World Health Organization (WHO) or guidelines on the safe management of wastes from health care activities and WHO Blue Book 2014 and then sent to the common bio-medical waste treatment facility for final disposal.

7. Conclusion

Biomedical waste generation is inevitable in health care settings, BTS being no exception. Biosafety is an integral part of any BTS to prevent harmful events from occurring in the various process areas of blood centres, including blood collection areas, laboratory areas, and other functional areas. Provisions have been made for ensuring biosafety in the regulatory norms, including both the Drugs and Cosmetics Act 1940 and Rule 1945, and BMW rules and responsibility lie with the generator of waste. Guidelines for biosafety based on regulatory norms for waste segregation, transportation, and disposal must be followed by the personnel involved in the day-to-day activities of BTS.

Key Points

- Biosafety is an important part of the management of the BTS.
- Levels of biosafety are related to microorganism risk levels, and Biosafety level 2 is to be adhered to in blood centres.
- The level involves moderate individual risk, and low community risk yet holds the vital key for prevention from infectious agents.
- Personal safety during work should be a part of departmental SOP and rigorously practised.
- It is the responsibility of the occupier (In-charge of BTS / Head of the institution) that the waste generated is segregated, transported, and disposed of as per the norms.
- The design and function of BTS directly affect biosafety measures which are mandatory to maintain a safe work environment.
- All laboratory staff must follow universal precautions.
- The use of safe blood bags, tube sealers, safe collection of samples, and stringent laboratory rules can result in appropriate biosafety in BTS.
- Disinfection, and spill management is required for ensuring biosafety in BTS.
- Biomedical Waste Management rules 2016 and the amendments are the statutory regulations to be followed along with the Drugs and Cosmetics Act 1940 and Rule 1945 by all BTS.

Bibliography

1. The Gazette of India Biomedical Wastes (Management and Handling) Rules. India: Ministry of Environment and Forests, Government of India; Notification Dated; 20th July 1998.
2. Bio-Medical Waste Management Rules. 2016 Published in the Gazette of India, Extraordinary, Part II, Section 3, Sub-Section (i), Government of India Ministry of Environment, Forest and Climate Change. Notification; New Delhi, the 28th March 2016.
3. WHO. Laboratory biosafety manual: World Health Organization; Geneva, 2004.
4. Drugs and Cosmetics Act and Rules 1940, and further amendment, 11 March 2020, Section XB and XIIB, Ministry of Health and Family Welfare Govt. Of India (https://cdsco.gov.in/opencms/opencms/system/modules/CDSCO.WEB/elements/download_file_division.jsp?num_id=NTc2MQ==
5. Ministry of Environment. Bio-Medical Waste Management (Amendment) Rules, 2018. Forest and Climate Change Notification New Delhi. 2018.

Section 16

Disaster Management

1. Introduction

Disaster is defined as a sudden, calamitous event that seriously disrupts the functioning of a community or society and causes human, material, and economic or environmental losses that exceed the community's or society's ability to cope using its own resources.

Disasters have existed ever since the history of mankind and had a long-lasting impact on people. They can be classified as natural disasters (e.g., earthquakes, tsunamis, volcanic eruptions, etc.) or man-made disasters (e.g., conventional wars, nuclear attacks, bombing, stampedes, fires, chemical warfare, biological warfare, etc.). Disasters result due to a combination of hazards, vulnerability, and the inability to reduce the potential negative consequences of risk. Thus, no country is immune from disasters, though vulnerability to disaster varies.

A 'Hazard' can be defined as any phenomenon that has the potential to cause disruption or damage to people and their environment.

$$\text{Disaster} = \text{Vulnerability} + \text{Hazard/Capacity} + \text{Capability}^{1}$$

A disaster happens when a hazard impacts the vulnerable population and causes damage, casualties, and disruption.

A Mass Casualty Event (MCE) may be defined as a 'single or simultaneous event(s) or other circumstances where the normal major incident response of one or several health organizations must be augmented by extraordinary measures to maintain an efficient, suitable and sustainable response'. It is an event that generates more patients at one time than locally available resources can manage using routine procedures. It requires exceptional emergency arrangements and additional or extraordinary assistance. MCE is considered a more appropriate term than a disaster as it is healthcare-specific and addresses the load on healthcare.[1-3] MCE implies that critical sufficiency and supply of resources, including blood, may be constrained.[4]

2. Disaster management

It can be defined as the organization and management of resources and responsibilities for dealing with all humanitarian aspects of emergencies to lessen the impact of disasters. Disaster management refers to the conservation of lives and property during a natural or man-made disaster.

In India, National Disaster Management Authority (NDMA) is an apex body (an agency of the Ministry of Home Affairs) whose primary purpose is to coordinate response to natural or man-made disasters and capacity-building in disaster resiliency and crisis response. NDMA is responsible for framing policies, laying down guidelines and best practices, and coordinating with the State Disaster Management Authorities (SDMAs).

3. Phases of disaster management

Disaster management is a cycle that can be classified into four continuous phases. These four major functional areas of the disaster management cycle include:

1) Disaster Mitigation
2) Disaster Preparedness
3) Disaster Response and
4) Recovery.

3.1 Disaster mitigation

Disaster mitigation measures are those that eliminate or reduce the impacts and risks of hazards (and hence, reduce loss of life and property) through proactive measures taken before an emergency or disaster occurs. Disasters can happen at anytime and anyplace; their human and financial consequences are hard to predict. Effective mitigation requires understanding local risks, addressing the hard choices, and investing in long-term community well-being. Examples of mitigation include planning and zoning, floodplain laws, capital improvement programs, open space preservation, stormwater management regulations, modifications of structures, structural retrofit, relocation, etc.

3.2 Disaster preparedness

Disaster preparedness refers to measures taken to prepare for and reduce the effects of disasters. Preparedness focuses on areas that cannot be addressed through mitigation efforts alone. This enables governments, organizations, communities, and individuals to respond rapidly and effectively to disaster situations. This includes the formulation of viable disaster plans, the maintenance of resources, and the training of personnel. Necessary legislation support preparedness. Organizing, planning, coordinating, resource planning and training are its major concerns. Once a plan is created, it should be practiced and improved upon, so flaws in the plan can be corrected and the best possible care for patients can be provided.

3.3 Disaster response

The response involves actions carried out immediately before, during, and after a hazard impact and aims to save lives, reduce economic losses, and alleviate suffering. The response phase includes the mobilization of the necessary emergency services and first responders in the disaster area. A well-rehearsed emergency plan developed as part of the preparedness phase enables efficient coordination of resources.

3.4 Recovery

Recovery efforts begin once the initial response actions have taken place. These efforts focus on restoring critical systems (e.g., communications, water, power, and sewage) to resume and maintain business operations. In addition, recovery efforts complete the cycle of disaster management by providing insights into additional mitigation strategies deployed for future emergencies.

Recovery differs from the response phase in its focus; recovery efforts are concerned with issues and decisions that must be made after immediate needs are addressed. Recovery efforts are primarily concerned with rebuilding destroyed property, re-employment, and the repair of other essential infrastructure.

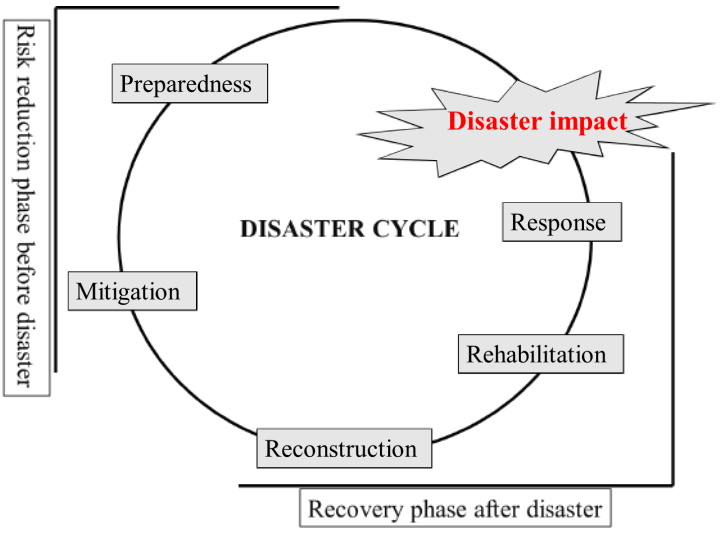


Figure 1: Disaster management cycle

4. Disaster management from a Transfusion Medicine perspective

The word disaster refers to any natural or man-made event/act that "suddenly requires a much larger amount of blood than usual OR temporarily restricts or eliminates a blood centre's ability to collect, test, process, and distribute blood OR temporarily restricts or prevents the local population from donating blood, or restricts or prevents the use of the available inventory of blood products and thus requires immediate replacement or resupply of the region's blood inventory from another region OR creates a sudden influx of donors, requiring accelerated drawing of blood to meet an emergent need elsewhere".[5]

Transfusion services are critical to most disaster situations as blood may be required to manage the injured after a disaster. In addition, transfusion services may also get affected by a disaster like flooding, fire, earthquake, or an act of terrorism, affecting their ability to function. Thus, disaster planning and management is an important element of the functioning of transfusion services which may be done as a part of a hospital disaster management plan in case of hospital-based blood centres or an independent disaster plan in case of stand-alone blood centres.

4.1 Disaster preparedness by blood transfusion services

Transfusion preparedness involves plans that have been developed and tested so that the blood service can respond successfully to the emergency. Disaster preparedness by blood transfusion services should be done keeping in mind the following aims

1. To maintain a sufficient inventory of blood components at a critical level and safeguard the existing stock of blood components
2. To be able to determine the blood need depending on the severity of the disaster
3. To maintain an adequate inventory of consumables required to operate the transfusion services in the event of a disaster
4. To coordinate with other hospital care areas as well as the hospital disaster management services in a smooth manner
5. To have an effective transport plan to provide and receive blood and blood components from other blood centres
6. To have an efficient communication plan with the public and blood donors to manage blood inventory
7. To safeguard the health, safety, and welfare of donors, staff, and visitor

Developing a disaster plan is a multistep process that includes determining the need for blood /components, routes of communication, how to mobilize hospital staff, how to procure more blood /components, etc.

To fulfill the aims described above, each transfusion service should develop a plan. Some of the points which may be considered while preparing an SOP for disaster planning are provided below.

1. Coordination with the hospital disaster management committee and list of people to be contacted in case of a disaster from the blood centre. Similarly, the contact details of the person in the disaster management committee who would coordinate with the blood centre should also be documented.
2. Blood centre should calculate their average daily requirement and should have plans to maintain a safety stock (buffer stock), and they should have defined critical inventory levels.
3. Sufficient storage facilities and alternative storage facilities should be made available and documented
4. Prepare a list of critical products and supplies related to collecting, processing, controlling–temperature transport, and storage.
5. Supplier information for critical consumables as well as alternative arrangements should be available. Efforts should be made to have one or two local suppliers to have an uninterrupted supply of consumables.
6. Documented contact details of the transport services and contact details of other blood centres in the vicinity should be kept handy.
7. A plan to spread correct information to society using pre-identified communication mediums should be available. Contact details of appropriate authorities responsible for handling messages to the public and blood donors should be made available
8. Contact details of emergency officials, including power, fuel, and water supplies, should be available.
9. Staff should be designated to perform specific roles in times of disaster, and periodic training and mock drills should be conducted.
10. Plan for staff safety and evacuation routes from the blood centres must be identified and displayed clearly.

Transfusion plans should be nested within wider emergency and healthcare planning and not considered in isolation.

4.2 Disaster response in blood transfusion services

The response involves putting plans into action, i.e., responding to the event as it unfolds and having the structures and processes in place to do this.

If a disaster is notified to the blood centre, it should activate the disaster management plan.

Whenever MCE is declared, the initial briefing is crucial. It should cover:

1. Where exactly incident occurred
2. Type of incident: bombing, shooting, major crash, earthquake, etc.
3. Disruption to transport, access, utilities, travel, etc.
4. Potential number of casualties and type of injuries
5. Destination of casualties, etc.

The following things may progress simultaneously:

1. The person designated to communicate with the hospital disaster management services should identify the severity of the event and its effect on the transfusion services. The same should be communicated to the blood centre Incharge. Communication with other services such as power, water, and emergency services should also be established. Additional staff should also be mobilized depending on the disaster-related expected admissions.
2. An assessment of blood inventory should be done, and additional requirements arising out of the event should be calculated as below.

Firstly, the total expected admissions in the hospital should be calculated.

Hospital admissions expected (disaster related) = Total current hospital admissions + Total potential for expected admissions

Secondly, an in-hand inventory of group O red blood cells that could be utilized for disaster-related services should be assessed. This may be calculated by subtracting the number of O group red cells required for non-disaster needs/ emergencies from the available inventory of O red cells.

Then calculate the expected number of red cells required for disaster needs by multiplying the number of disaster-related expected admissions by three. Several studies have identified that an average of 3 red cell units may be required per admitted patient in case of disaster.[6,7]

Once the required units are calculated, one should subtract the available units of the O group from the required units to calculate the additional units that may be required.

Blood planning should include a pre-determined first-hour capability (total of P1 and P2s), which can be used to support transfusion planning for the hospital and the blood service. For demand planning, the estimated transfusion requirement (in the first 24 hours) for the P2 patient is 2-4 units of red cells and for the P1 patient is approx. 8 units of red cells, 8 units of FFPs, and 2 units of adult therapeutic dose platelets (pool of 4 each).[8]

3. The blood centre should then contact other unaffected blood centres to arrange additional inventory. Contact with transport facilities should be made for the same.
4. The blood centre should also assess critical supplies depending on the additional needs. Suppliers should be informed to be ready to supply additional inventory of consumables and critical supplies.
5. Blood donors should be communicated with depending on the calculated needs using mass communication mediums. Correct dissemination of information is necessary as incorrect information may lead to crowding, leading to disruption of donation services. Over-collection of blood components during a disaster may also lead to wastage.
6. In addition to managing inventory and donors, the blood centre should also focus on transfusion-related problems. For example, patient identification is a big challenge in mass casualty events and may lead to mis-transfusion if not addressed. Details on how to manage patient identification issues and massive transfusion protocols are available in section 8.

5. Business operations planning

Blood services must plan for both emergency services as well as business operations. Blood services must have appropriate and well-tested business continuity plans in place so that they can return to normal operations as soon as possible. Each BTS must ensure that it's critical functions of manufacturing, testing and supply management have contingency arrangements in place. Continuity of operations plan (COOP) is designed to ensure the continuity of operations of essential functions in an emergency or disaster. AABB task force has provided the list of essential functions to consider in a COOP.

Essential Elements of COOP:[5]

- Identify essential processes and functions required for continued operations: e.g., hospitals must ensure that they can transfuse blood to patients in need.
- Develop decision trees for the implementation of the COOP.
- Consider alternative facility options.
- Establish safety and security plans for staff facilities and fleet vehicles.
- Identify and ensure protection and survivability of vital records and databases: Periodic backup of computerized records should be taken on a duplicate server.
- Review insurance coverage and ensure adequacy for potential risks.
- Establish minimum cash reserves necessary to continue operations for 90 days.

- Develop an emergency communications plan (ECP): In the event of a disaster, telecommunication systems may become overloaded or jammed. In addition, computer systems may get disrupted due to loss of power. This may cause generalized confusion and an inability to take rapid decisions. Therefore, ECP should be in place to ensure that staff can communicate both internally and externally.

Communication should also be established with local/ regional/ state/national emergency response organizations.

- Establish a chain of command and order of succession for decision-making authority.
- Develop a plan to handle media issues.
- Identify spokespersons and train them in risk and emergency communications.
- Plan to maintain supplies and logistical support for operations.
- Evaluate utility needs and develop contracts or memorandums of an agreement to ensure replenishment and restoration of essential utilities.
- Review information technology systems and develop redundancies to ensure that vital systems and their supporting subsystems remain operational during an emergency.
- Identify staffing issues, including essential personnel and key contacts necessary to carry out essential functions and human resource considerations of employee compensation and benefits during and after the disaster.
- Develop procedures for transitioning back to normal operations.
- Identify and maintain key contact lists

6. Managing donor reaction in disasters

There is an increased willingness of donors to donate blood in events of disaster or mass casualties. This is a natural human response as people want to help by donating blood in times of need. Past experience has shown that immediate blood needs can mostly be met by stock already in place, and there is no immediate need for excessive collection.[5]

It is very important to appropriately manage the surge in donor response, as blood services need to ensure adequate blood supply. At the same time, unnecessary and exaggerated donor responses can lead to the wastage of blood and resources; and put pressure on already stressed blood services.[4-6]

It is recommended that there should be a pre-prepared and tested 'Donor surge plan' (in the preparation phase of disaster management), which should be activated at the time of disaster. Predetermined strategies should be used to manage large numbers of donors.[8] Effective communication with donors is very important.

Blood collection should be guided by the medical need for blood, collection capacity of blood services (including staffing, collection, testing, storage, etc.), priority blood groups required, etc. Blood services should work closely with donors to ensure a safe and sustainable supply of blood. Clear and precise messages should be communicated to the public through media on the actual blood requirement. Communication should emphasize enrolment as regular voluntary blood donors to ensure the sufficiency of blood at all times rather than excessive collections in the immediate post-disaster phase.

Key points

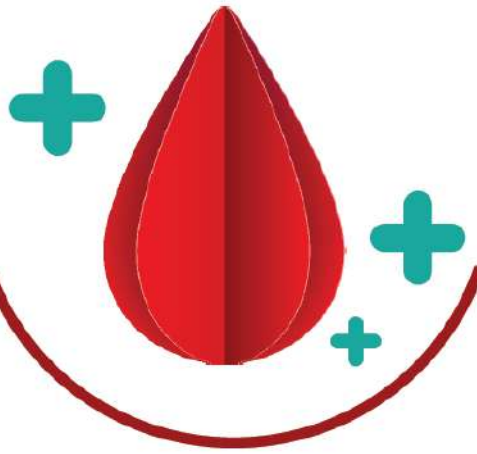
- Disaster is defined as a sudden, calamitous event that seriously disrupts the functioning of a community or society and causes human, material, and economic or environmental losses that exceed the community's or society's ability to cope using its own resources.
- Disaster Management can be defined as the organization and management of resources and responsibilities for dealing with all humanitarian aspects of emergencies; it includes disaster mitigation, preparedness, response, and recovery.

- Transfusion planning is an important and integral part of disaster planning. It involves plans that have been developed and tested so that the blood service can respond successfully to the emergency.
- Transfusion plans should be holistic and work within wider emergency and healthcare planning, including business continuity. In addition, plans should work within the regulatory and legislative framework.
- Regular staff training and robust interdepartmental communication are very important for successful disaster management.
- Blood services must have robust business continuity plans in place to ensure that their critical functions are not affected.
- The surge in donor response should be managed appropriately.

References:

1. Doughty H, Strandenes G. Whole blood in disaster and major incident planning. ISBT Science series;(2019) 14, 323–331
2. Glasgow SM: Modelling Red Blood Cell Provision in Mass Casualty Events. PhD Thesis Queen Mary Univ Lond. 2015.
3. WHO. Mass Casualty Management Systems: Strategies and guidelines for building health sector capacity. 2007. Available at https://www.who.int/hac/techguidance/MCM_inside_Jul07.pdf accessed on 20/12/2020.
4. Doughty H, Rackham R. Transfusion emergency preparedness for mass casualty events. ISBT Science Series 2019; 14: 77–83
5. Disaster Operations Handbook. AABB 2008, 2nd ed. Bethesda available at https://www.aabb.org/docs/default-source/default-document-library/about/disaster-operations-handbook.pdf?sfvrsn=619c092c0 accessed on 23/11/2020
6. Bala M, Kaufman T, Keidar A, et al. Defining the need for blood and blood products transfusion following suicide bombing attacks on a civilian population: a level I single centre experience. Injury 2014; 45:50-5.
7. Dann EJ, Bonstein L, Arbov L, et al. Blood Bank protocols for large scale civilian casualty events: experience from terrorist bombing in Israel. Transfusion Med 2017; 135-9.
8. Clinical guidelines for Major Incidents and Mass casualty events. NHS England 2018 v1.0. available at: https://www.england.nhs.uk/publication/clinical-guidelines-for-major-incidents-and-mass-casualty-events.

Section 17



Stem Cells and Cord Blood Banking

1. Introduction

Haematopoietic stem cells (HSC) are primitive pluripotent cells capable of self-renewal and differentiation into any cells of haematopoietic lineage (lymphocytes, monocytes, granulocytes, erythrocytes, and platelets). Clinically, HSCs can fully reconstitute the functions of marrow when transplanted into susceptible recipients. Therefore, HSC transplantation has been increasingly utilized to treat different haematologic and non-haematologic conditions.

2. Sources of stem cells:

The choice of the source of stem cells is determined by several variables, including the availability of an adequately matched donor.

The sources of stem cells for haematopoietic stem cell transplantation (HSCT) areas are listed in Table 1.

Table 1: Sources of stem cells

Bone marrow	Peripheral blood	Umbilical cord blood (UCB)
• The traditional source of progenitor cells • The collection is done under general anaesthesia • Relatively low T cell content • A higher level of human leukocyte antigen (HLA) matching is required • Rapid engraftment as compared to UCB transplant	• Commonly used source for haematopoietic stem cells. • Requires mobilization of donors or patients with chemotherapy, haematopoietic growth factors, or both. • Predominantly used in allogeneic transplants • A higher level of human leukocyte antigen (HLA) matching is required • Rapid engraftment as compared to UCB transplant	• Collected from the placenta after delivery (approximately 50-200 ml) • Stored either for use by the neonate in later life or cryopreserved after HLA typing for use by another recipient • A lower level of human leukocyte antigen (HLA) matching is required[1] • UCB transplant may be the best alternative when only 1 and 2 allele-mismatched donor units are available • Relatively rapid availability of UCB units compared to the marrow and peripheral blood products

3. Collection of haematopoietic stem cells:

Haematopoietic stem cells are normally found in the marrow, from peripheral blood or cord blood. According to International Bone Marrow Transplant Registry (IBMTR), peripheral blood stem cells (PBSCs) are used in

most autologous and allogeneic stem cell transplantation. However, in some cases, marrow stem cells are preferred.

3.1. Collection of bone marrow:

Typically, marrow is harvested in a steady-state (i.e., without growth factors or chemotherapy priming). Marrow donors must be physically suitable for donation after undertaking the donor's medical history. Relevant donor screening, infectious disease testing, and HLA compatibility testing must be done. Marrow harvest is an invasive procedure performed under sterile conditions in the operation theatre under general anaesthesia. The donor must be able to tolerate the volume loss.

The volume of marrow collected is based on the dose of cells desired for transplant, which is based on the size/ weight of the patient with a minimum of 2.0×10^8 nucleated cells/kg needed to facilitate efficient engraftment. The "Be the Match" registry limits the volume collected from a marrow donor to 20 mL/kg.[2]

3.1.1. Marrow harvest technique:

The marrow harvest technique varies considerably depending on the institutional practice.

- In general, an 11-14 gauge needle on a syringe flushed with anticoagulant is recommended. It is inserted into the posterior iliac crest, and approximately 5 mL of marrow is aspirated.
- The needle and syringe are then rotated to a different vector, and the aspiration is repeated.
- Vigorous aspiration is avoided to prevent significant peripheral blood contamination of the product. Instead, the aspirated marrow is collected into a large collection bag containing anticoagulant and media and/or an infusible-grade electrolyte solution.
- The process is repeated utilizing different bone sites until the collection volume target, based on the total nucleated cell (TNC) count or donor volume limit, is reached.

3.1.2. Complications of bone marrow harvest:

- Minor: Pain at the site of harvest, fatigue, insomnia, nausea, dizziness, and anorexia occur frequently but resolve in most donors by 1 month.
- Serious complications are rare.

3.1.3. Limitations for bone marrow harvest:

- Previous radiation to the pelvis may limit the amount of marrow available for harvest.
- Previous chemotherapy may limit the number of nucleated cells that can be aspirated from the marrow space.
- A significant tumour burden in marrow space may contaminate the graft by tumour cells.

3.2. Collection of peripheral blood stem cells (PBSC):

It is a known fact that primitive haematopoietic stem cells can restore haematopoiesis after lethal myelosuppression. Initially, stem cell mobilization was achieved by using chemotherapy alone. Presently, recombinant growth factors such as granulocyte-colony stimulating factors (G-CSF) are considered the standard mobilizing agent. Mobilization of stem cells into the peripheral blood is defined as the increased release of immature and mature haematopoietic cells from the marrow into the blood circulation. Haematopoietic progenitor cells are measured using the $CD34^+$ cell surface marker. The dose of stem cells in the graft has a critical effect on haematopoietic recovery. A dose of at least 2×10^6 $CD34^+$ cells/kg is required to ensure neutrophil and platelet recovery.

3.2.1. Mobilization of PBSC:

The collection of PBSCs involves its mobilization followed by collection using the apheresis method. Poor mobilization of PBSCs might occur due to old age, female gender, prior radiation to active marrow sites, prior treatment with purine analogues (especially fludarabine), or due to increasing cycles and regimens of chemotherapy. Mobilizing agents can be divided into chemotherapy, cytokines, or chemokines.

a. **Chemotherapy:** Chemotherapy is used as a mobilization agent only in autologous transplant settings and not in allogeneic donors. The chemotherapy regimen to be used for stem cell mobilization depends on disease entity and drug selection, which is influenced by the drug's ability to mobilize and its activity against the underlying disease.
   - ➢ Typical chemotherapy agents used for mobilization include:
     - Single-agent cyclophosphamide, especially in patients with Multiple myeloma
     - Combination chemotherapy regimens like ifosfamide, carboplatin, and etoposide (IEC) or etoposide, methylprednisolone, cytarabine, and cisplatin (ESHAP), are typically used in lymphoma patients.
   - ➢ Advantage: It can be coordinated as a part of a salvage chemotherapy regimen
   - ➢ Disadvantage: The timing of apheresis is not as predictable as it is with G-CSF alone
   - ➢ Side effects:
     - Side effects of individual chemotherapies
     - Risk of myelosuppression
     - The potential need for transfusions
     - Risk of infections

b. Cytokines: G-CSF has become the standard agent for cytokine mobilization. G-CSF has been shown to mobilize more $CD34^+$cells with less toxicity than other growth factors. A standard dose of G-CSF is 10-15 μg/kg/day given subcutaneously. Peripheral blood $CD34^+$ cells levels are ascertained on day 4 or day 5 of G-CSF, and if they are above the institution's cut-off level (typically 5-20 $CD34^+$ cells/ μL), then PBSC collection is begun on the same day/following day.
   - ➢ Advantage: Predictability of apheresis scheduling is easier as $CD34^+$ cells peak by day 4/day 5 of G-CSF mobilization
   - ➢ Disadvantage: Sometimes, G-CSF mobilization fails to provide an adequate collection and is termed as poor mobilization
   - ➢ Side effects:
     - Injection site erythema
     - Bone pain
     - Headache
     - Fever
     - Splenic rupture (rarely)

c. **Chemokines:** A new chemokine has been shown to mobilize stem cells. Plerixafor is a reversible bicyclam inhibitor of haematopoietic stem cell binding to stromal cell-derived factor-1 alfa (SDF-1) on marrow stromal cells via C-X-C chemokine receptor type 4 (CXCR4). It has been shown to mobilize stem cells when used alone or in combination with G-CSF. The regimen that has been studied mostly includes G-CSF at a standard subcutaneous dose of 10 μg/kg/day for 4 days. On the evening of day 4, plerixafor (240 μg/kg given subcutaneously) is administered approximately 10-11 hours before apheresis, followed by apheresis on day 5. Non-responders to G-CSF often respond to a dose of plerixafor administered on the previous night of collection.
   - ➢ Side effects:
     - Injection site erythema
     - Vomiting
     - Flatulence
     - Diarrhoea

3.2.2. Collection of PBSCs by apheresis:

PBSC collection can be either started when peripheral leukocyte counts rise to $\geq$ 1.0 x $10^9/\mu$l or when

peripheral blood CD34 level is above the centre's cut-off (typically 5-20 $CD34^+$ cells/ μL). Because PBSCs segregate in the mononuclear cell fraction of blood, apheresis devices capable of mononuclear cell concentration and harvest may be used to collect these cells. Continuous flow centrifugation devices are most commonly preferred because of their shorter processing time than discontinuous flow centrifugation devices. If whole blood is processed at a typical rate of 50-70 mL/minute, processing 2-3 blood volume (10-15 L) per procedure requires approximately 3-5 hours. PBSCs collected in any given procedure depend on their mobilization from the marrow into peripheral blood, duration of procedure, and collection efficiency of the apheresis device.

3.2.3. Adverse effects of PBSC Collection:

Procedures for mobilization and collection of PBSCs from patients and normal donors are well-tolerated by most of them. Common adverse effects of mobilization with growth factors include bone pain, myalgia, headache, and fatigue. These symptoms either subside on their own or sometimes require medication such as non-steroidal anti-inflammatory drugs (NSAIDs). However, these symptoms subside completely when growth factors are stopped. Aheresis's adverse effects for PBSC collection largely consist of complications related to citrate toxicity (hypocalcaemia symptoms like paraesthesia, tingling, shivering, tetany, etc.). Citrate toxicity is usually manageable by a reduction in flow rate. Intravenous infusion of calcium solution is often required. Oral or intravenous (IV) calcium is used prophylactically by many centres to prevent citrate toxicity during PBSC procedures by apheresis. Sometimes complications can result from peripheral/central line placement (including haematoma, thrombosis, infection, etc.).

3.3. Collection of Umbilical cord blood (UCB):

The objective is to maximize the volume of blood harvested from the placenta while reducing the risk of contamination from micro-organisms (bacteria, fungi), maternal blood, and secretions without influencing the routine delivery process. In order to maximize the volume harvested and provide UCB units suitable for clinical use, the collection should continue until the blood stops flowing. UCB can be collected by in-utero or ex-utero method. Nevertheless, both methods require adherence to aseptic techniques to minimize contamination. UCB collection methods are:

a. **In-utero collection:**
   - Performed by trained obstetricians after the delivery of the infant
   - The umbilical cord is clamped and cleaned, and blood is aspirated from the placental vein while the placenta is still in-utero
   - Advantages:
     - o Avoids the possibility of failed collection resulting from damage to placenta during delivery
     - o Reduces delay in collection
     - o An increase in volume collected
     - o Reduces incidence of clotted collections

b. **Ex-utero collections:**
   - Performed by trained UCB bank staff after the infant and placenta are delivered.
   - Following delivery, the placenta is passed to UCB bank staff to harvest the blood following robust cleaning of the umbilical cord and aspiration of blood from a placental vein.

4. Processing and storage of haematopoietic stem cells (HSCs)

4.1. Processing methods:

Processing methods for HSCs can be divided into routine methods, which are usually centrifugation based, and specialized methods that involve a variety of technologies.

4.1.1. Routine methods:

It includes volume (plasma) reduction, red cell reduction, buffy coat preparation, thawing/washing, and filtration. These procedures do not amount to the manipulation of stem cells.

a. **Volume reduction**: It is performed in settings of minor ABO-mismatched allograft (marrow or peripheral blood) transplantation to reduce the amount of incompatible plasma and to prevent fluid overload in small patients and/or patients with renal disease or cardiac failure. Volume reduction may also be performed before cryopreservation (e.g., for UCB banking with limited storage space or during cell concentration optimization).

b. **Red cell reduction:** Classically, red cell reduction employed sedimenting agents (e.g., hydroxyethyl starch) to reduce red cell content. Recently red cell reduction from either bone marrow or harvested HSC has been made using apheresis machines and may be a better alternative to sedimentation methods. Red cell reduction methods are used to prevent haemolytic transfusion reactions when major ABO-incompatible marrow or HSC allografts and allografts with other clinically relevant red cell antigens (e.g., Kell, Kidd) are transplanted. Red cell reduction before freezing also limits the amount of infusion of lysed red cell fragments and free haemoglobin, which may be particularly important for patients with renal failure. Red cell reduction is also useful when storage space is limited. Peripheral blood-derived HSCs generally do not require red cell depletion because apheresis instruments collect mononuclear cells efficiently with very little red cell content.

c. **Buffy coat preparation:** Buffy coat concentration of marrow involves centrifugation and harvesting of the white cell fraction and can be performed with an apheresis or cell-washing device. Manual centrifugation may be used when product volume is too low for apheresis or cell washing devices. Buffy coat preparation is usually used to reduce the unit volume for cryopreservation or as a method of red cell reduction. Following this, further manipulation (e.g.,immunomagnetic selection) may be required in selected cases.

d. **Thawing:** The thawing procedure for all HSCs, regardless of source, is similar. Although this procedure is straightforward, it should be done carefully because frozen plastic containers are prone to break for a variety of reasons.[3] While the product is being verified to determine the product's identity, it should be handled with care and ensure the bag's integrity. The product is then placed into a clean or sterile plastic bag and submerged in a 37°C water bath. Gentle kneading allows the thaw procedure to proceed relatively quickly while preventing recrystallization and consequent cell damage/death. If the freezing bag breaks, the product may be recovered using this approach, but a risk-benefit discussion with the patient's physician should take place to determine the course of the patient's care. A haemostat should be used to prevent loss of the product if the bag breaks, and the contents should be aseptically diverted into a transfer bag. A sample should also be sent for culture.

e. **Washing:** Washing removes lysed red cells, haemoglobin, and cryoprotectant [dimethyl sulfoxide (DMSO)]. Although UCB is typically red cell-depleted before cryopreservation, it remains the primary HSC product that is routinely washed. Briefly, the thawing process involves slow, sequential addition of a wash solution (e.g., 10% dextran followed by 5% albumin), transfer into an appropriately sized bag for centrifugation and resuspension of cell pellet(s) before delivery to the patient care unit for infusion. Many laboratories perform two centrifugation steps, removing the supernatant from the first spin and centrifuging that portion a second time before combining the two cell pellets. This approach optimizes cell recovery.[4]

   However, opinion regarding the washing of cryopreserved HSC is variable. Since washing invariably leads to the loss of some HSCs, some centres prefer to dilute the HSCs in normal saline at the time of infusion to avoid the side effects of DMSO.

f. **Filtration:** Marrow harvest typically involves sequential filtration in the operating room or laboratory to remove bone spicules, aggregates, and debris. However, opinions regarding the use of standard blood filters upon infusion of HSCs vary. The decision to use a standard blood filter (>170 microns) lies upon the individual cell processing laboratory and/or transplant centre. If an institution opts to use a standard blood filter, the laboratory should validate its filtration process.

4.1.2. Specialized cell processing:

Specialized cell processing methods are used to optimize product purity and potency beyond levels obtained through routine methods. These include elutriation, cell selection system, and cell expansion.

a. **Elutriation:** Counter-flow centrifugal elutriation is a specialized method that separates cell populations based on two physical characteristics— size and density (sedimentation coefficient). A centrifuge is used to separate the cell populations of a cell product based on density alone. In elutriation, fluid/media is passed through the chamber in a direction opposite (counterflow) to the centrifugal force. Adjustment of flow rate and/or centrifugation speed allows the separation of cell populations based on size as well. Through this process, cells with "signature" size/density profiles can be separated from the rest of the cells. Historically, this method was used for the T-cell depletion of HSC grafts. In recent years, the method has been used to enrich monocytes for the preparation of dendritic-cell vaccines.

b. **Cell selection systems:** Immunomagnetic cell selection systems incorporating monoclonal-antibody-based technologies to target cell-surface antigens (e.g., CliniMACS system, Miltenyi Biotec GmbH, Bergisch, Gladbach, Germany) have become widely used methods of cell depletion/enrichment at many institutions. These methods involve the isolation of the cell type of interest by either positive selection (target cells retained) or negative selection (target cells depleted). Monoclonal antibodies (e.g., anti-CD34 for HSC isolation) are coupled to 50-nm ferromagnetic particles. Magnetically labelled target cells are retained in the process as the cell suspension pass through a column in which a magnetic field is generated. Unlabelled cells pass through the column and are collected in a negative-fraction bag. Target cells are then released from the column by removing the magnetic field from the column, which allows the passage of the cells into a separate collection bag.

c. **Cell expansion:** Because the dose of nucleated, $CD34^+$, and colony-forming cells positively correlates with the patient outcome, much effort has been focused on the ex-vivo expansion of HSCs and progenitors. It is thought that successful expansion enhances haematopoietic engraftment while reducing transfusion dependence, risk of infection, and duration of hospitalization. In recent years, UCB has become the focus of expansion trials due to the higher proliferative and self-renewal capacity of HSCs. Most expansion cultures contain a cytokine cocktail that includes stem cell factor, FMS-like tyrosine kinase 3 (FLT-3) ligand, and thrombopoietin, along with novel and/or proprietary ingredients. The media, culture vessels, and culture duration used vary from protocol to protocol.

4.2. Cryopreservation and storage:

Methods for cryopreservation must be used because HSCs may need to be stored for weeks to years before being transplanted.[5] Most cell-processing laboratories use the cryoprotectant DMSO, usually at 10% final concentration, and a source of plasma protein for cryopreservation of HSCs. DMSO is a colligative cryoprotectant; it diffuses rapidly into the cell, reducing the osmotic stress on the cell membrane. DMSO prevents dehydration injury by moderating the non-penetrating extracellular solutes that form during ice formation. It also slows extracellular ice crystal formation. Some laboratories add hydroxyethyl starch (HES), which allows the use of a decreased concentration of DMSO (e.g., 5% DMSO and 6% HES). HES is a non-penetrating (extracellular) macromolecular cryoprotectant. This high molecular-weight polymer likely protects the cell by forming a glassy shell or membrane around the cell, retarding the movement of water out of the cell and into the extracellular ice crystals. The HSCs may be frozen at a controlled rate or a non-controlled rate, in which the HSC product is simply transferred into a freezer bag and placed in a –80°C mechanical freezer. Controlled-rate freezing is favoured in the clinical laboratory setting. It utilizes computer programming to incrementally decrease HSC product temperature in a closely monitored fashion. The controlled rate freezing protocols used vary from institution to institution. In general, the HSC product is placed in a chamber and initially cooled at a rate of 1°C/minute. When the temperature decreases to approximately –14°C to –24°C, the HSC product begins to transit from a liquid to a solid state. At this time, the freezer undergoes a period of supercooling to counteract the latent heat of fusion that is released by the phase change. Following the solidification of the HSC product, cooling proceeds at the rate of 1°C/ minute until the product has reached –60°C. At this point, the product is cooled at a controlled rate determined by the institution until it reaches –100°C. Following both controlled-rate and non-controlled-rate freezing, the HSC product is transferred to a

storage freezer. An increasing number of laboratories store HSCs in the vapour phase of liquid nitrogen (LN2) at temperatures below –150°C; however, some laboratories do store cells in the liquid phase of LN2.

5. Flow cytometry:

Flow cytometry is a technique for counting and examining microscopic particles like cells by suspending them in a stream of fluid and passing them through an electronic detection apparatus. Flow cytometry renovated the analysis of cell populations.

5.1. Principle:

- Antibodies against cell-surface molecules are labelled with fluorescent tags.
- Cells are incubated with the fluorescent tag antibodies, and these "stained" cells are then passed through a flow cytometer.
- Laser exposure of the individual cells excites the fluorochromes, causing fluorescent emissions which are detected by sensors in the flow cytometer.
- As the amount of fluorescence is determined on a cell-by-cell basis, quantifying the number of cell-surface molecules and the visualization of small populations of cells is possible.

5.2. Use of flow cytometry in the HSCT setting:

a. Determination and quantification of haematopoietic stem cells
b. Determination of T-cells
c. Quantification of contaminating leukocytes
d. Chimerism

a. Determination and quantification of haematopoietic stem cells: The expression of CD34 can identify haematopoietic stem cells in bone marrow, peripheral blood, and umbilical cord. Expression of CD34 antigen on the cell membrane has been correlated with colony-forming units in cell cultures, which is considered the gold standard for stem cell quantification.[6] But quantification of $CD34^+$ cells by flow cytometry is widely used in clinical practice as an indirect indicator of haematopoietic progenitor cells. Most centres utilize flow cytometry to quantitate stem cells contained in autografts, allografts, or T-cells present in donor lymphocyte infusions and in investigations related to stem cell mobilization or immune reconstitution.[7,8]

The most important flow cytometry analysis is to define the content of CD34 positive haematopoietic progenitor cells in stem cell grafts and to measure the T-cell content before allogeneic transplantation as per the guidelines issued by the Joint Accreditation Committee of the International Society for Cell and Gene Therapy (ISCT) and European Society for Blood and Marrow Transplantation (EBMT). It would be beneficial for operational and economic efficiency if sufficient stem cells could be obtained and optimal timing of the leucapheresis could be reliably predicted. For obtaining a yield of $>1 \times 10^6$/kg $CD34^+$ cells in a single apheresis procedure, a peripheral blood CD34 cell count must be $>20 \times 10^3/\mu l$.[9] $CD34^+$ progenitor cells were quantified by flow cytometry using the International Society of Haematotherapy and Graft Engineering (ISHAGE) protocol.[10]

b. Determination of T-cells: Applications of flow cytometry in bone marrow transplantation also include haematopoietic stem cell graft manipulation in the pre-transplantation phase by determination of the efficacy of ex vivo T-cell graft depletion. In the post-transplantation phase, it can assist in the evaluation of immune recovery, graft rejection, graft-versus-host disease (GVHD), and graft-versus-leukaemia effect (GVL).[11,12]

c. Quantification of contaminating leukocytes: Flow cytometry is a sensitive method to determine the number of leukocytes in leucofiltered components like red cells, platelets, etc. This is important as contaminating leukocytes in transfused blood components may cause several adverse effects, which include febrile non-haemolytic transfusion reaction (FNHTR), graft-versus-host disease (GVHD), cytomegalovirus transmission (CMV), alloimmunization to HLA antigens, etc.

d. **Chimerism:** Chimerism is a state when two or more different cell populations, are evident during serology testing. This can occur after a recent transfusion, post-transplantation, or due to certain clonal disorders. Chimerism analysis by flow cytometry has been extensively reviewed by Bluth et al.[13]

6. Requirements for cord blood banking:

Umbilical cord blood (UCB) is the whole blood, including haematopoietic progenitor cells collected from placental and or umbilical cord blood vessels after the umbilical cord has been clamped.

6.1. Cord blood bank:

A place or organization or unit for carrying out and responsible for operations of collection, processing, testing, banking, selection, and release of cord blood units.

The Drug Controller General of India (DGCI) within the Central Drugs Standard Control Organization (CDSCO) established guidelines for cord blood banking by amending the Drugs and Cosmetics Rules of, 1945. These cord blood banking guidelines, which became effective in 2012, are applicable to cord blood units intended for autologous and allogeneic use and are designed to provide a framework for facilities to obtain a license to manufacture and distribute cord blood in India. The guidelines outline collecting, processing, testing, storage, banking, and release requirements for umbilical cord blood. Concerning building and room requirements, the regulations set forth measures to minimize the risk of contamination from external environmental factors. The cord blood regulations also define screening requirements, including hepatitis B, hepatitis C, human immunodeficiency virus 1 and 2 (HIV 1 and 2), syphilis, malaria, cytomegalovirus, and human T-lymphotropic virus I and II. The regulations also require that the cord blood unit be tested for total nucleated cell count, total mononuclear cell count, $CD34^+$ count, cell viability, ABO group and Rh type, sterility tests for bacterial and fungal contamination, and HLA matching (for allogeneic units only).

6.2. Regulatory requirements for umbilical cord blood banking:

Under part XII D of the Drugs and Cosmetics Act, 1940 and Rules 1945 (As amended up to the 31st December 2016), the following are the requirements for cord blood banking.[14]

6.2.1. General requirements

a. **Location and surroundings:** The building(s) for storage of UCB shall have measures to avoid the risk of contamination from the external environment, including open sewage, drain, public lavatory, or any factory which produces disagreeable or obnoxious odour or fumes, excessive soot, smoke, chemical or biological emissions.

b. **Building and premises:** The premises used for processing and storage shall be designed, constructed, adapted, and maintained to ensure that the above operations and other ancillary functions are performed smoothly under hygienic conditions and in sterile areas wherever required. They shall also conform to the conditions laid down in the Factories Act, 1948 (63 of 1948).

   The premises shall be:

   - Adequately provided with working space to allow logical placement of equipment, material, and movement of personnel to maintain safe operations and prevent contamination.
   - Designed/constructed/maintained to prevent insects, pests, birds, vermin, and rodents entry.
   - Interior surfaces (walls, floors, ceilings, and doors) shall be smooth and free from cracks and permit easy cleaning, painting, and disinfection. In aseptic areas, the surfaces shall be impervious, non-shedding, non-flaking, and non-cracking.
   - Flooring shall be unbroken and provided with a cove both at the junction between the wall and the floor and the wall and the ceiling.
   - Provided with light fitting and grills, which shall flush with the walls and not hang from the ceiling to prevent contamination.
   - If provided with fire escapes, these shall be suitably installed in the walls without any gaps.

- Provided with the furniture in aseptic areas which is smooth, washable, and made of stainless steel or any other appropriate non-shedding material other than wood.
- Provided with separate areas for processing and storing products to prevent mix-ups, product contamination, and cross-contamination.
- Provided with defined environmental conditions for temperature, humidity, ventilation, and air filtration.
- A periodical record of cleaning and renovating the premises shall be maintained.

c. Disposal of waste and infectious materials:

- Waste materials awaiting disposal shall be stored safely.
- The disposal of sewage and effluents from the facility shall be in conformity with the requirements of the Pollution Control Board.
- All bio-medical waste shall be dealt with following the provisions of the Bio-Medical Waste Management Rules, 2016 AND Bio-Medical Waste Management (Amendment) Rules, 2018

d. Health, clothing and sanitation of personnel:

- All personnel shall undergo a medical examination before employment and shall be free from infectious and contagious diseases, and after that they should be medically examined periodically at least once a year, and for this purpose, records shall be maintained thereof.
- All personnel, before and during employment, shall be trained in practices that ensure personal hygiene, and a high level of personal hygiene shall be observed by all those engaged in the collection, processing, and banking of umbilical cord blood.
- All persons shall wear clean body coverings appropriate for their duties before entering the Processing Zone, and the Change Rooms with adequate facilities shall be provided before entrening into any specific zone.
- Smoking, eating, and drinking are prohibited inside the laboratory.
- All personnel working in the laboratory shall be protected against virus infections.

6.2.2. Requirements for processing, testing and storage areas for UCB:

Separate dedicated areas specifically designed for the purpose and the workload shall be provided. There shall be separate areas for designated work purposes, namely: -

a. Cord blood reception: A cord blood reception area with space for the temporary storage of units and physical examination shall have adequate registration, data entry, and generation of bar-coded labels. An air-conditioned area of at least 10.00 sq. meters shall be provided.

b. Cord blood processing area: The room shall be clean and have air handling. System to provide a Class 10,000 environment. Entry to this area shall be through the airlock. The room will house Class 100 biological safety cabinets for UCB processing. The clean room temperature shall be maintained from 20ºC to 25ºC with a positive differential pressure of 10-15 Pascal and relative humidity of 50-60%. The minimum area shall be 10.00 sq. meters for the activity.

c. Haematology and Serology Laboratory: The laboratory shall be equipped and utilized for independent testing of UCB for ABO grouping and Rh typing, total nucleated cell count, progenitor cell count, and viability test. The room shall be air-conditioned, and an area of at least 10.00 sq. meters shall be provided.

d. Transfusion transmissible infectious disease screening laboratory: The laboratory shall be equipped and utilized for screening tests on maternal blood for infectious diseases viz. HIV I & II; Hepatitis B & C virus, syphilis, malaria, CMV, and HTLV. The room shall be air-conditioned, and an area of at least 10.00 sq. meters shall be provided.

e. Sterility testing laboratory: The laboratory shall be used for performing sterility tests on the UCB unit. The premises may be classified depending on the testing method used. The room shall be air-conditioned with an adequate and ancillary area for media preparation, sterilization, incubation and decontamination. An area of at least 10.00 sq. meters shall be provided.

f. HLA typing laboratory: The UCB unit shall have arrangements for HLA typing and genetic disease testing. In-house testing can be done by providing a well-demarcated laboratory from the processing area for

evaluation of possible genetic disease and HLA typing. The area shall have a class 100,000 environment, and air-conditioned and an area of at least 10.00 sq. meters shall be provided.

g. **Sterilization-cum-washing:** Appropriate facilities shall be provided within the premises for proper washing and sterilization. This facility would be optional for laboratories using entirely disposable items.

h. **Records and store rooms:** There shall be designed record room(s) and store room(s) of at least 10.00 sq. meters each. Access to the record room shall be permitted only to the authorized person. The room will have adequate protective facilities as the documents and records are to be preserved for long years.

i. **Cryogenic storage room:** A minimum space of 20.00 sq. meters shall be provided by the licensee. The cryogenic storage room shall have provision for temperature monitoring of storage vessels, liquid nitrogen level in storage vessels, and oxygen meter. The service space between each liquid nitrogen storage vessel, supply cylinders, and connecting hose should be a minimum of 1.00 sq. meters. In addition, separate storage space for other accessories required shall be provided. The room shall be air-conditioned.

j. **General storage area:** General storage area shall be provided to store all the consumables under conditions deemed optimum for storage by manufacturers.

6.3. Collection and storage of processed UCB component

6.3.1. Collection:

a. UCB unit specific for an individual will be collected after consent from the parents, whose child 's UCB is to be collected. Private and public UCB banking have different agreements.

b. UCB shall be collected from hospitals, nursing homes, birthing centres, and any other place where a consenting mother delivers under the supervision of the qualified registered medical practitioner responsible for the delivery.

c. UCB shall be collected aseptically in a disposable PVC bag, containing an adequate quantity of sterile, pyrogen-free anti-coagulant and sealed effectively. Such PVC bags shall be procured from a licensed manufacturer.

d. UCB would be collected from a premise operating in hygienic conditions to allow proper operation, maintenance, and cleaning.

6.3.2. Transportation:

a. UCB unit shall be transported from the birthing centre to the designated laboratory under and as per the procedure prescribed by the cord blood bank.

b. The transportation procedure shall be validated to ensure the optimum survival of the stem cells.

c. The transportation temperature should be between 18ºC to 28ºC.

d. The time period between collection and processing shall not exceed 72 hours.

6.3.3. Storage:

a. The UCB shall be stored at room temperature between 20ºC to 25ºC in the reception area before processing.

b. Samples pending tests for specific transfusion transmittable infectious diseases shall be stored in a segregated manner.

Note: Temperature range between 4ºC to 37°C, for the whole period of transit, may be allowed beyond 18ºC to 28ºC in exceptional cases. The licensee shall adequately explain the effects of deviation of transit temperature on the product in the client education booklet.

5.4. Personnel:

Cord blood bank shall have the following categories of whole-time competent technical staff, namely: -

a. **Medical director:** The operation of the cord blood bank shall be conducted under the active directions and supervision of a medical director who is a whole-time employee and possesses a post-graduate degree in medicine – MD [Pathology/Transfusion Medicine/Microbiology] and has experience/training in cord blood processing and cryogenic storage.

b. Laboratory in-charge: The laboratory incharge shall have a post-graduate qualification in Physiology or Botany or Zoology or Cell Biology or Microbiology or Biochemistry or Life Sciences or Graduate in Pharmacy and one-year working experience in a pathological laboratory licensed by the local health authority or any microbiology laboratory of a licensed drug manufacturing/testing unit and or experience/ training in cord blood processing and cryogenic storage.

c. Technical supervisor (cord blood processing): - The technical supervisor shall have a:

i. Degree in Physiology or Botany or Zoology, Pharmacy or Cell Biology or BioSciences or Microbiology or Biochemistry or Medical Laboratory Technology (M.L.T.) with a minimum of three years of experience in the preparation of blood components and/or experience or training in cord blood processing and Cryogenic Storage; or

ii. Diploma in Medical Laboratory Technology (M.L.T.) with five years' experience in preparing blood components and experience or training in cord blood processing and Cryogenic Storage shall be essential.

d. Cord blood bank technician(s): - The technicians employed shall have a:

i. A degree in Physiology or Botany or Zoology or Pharmacy or Cell Biology or Bio Science or Microbiology or Biochemistry or Medical Laboratory Technology (M.L.T.) with six months' experience and or training in cord blood processing and cryogenic storage or

ii. Diploma in Medical Laboratory Technology (MLT) with one year experience in testing blood and/or its components and/or experience or training in cord blood processing and cryogenic storage.

6.5. Air handling system:

Air handling for sterile areas shall be different from those for other areas. The filter configuration in the air handling system shall be suitably designed to achieve the grade of air as given in Table 2. The environmental microbiological monitoring of clean areas shall be following the recommended limits given in Table 3. The processing area shall have HVAC (heating, ventilation, and air conditioning) system and be fitted with HEPA (High-Efficiency Particulate Air) filters having Grade C (Class 10,000) environment as given in Table 2. The entire processing shall be done conforming to the Grade A (Class 100) Standard of air quality.

Table 2: Airborne particulate classification for the manufacture of sterile products

Grade	Maximum number of permitted particles per cubic meter equal to or above			
	At rest (b)		In Operation (a)	
	0.5μm	5μm	0.5μm	5μm
A	3,500	0	3,500	0
B(a)	3,500	0	3,50,000	2000
C(a)	3,50,000	2000	35,00.000	20,000
D(a)	35,00.000	20,000		

Notes: -

(a) To reach the B, C, and D air grades, the number of air changes shall be related to the size of the room and the equipment and personnel present in the room. The air system shall be provided with the appropriate filters such as HEPA for grades A, B, and C. The maximum permitted number of particles in the at-rest condition shall approximately be as under - [Grade A and B correspond with class 100 or M 3.5 or class 5]; Grade C with Class 10,000 or M 5.5 or International Organization for Standardization (ISO) Class 7; Grade D with Class 1,00,000 or M 6.5 or ISO Class 8.

(b) The requirement and limit for the area shall depend on the nature of the operation carried out.

Table 3: Recommended limits for microbiological monitoring of clean areas "In Operation"

Grade	Air sample cfu/m3	Settle plates (dia 90mm) cfu/2hrs	Contact plates (dia 55 mm) cfu per plate	Glove points (Five fingers) cfu per glove
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	50	-
D	500	500	500	-

Notes: -

(a) These are average values.

(b) Individual settle plates may be exposed for not less than two hours in Grade B, C, and D areas, and not less than thirty minutes in Grade A.

6.6. Quality control:

Facilities shall be provided for quality control such as haematological, microbiological, and instrumental testing. Following duties shall be performed under the function of quality control:

a. To prepare detailed instructions for carrying out such tests and analysis.

b. To approve or reject raw materials and consumables used in any step based on approved specifications.

c. Haematological tests like total nucleated cell counts, mononuclear cell count, enumeration of the population of stem cells, and stem cell viability shall be performed on samples of processed UCB unit.

d. Microbiological tests shall be done on maternal blood samples for freedom from Hepatitis B surface antigen, Hepatitis C virus antibody, HIV I and II antibodies, Syphilis, Malaria, CMV, and HTLV. Bacterial and fungal cultures shall be done on the UCB samples.

e. Instruments that would be used to process, test and store the UCB unit would before commissioning and calibrated from time to time to check their conformity to specific standards according to an approved and valid protocol.

f. According to an accepted and validated protocol, the environmental monitoring of the clean rooms would be done at periodic intervals.

g. All tests mentioned above shall be done in-house except tests under item numbers (e), (f) and test for enumeration of the stem cell population, HLA typing and genetic disease testing, which may be outsourced to a competent third party approved by the licensing authority.

6.7. Screening tests

a. Maternal blood sample: Hepatitis B, Hepatitis C, HIV 1 & 2, Syphilis, Malaria, CMV, HTLV.

b. The Umbilical cord blood sample: Total nucleated cell count, total mononuclear cell count, progenitor cell ($CD34^+$) enumeration, cell viability, ABO group and Rh type, sterility test, HLA matching (only for allogeneic cord blood units).

6.8. Storage:

The UCB shall be cryopreserved using a controlled rate freezing, or equivalent validated procedures. The frozen storage shall be at minus 196°C and shall not be warmer than minus 150°C. There will be no shelf life for this class of product.

6.9. Reference samples:

a. At least two reference samples shall be collected from cord blood unit product before cryopreservation and stored at minus 196°C and shall not be warmer than minus 150°C.

b. At least one additional reference sample shall be stored at minus 76°C or colder for purposes other than viability analysis.

6.10. Labelling:

a. Initial label placed during collection shall specify:
   i. Human Umbilical Cord Blood
   ii. Approximate Volume or weight of contents in the collection bag [UCB+ Anticoagulant]
   iii. Mother's name
   iv. Date, time and place of collection
   v. To be labelled in bold, —ROOM TEMPERATURE ONLY– DO NOT REFRIGERATE, DO NOT IRRADIATE
   vi. Manufacturing license number
b. Label at the completion of processing and before issue - Cryogenic Storage Label [Statutory label] shall indicate the following: -
   i. Name of product: Human Progenitor Cell [HPC] – Cord Blood
   ii. Volume or weight of contents
   iii. Percentage of cryoprotectant [DMSO]
   iv. Percentage of any other additive
   v. Date of collection [birth]
   vi. Date of processing
   vii. Name of manufacturer
   viii. Manufacturing license number
   ix. Storage temperature – not less than - 196ºC and shall not be warmer than minus 150ºC
   x. Unique Traceability Number and/or bar code
c. Issue label at the time of the release of UCB unit shall indicate the following, namely:
   i. Name of manufacturer
   ii. License number
   iii. All details of the cryogenic storage label
   iv. The results of Total Nucleated Cells, Progenitor Cell percentage [$CD34^+$], Viability
   v. Results of transfusion transmittable diseases testing on maternal blood
   vi. ABO and Rh Group and HLA typing (allogeneic)
   vii. Date of processing
   viii. A statement indicating: - "Do not use leucoreduction filters" and "Do not irradiate"
   ix. Name and address of receiving hospital

5.11. Records or Documentation

a. Records:
   i. Client / donor enrolment / agreement record
   ii. Collection of unit and transportation record
   iii. Master record of the stored unit
   iv. HLA matching record
   v. Unit release register
   vi. Stock register for blood collection bag cryoprotectant and preservant, RBC sedimentation enhancer
   vii. Stock Register for Diagnostic Kits, Reagents and other consumables
   viii. Record on feedback after use of cord blood / adverse reaction record

b. Standard Operating Procedures:
   i. UCB collection
   ii. Transportation of the collected UCB unit

iii. Processing of UCB unit
iv. Cryogenic storage of processed UCB unit
v. Testing of maternal blood for transfusion transmittable infections
vi. Testing of UCB for ABO Grouping and Rh Typing
vii. Testing of UCB unit for Total Nucleated Cell Count, Mononuclear Cell Count, Progenitor Cell ($CD34^+$) enumeration, and viability
viii. Testing of UCB stem cell unit for sterility
ix. Disposal of biomedical waste
x. Dispensation of UCB unit
xi. Preventive maintenance protocol for all Instruments
xii. Acceptance / Rejection procedure of consumables
xiii. Environment monitoring of classified areas
xiv. Any other standard operative procedure as per requirements

6.12. UCB release:

There shall be a designated area with adequate space for procedures and records related to UCB unit selection and release. The UCB bank shall obtain a written or electronic request from the transplant physician or designee for shipment of the UCB unit. Accompanying documentation at the time of issue from the UCB bank shall include indications, contra-indications, caution, and instruction for handling and use of the UCB unit, including short-term storage and preparation for transplantation. The procedure for transportation of cryopreserved UCB unit within the facility shall be designed to protect the unit's integrity and the health and safety of the personnel. Cryopreserved UCB unit stored at -150°C or colder shall be transported in a liquid nitrogen-cooled dry shipper that contains adequate absorbed liquid nitrogen and has been validated to maintain the temperature below -150°C for at least 48 hours beyond the expected time of arrival at the receiving facility.

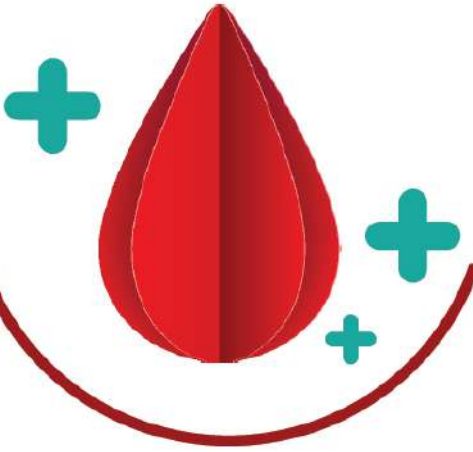
Key Points

- Sources of stem cells for haematopoietic stem cell transplantation include bone marrow, peripheral blood, and umbilical cord blood.
- Bone marrow harvest is an invasive procedure performed under general anaesthesia, while mobilization is required to collect peripheral stem cells.
- Umbilical cord blood can be collected by In-utero or Ex-utero methods.
- Routine stem cell processing methods include volume reduction, red cell reduction, buffy coat preparation, thawing/washing, and filtration.
- Specialized processing of stem cells includes elutriation, cell selection system, and cell expansion.
- Cryopreservation of stem cells is done by using a penetrating cryoprotectant (DMSO) or non-penetrating cryoprotectant (HES).
- Stem cells may be frozen at a controlled rate or a non-controlled rate. However, controlled rate freezing is favoured in a clinical laboratory setting.
- Flow cytometry is widely used in HSCT setting to determine and quantify stem cells, determination of T-cells, quantification of contamination of leucocytes, and chimerism.
- The requirements for cord blood banking are mentioned under part XII D of the Drugs and Cosmetic Act, 1940 and Rules, 1945.

References:

1. Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, Technical Manual. 18th edition. Bethesda MD. AABB press. 2014
2. National Marrow Donor Program, NMDP Standards, 24th Edition, Jan 2018.
3. Khuu HM, Cowley H, David-Ocampo V, Carter CS, Kasten-Sportes C, Wayne AS, et al. Catastrophic failures of freezing bags for cellular therapy products: Description, cause, and consequences. Cytotherapy 2002;4:539-49
4. Laroche V, McKenna DH, Moroff G, Schierman T, Kadidlo D, McCullough J. Cell loss and recovery in umbilical cord blood processing: A comparison of postthaw and postwash samples. Transfusion 2005; 45:1909-16.
5. Fleming KK, Hubel A. Cryopreservation of haematopoietic and non-haematopoietic stem cells. Transfus Apher Sci 2006; 34:309-15.
6. Lemos NE, Farias MG, Kubaski F, Scotti L, Onsten TGH, Brondani LA, et.al. Quantification of peripheral blood $CD34^+$ cells prior to stem cell harvesting by leucapheresis: a single centre experience. HematolTransfus Cell Ther. 2018; 40:213-8.
7. Souza MH, Diamond HR, Silva ML, Campos MM, Bouzas LF, Tabak D, et.al. Immunological recovery after bone marrow transplantation for severe aplastic anaemia: A Brazilian experience. Eur J Haematol 1994; 53 (3): 150-5.
8. Diamond HR, Souza MH, Bouzas LF, Tabak DG, Campos MM, Rumjanek VM. Deficit of T-cell recovery after allogeneic bone marrow transplantation in chronic myeloid leukaemia patients. Anticancer Res 1995; 15: 1553-60.
9. Sawant RB, Rajadhyaksha SB. Correlation of $CD34^+$cell yield in peripheral blood progenitor cell product with the pre-leucapheresis cell counts in peripheral blood. J Assoc Physicians India 2005; 53:1031-5.
10. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for $CD34^+$ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. J Hematother. 1996; 5:213–26
11. Storek J, Joseph A, Espino G, Dawson MA, Douek DC, Sullivan KM, et al. Immunity of patients surviving 20 to 30 years after allogeneic or syngeneic bone marrow transplantation. Blood 2001; 98:3505-12.
12. Bordin JO, Heddle NM, Blajchman MA. Biologic effects of leukocytes present in transfused cellular blood products. Blood 1994; 84:1703-21.
13. Bluth MH, Reid ME, Manny N. Chimerism in the immunohaematology laboratory in the molecular biology era. Transfus Med Rev 2007; 21:134-46.
14. Drugs and Cosmetics Act, 1940 and Rules, 1945 as Amended up to 31st December 2016. Available from: www.cdsco.gov.in Last accessed 30th September 2019

Section 18



Quality Assurance in Blood Transfusion

1. Introduction:

The blood transfusion services should provide blood and blood products that are safe, potent, and effective. To provide a high level of assurance of safe blood and transfusion practices to blood donors, physicians, patients, and their families, a quality philosophy must be evolved in blood transfusion services. Bringing this quality philosophy into operation includes quality control, quality assurance, and continuous quality improvement.

Assurance of a quality product includes issues of blood components for patient safety and blood donors, reagent quality control, monitoring of equipment repair and maintenance, the competence of personnel, and testing of a defined number of units of each product for the appropriate parameters, and hazardous waste management. Inherent to this goal is the provision of a safe work environment.

Blood and blood components are biological products and considered drugs. Blood centre compliance with federal regulations and standards prescribed in the Drugs and Cosmetic Acts, 1940 and rule 1945 is the statutory requirement of the Drugs Controller General of India (DCGI).[1]

Quality control, quality assurance, and continuous quality improvement are complementary yet distinctly different concepts.

1.1. Quality control:

Quality control (QC) is the management of the testing process.[2]

It includes:

- Assessment of the accuracy and reproducibility of a test.
- Equipment or instruments used to perform tests must be monitored to determine if they are functioning properly.
- Reagents must be tested to determine if they have maintained their specificity and sensitivity.
- Quality control of the components.

1.2. Quality assurance:

Quality assurance is the term used for all the measures from the recruitment of donors to transfusion of blood or blood products to ensure that the products are of the desired quality required for their intended use and that the laboratory results are reliable. This ensures that the patient receives a positive, defined advantage from a particular product, so that donor, patient, and staff are not harmed. Some activities are entirely within the control of blood transfusion services, while others (e.g., collection of blood sample and administration of blood) may be outside of its immediate control, although even these should meet the requirements set by the transfusion service.[2]

Quality assurance entails defining, controlling, and documenting every aspect of a process or procedure so that compliance with determined standards can be predicted. Thus, quality control is an integral part of quality

assurance.

1.3. Continuous quality improvement:

Continuous quality improvement is a broad term that involves reviewing whether appropriate and efficient care was provided to the patient. It also involves the process of reducing rework, inappropriate patient care, and waste.

2. Principles of quality assurance in blood transfusion:

For safe transfusion practice, quality assurance requires an organized management scheme, with clearly identified levels of individual responsibility, properly trained staff, and adequately designed and equipped laboratories. Depending on the level of operation, a section with specific responsibility for quality control under one senior trained technologist may be created.

The main areas requiring attention in the quality assurance programme include:

- Premises
- Personnel
- Specifications and quality control of
  - Blood and blood components
  - Reagents
  - Equipment
- Documentation
- Educational, research and development programmes.
- Introduction of automation and computing (where possible)
- Participation in the external quality assurance (EQAS) programme

2.1. Premises:

A nicely designed, properly constructed, ventilated, air-conditioned, with adequate space for working and movement of staff is required for a good quality blood centre.[1]

- The working place and floor of the lab should be suitable for easy and effective cleaning.
- Adequate power and water supply with backup, facilities for waste disposal and strict adherence to sanitation standards are essential.

2.2. Personnel:

There must be adequate personnel with appropriate educational qualifications, training, and experience to ensure the competent performance of assigned duties applicable to all categories of the staff working in the blood centre, including medical officers, laboratory staff, nursing staff, counsellors, and social workers. The job description should exist for all personnel.

3. Quality assessment review:

Quality assessment of a blood centre can be periodically reviewed by the following organizations:

3.1. Director of blood transfusion service:

Ensures that the technicians, counsellors, and nurses do procedures according to the standard operating procedures and quality control.

3.2. State/Central drugs controller:

Ensures that the blood transfusion service follows the rule and regulations laid down in Part XII-B of Drugs and

Cosmetics Acts 1940 and Rules 1945 (DCA) notified by the Drugs Controller General (India).

3.3. Accreditation society:

Blood centres can participate in any national or international accreditation programme like the National Accreditation Board for Hospitals & Health care Providers (NABH), India. These accreditation societies reviewed the quality standards of the blood centre periodically.

3.4. Haemovigilance programme of India (HvPI):

Blood centres can register themselves and be a part of the Haemovigilance programme of India (HvPI). Currently, it is voluntary participation in India. In HvPI, the blood centre actively reports adverse blood transfusion reactions and adverse blood donor reactions.

4. Quality control of reagents:

Standardization of blood group reagents is carried out by the manufacturers, and they should meet established requirements. The quality control at the user laboratory should be done to check new batches to ensure that they comply with all biological standards. A vial from each new batch/ lot should be checked for its appearance, specificity, avidity, reactivity, and potency (titre) using known positive and negative control. Appearance, specificity, and avidity of reagents should be checked daily. No reagents should be used after the date of expiry.

4.1. General principles:

- The reagents must have a shelf-life of at least one year for use.
- All anti-sera must be complying with all the quality control criteria as mentioned in DCA.
- All blood grouping and anti-human globulin (AHG) reagents should contain a preservative to minimize bacterial and fungal growth.
- They should be kept in refrigerators at 2^0C-6^0C. However, freezing is not recommended. Instead, the manufacturer's instructions should be followed for storage.
- All reagents should be clearly labelled with the batch number, expiry date, and storage temperature; instructions for use are enclosed with each reagent packing.
- All reagents should be used according to the manufacturer's instructions. They must be re-standardized if they are to be used by alternative techniques or in a diluted form (e.g., ABO and Rh typing in microplates or automatic system).
- New reagents should not be introduced into routine work until an internal serological assessment has validated that the new reagents are satisfactory.
- Polyclonal (human serum) reagents should carry a statement saying that the individual donations used to prepare the product have been tested and found negative for HIV 1 & 2 antibody, HBsAg, and HCV antibody. However, since no test offers complete assurances that products derived from human blood will not transmit infection, it should be handled with universal precautions.

4.2. Quality control of reagent red blood cells

Reagent red cells shall be prepared daily from a minimum pool of 3-5 individual cells for each group (A, B, and O). A pool of reagent red cells should be washed at least 3 times with normal saline and prepared at 40-50 % concentration for slide testing, and 3-5% concentration for tube testing. If reagent red cells are slightly haemolysed, the cells can be washed once with saline. If the supernatant becomes clear after one wash and cells are reactive, they are acceptable for use. Quality requirements of reagent red blood cells should be as per Table 1, and haemolysed and discoloured red cells should be discarded.[4]

Table 1: Quality requirements of reagent red blood cells

Parameters	Quality requirements	Frequency of QC
Appearance	No haemolysis or turbidity in the supernatant by visual inspections	Each day
Reactivity and Specificity	Clear cut reactions with known sera against red blood cells antigens	Each day

4.3. Quality control of antibody reagents:

Antibody reagents like ABO antisera, Rh (D) antisera, antihuman globulin reagent, enzyme reagent, etc. has to be checked for their quality requirements at the timing of receiving new lot of reagents as well as daily or in periodic intervals to know their potency and consistency.

The main five criteria, appearance, specificity, avidity, reactivity and potency (titre) should be checked to assess the quality of antibody reagents.

a. **Appearance:** The antisera reagent should be clear on visual inspection. There should be no turbidity, precipitate or gel formation, discolouration and no particles observed in the antisera reagent vial.
b. **Specificity:** It denotes that antisera reagent should react only with red cells having the corresponding antigen while it should not react with other antigens on red cells. For specificity testing, the antisera reagent should be tested with three different red cells of the corresponding antigen and three different red cells, which lack the corresponding antigen.
c. **Avidity:** It denotes the speed (time) and strength of agglutination, or in other words, it denotes reactivity time (in seconds) taken by the antibodies to show the agglutination. The test is done by mixing two drops of anti-serum with one drop of 40-50% cell suspension on a slide or tile and rocking gently at room temperature (RT). The time for a visible reaction (+1) and then for a strong (+4) reaction to occur is recorded with a stopwatch's help.
d. **Reactivity:** It denotes that antisera should not cause immune haemolysis due to excessive antibodies (prozone phenomenon) when it reacts with the corresponding antigen. It also shows that there should not be rouleaux formation by antisera due to excessive protein contents.
e. **Potency (Titre):** It checks the capacity of antisera to react with the corresponding antigen in serial dilution. Undiluted antisera should give the highest reaction strength, while with serial dilution, reaction strength should decrease gradually. Titre is defined as the reciprocal of the highest dilution of antibodies which gives agglutination.

4.3.1. Quality control of ABO antisera reagents:

Quality control of monoclonal Anti-A, Anti-B and Anti-AB along with A1 and H lectin antisera is defined in Table 2 to 5.

Table 2: Quality requirements of ABO reagent (anti-A, anti-B, and anti-AB)

Parameters	Quality requirements	Frequency of QC
Appearance	No turbidity, precipitate, particles, or gel formation by visual inspection	Each day
Specificity	Anti-A should react with A_1, A_2, A_1B and A_2B red cells, and Anti-B should react with B and AB red cells. At the same time, both of these antisera should not react with O group red cells.	Daily and each new lot.
Avidity	Macroscopic agglutination with 40-50% red cells suspension in homologous serum/normal saline using the slide test;	Daily & each new lot
Reactivity	No immune haemolysis or prozone, and rouleaux formation.	Each new lot
Potency	Undiluted serum should give +++reactions in saline tube test using 3% red cells suspensions at room temperature	Each new lot

Table 3: Acceptable titre and avidity of ABO antisera reagents

Antisera	Colour	Type of the reagent	Type of red cells (2-3% cells suspension)	Titre	Avidity Time	Intensity (Agglutination strength)
Anti-A	Yellow	Polyclonal	A_1	≥ 1:256	10-12 sec	+++
			A_2	≥ 1:128	15-18 sec	++ to +++
			A_2B	≥ 1:64	15-18 sec	++
			O	-	-	-
			B	-	-	-
		Monoclonal	A_1	≥ 1:256	3-4 sec	+++ to ++++
			A_2	≥ 1:128	5-6 sec	++ to +++
			A_2B	≥ 1:64	5-6 sec	++++
			O	-	-	-
			B	-	-	-
Anti-B	Blue	Polyclonal	B	≥ 1:256	10-12 sec	+++
			A_1B	≥ 1:128	10-15 sec	+
			O	-	-	-
			A_1	-	-	-
		Monoclonal	B	≥ 1:256	3-4 sec	++++
			A_1B	≥ 1:128	5-6 sec	+++ to ++++
			O	-	-	-
			A_1	-	-	-
Anti-AB	Mostly colourless or Cherry red coloured	Polyclonal	A_1	≥ 1:256	10-12 sec	+++
			B	≥ 1:256	10-12 sec	+++
			A_2	≥ 1:64	15-18 sec	++ to +++
			O	-	-	-
		Monoclonal	A_1	≥ 1:256	3-4 sec	++++
			B	≥ 1:256	3-4 sec	++++
			A_2	≥ 1:128	5-6 sec	+++
			O	-	-	-

Table 4: Quality requirement of Anti -A_1 Lectin

Parameter	Quality requirement	Frequency of QC
Appearance	No turbidity, precipitation, particles or gel formation by visual inspection	Each day
Specificity	Clear cut reaction with A_1 and A_1B cells. No reaction with A_2, A_2B, B, O cells and cells with other subgroups of A antigen.	On the day of use and each new lot
Avidity	Visible agglutination with 40-50% A_1 red cells suspension in homologous serum using the slide test in 5-20 seconds.	On the day of use and each new lot
Reactivity	No immune haemolysis or prozone phenomenon, and rouleaux formation.	Each new lot
Potency	Titre should be at least A_1 cells: 1:32, A_1B cells: 1:16	Each new lot

Table 5: Quality control of Anti-H (H lectin)

Parameter	Quality requirement	Frequency of QC
Appearance	No turbidity, precipitation, particles or gel formation by visual inspection	Each day
Specificity	Clear cut reaction with cells of all ABO groups. But no reaction with Bombay phenotype cells.	Each new lot
Reactivity	No immune haemolysis or prozone phenomenon, and rouleaux formation	Each new lot

4.3.2. Quality control of Anti-D antisera reagent:

Rh(D) is the most immunogenic and has a higher frequency distribution among all the five varieties of Rh. Quality control of anti-Rh (D) regents is more complex than ABO reagents because of the great variety of reagents, methods of use and the necessary control procedure. The quality control of anti -D reagents is given in Table 6 and 7.

For reliable Rh(D) typing, the following requirements must be met:

1. Use two distinct anti-Rh(D) antisera reagents from two different manufacturers, or use two distinct anti-Rh (D) reagents of two different batches from the same manufacturer.
2. Incorporate Rh (D) positive (group O-R_1r) and Rh (D) negative control cells with the test.
3. Each test sample must give a negative 'auto' test (own cells and own serum).
4. Monoclonal Rh (D) antisera reagents are unreliable for the detection of Weak D antigen.
5. Blend of IgM and IgG monoclonal or blend of monoclonal IgM and polyclonal (Human) IgG can be used for the AHG test to identify Weak D antigen.

Table 6: Quality requirements for Anti-D antisera reagent

Parameter	Quality requirement	Frequency of QC
Appearance	No turbidity, precipitation, particles or gel formation by visual inspection	Each day
Specificity	Clear cut reaction with R_1r cells and no reaction with rr (D negative) cells.	Each day and each new lot
Avidity	Visible agglutination with 40-50% red cells suspension in homologous serum using the slide test.	Each day and each new lot
Reactivity	No immune haemolysis or prozone phenomenon, rouleaux formation	Each new lot
Potency	Titre 32-64 for anti-D using R_1r, R_2r red cells	Each new lot

Table 7: Acceptable titre & avidity of Anti-Rh (D) antisera Reagent

Type of reagent	Type of red cells		Titre		Avidity	Intensity (Agglutination strength)
	ABO phenotyping	Rh Phenotyping	Immediate spin	After 30-45 min incubation at 37^0C		
IgM Monoclonal	O cells	R_1r or R_1R_2	1:64-1:128	1:128-1:256	5-10 Sec	+++

Type of reagent	Type of red cells		Titre		Avidity	Intensity (Agglutination strength)
Blend of IgM +IgG monoclonal	O cells	R_1r or R_1R_2	1:32-1:64	1:128-1:256	10-20 Sec	+++
Blend of monoclonal IgM+ Polyclonal (human) IgG	O	R_1r or R_1R_2	1:32-1:64	1:128-1:256	10-20 Sec	+++
Polyclonal (Human) anti-Rh(D)	O-	R_1r or R_1R_2	-	1:32-1:64 In Albumin/ Enzyme/ AHG test	60 Sec	+++

4.3.4. Polyspecific Anti-Human Globulin (AHG) reagents:

Polyspecific anti-human globulin reagents have anti-complement C3b and C3d and anti-IgG activity.

For quality control testing:

- Each vial of a new batch is tested for its specificity and sensitivity with IgG (anti-D) coated 'O' R_1r red cells as a positive control and non-sensitized 'O' R_1r cells as a negative control.
- For complement activity, check with red cells coated with C3b, C3d or red cells sensitized with complement binding (anti-Le^a).
- The potency of anti-IgG of AHG reagents can be estimated by titration using IgG (anti-D) sensitized O-R_1r cells.

Table 8: Quality requirements for Anti-Human Globulin (AHG) antisera reagent:

Parameter	Quality requirement	Frequency of QC
Appearance	No precipitate, particles or gel formation by visual inspection	Each day
Specificity	Agglutination of red cells sensitized with anti-D serum-containing not more than 0.2 mg/ml antibody activity	Each day and each new lot
	Agglutination of red cells sensitized with a complement binding antibody (e.g., anti-Le^a)	Each new lot
	Agglutination of red cells coated with C3b and C3d, and no/ weak agglutination with C4 coated red cells	Each new lot
Reactivity	No prozone phenomenon and No haemolysis or agglutination of unsensitised red cells	Each new lot
Potency	Anti-IgG - 1:64 with IgG (anti-D) coated O-R_1r red cells Anti-C3/C4 - 1:4 with red cells sensitized with complement binding antibodies (anti-Le^a)	Each new lot

Table 9: Quality requirements for ABO and Rh (D) grouping cards (Column agglutination technology)

Parameter	Quality requirement	Frequency of QC
Appearance	Should not show any signs of drying, discolouration, bubbles, crystals, cracked gel, gel with fissures, or loss of visible fine line of supernatant by visual inspection	Each day

Parameter	Quality requirement	Frequency of QC
Specificity	Reaction in respective anti-A, anti-B, and anti-D incorporated corresponding gel columns in case of A, B or AB Rh(D) positive blood group and No reaction in any of the gel columns incorporated with anti-A, anti B and anti-D antisera in case of O Rh(D) Negative red cells	Each day and each new lot

Table 10: Quality requirements for Anti-Human Globulin (AHG) cards (Column Agglutination Technology)

Parameter	Quality requirement	Frequency of QC
Appearance	Should not show any signs of drying, discolouration, bubbles, crystals, cracked gel or gel with fissures, or loss of visible fine line of supernatant by visual inspection	Each day
Specificity	Reaction with O Rh(D) +ve IgG coated cells O Rh(D) +ve C3d coated cells Negative reaction with unsensitised O Rh(D)+ve cells	Each day and each new lot

4.3.6. Enzyme Reagents:

Protease enzymes (e.g., papain and bromalin) are widely used for antibody detection. Their suitability is generally based on the demonstration of satisfactory serological activity with weak IgG anti-Rh(D). For quality control of protease (enzymes) see Table 11.

Table 11: Quality requirements for enzymes reagents

Parameter	Quality requirements	Frequency of QC
Appearance	No precipitate, particles or gel formation by visual inspection	Each day
Reactivity	No agglutination or haemolysis using inert AB serum	Each day of use
Specificity	Agglutination (+++/C) of cells sensitized with a weak IgG (Anti-D)	Each batch
Potency	An IgG antibody, preferably anti-D standardized to give a titre about 32-64 by the enzyme technique, should show the same titre on repeated testing with different batches	Each batch
	The 2-stage enzyme titre should at least be equal to the titre obtained with IgG(anti-D) by AHG test	Each batch

The one-stage technique, in which the enzyme, antibody and red cells are all mixed is satisfactory but not as sensitive as the 2-stage technique.

4.3.7. Bovine serum albumin (BSA):

It is usually used as:

1. 22% albumin as an enhancer of agglutination.
2. 1-7 % albumin as a stabilizer in other reagents especially those to be stored at 4° C.

Table 12: Quality requirements for 22% bovine serum albumin (BSA)

Parameter	Quality requirement	Frequency of QC
Appearance	No precipitate, particles or gel formation by visual inspection	Each day of use
Purity	> 98% albumin, as determined by electrophoresis	Each new lot

Reactivity	No agglutination of unsensitised red cells; no haemolytic activity; no prozone phenomenon	Each new lot
Potency	IgG anti-D should give a titre of 32-64 with O pooled red cells R_1r/R_1R_1	Each new lot

4.3.8. Quality control of saline reagents:

As mentioned in Table 13 and 14.

Table 13: Quality requirements for LISS (Low Ionic Strength Saline)

Parameter	Quality requirement	Frequency of QC
Appearance	No turbidity or particles by visual inspection	Each day
Osmolarity	270-285 millimoles	Each new batch
pH	6.65-6.85	Each new batch
Haemolysis	A mixture of 0.1 ml saline and 0.1 ml of 5% red cell suspension is centrifuged after 10 min and no haemolysis is observed	Each new batch
Conductivity	3.6-3.7 millimoles/cm at 23^0 C	Each new batch
Reaction	+/++ reaction with R_1r Red cells and 0.25IU/ml IgG anti D (weak) with routine AHG test	Each new batch

Table 14: Quality requirements for normal saline

Parameter	Quality requirement	Frequency of QC
Appearance	No turbidity or particles by visual inspection	Each day
NaCl content	0-154 mol/l(=9g/l)	Each new batch
pH	6.0-8.0	Each new batch
Haemolysis	A mixture of 0.1 ml saline and 0.1 ml of 5% red cell suspension is centrifuged after 10 min and no haemolysis is observed	Each new batch

4.3.9. Quality control of distilled water:

As mentioned in Table 15.

Table 15: Quality Requirements for distilled water

Parameter	Quality requirement	Frequency of QC
Appearance	Clear, no particles on visual inspections	Each day
pH	6.0-7.0	Each new batch

4.3.10. Quality control of Copper Sulphate (CuSO4):

As mentioned in Table 16.

Table 16: Quality requirements for Copper Sulphate (CuSO4)

Parameter	Quality requirement	Frequency of QC
Appearance	Blue in colour, and there should be no particles, precipitates, or contamination on visual inspections	Each day

Parameter	Quality requirement	Frequency of QC
The specific gravity of the stock solution	1.100	On the day of preparation
The specific gravity of the CuSo4 working solution	1.053 + 0.0003	Each day of use
Validity	To be checked with blood samples of known haemoglobin level (12-13.5 g/dl)	Each new batch

4.4. Quality control of blood and blood components

Quality control of blood and its components depends upon:

- Selection of donor
- Quality of the container and anticoagulant preservative solution
- Phlebotomy
- The technique of component preparation
- Storage temperature

The number of units to be tested for quality control of various blood components should be either 1% of the total collection per month or 4 units per month, whichever is greater. Out of the total tested units, ≥ 75% of blood components should fulfill the quality requirements.

All blood component units should be non-reactive for HIV 1 & 2, HBV, HCV, Malaria, and Syphilis.

4.4.1. Quality control of whole blood:

Whole blood is collected in either 350- or 450-ml volume in single, double, triple, quadruple, or pentavalent blood bags.

Table 17: Quality requirements for whole blood

Parameter	Quality requirement	Frequency of QC
Appearance	No haemolysis, No turbidity, No visible clot No frothy appearance	All units
Volume	350/450 ml ± 10 %	1 % of all units or 4 units per month.
Anticoagulants*	49/63 ml	All units
PCV(Hct)	30 to 40%	4 units per month
Sterility	By culture	Periodically (1% of all units)

* Volume of anticoagulant should be proportionate to the volume of blood.

14 ml of CPD/ CPDA -1 anticoagulant solution is required for 100 ml of blood.

The volume of whole blood should be collected from the formula given below:

Volume (ml)= weight of bag (gm) + Blood (gm) – weight of empty bag (gm)/1.053

4.4.2. Quality control of red blood cell (RBC) components:

There are various types of red cell components like red blood cell concentrate (RBC) without additive solution, RBC with additive solution, leucoreduced RBC (Buffy coat reduced and leucofiltered), washed RBC, and cryopreserved RBC. The quality requirements of these red blood cell components are mentioned in Table 18, 19, and 20.

Table 18: Quality requirements of red cell concentrate without additive solution (Prepared from 350 and 450ml Blood)

Parameter	Quality requirement	Frequency of QC
Appearance	No haemolysis, No turbidity, No visible clots, No frothing	All units
Volume	250 ml ±10% for 450 ml 150 ml ±10% for 350 ml	4 units per month/ 1 % of all units
PCV(Hct)	65-70%	4 units per month/ 1 % of all units
Sterility	By culture	Periodically (1% of all units)

Table 19: Quality requirements of red cells concentrate in additive solution (Adsol/SAGM), prepared from 450 ml /350 ml whole blood

Parameter	Quality requirement	Frequency of QC
Appearance	No haemolysis, No turbidity, No visible clots, No frothing	All units
Volume	350±10% for 450 ml 250±10% for 350 ml	4 units per month / 1 % of all units
PCV(Hct)	50-60%	4 units per month / 1 % of all units
Sterility	By culture	Periodically (1% of all units)

Table 20: Quality requirements of leucocyte reduced red blood cell concentrate

Parameter		Quality requirement	Frequency of QC
Appearance		No haemolysis, No turbidity, No visible clot, No frothing	All units
Volume	As mentioned in Table 19		
PCV (Hct)	As mentioned in Table 19		
Leukocyte count	Buffy coat reduced PRBC	$< 5 \times 10^8$	4 units a month
	Leucofiltered PRBC	$< 5 \times 10^6$	4 units a month
	Washed RBC	$< 5 \times 10^6$	4 units a month
Saline washed red cells	Plasma removed	>99%	4 units a month
	RBCs loss	<20%	4 units a month

4.4.3. Quality control of platelet components:

Different types of platelet components are prepared in blood centre like random donor platelet concentrates (prepared by platelet-rich plasma (PRP) method or buffy coat depletion method), pooled platelet concentrates, and single donor apheresis platelet concentrate. Quality control of these platelet components is mentioned in Table 21 to Table 25.

Table 21: Quality requirements of random donor platelet concentrate prepared by PRP method from 350ml/450 ml of whole blood

Parameter	Quality requirement	Frequency of QC
Appearance	Swirling present, No visual RBC contamination, No discolouration	All units
Volume	50-70 ml from 350 ml whole blood 70-90 ml from 450 ml whole blood	All units
Platelet count	3.5 x 10^{10} from 350 ml whole blood 4.5 x 10^{10} from 450 ml whole blood	4 units per month/ 1% of all units
pH	>6.0 (at the end of permissible storage period)	4 units per month/ 1% of all units
RBC contamination	Traces to 0.5 ml	4 units per month/ 1% of all units
WBC contamination	5.5 x 10^{7} to 5.5 x 10^{8}	4 units per month/ 1% of all units
Sterility	By culture	Periodically (1% of all units)

Table 22: Quality requirements of random donor platelet concentrate prepared from buffy coat depletion method from 450 ml whole blood

Parameter	Quality requirement	Frequency of QC
Appearance	Swirling present, No visual RBC contamination, No discolouration	All units
Volume	70-90 ml	4 units per month/ 1% of all units
Platelet count	>6 x 10^{10}	4 units per month/ 1% of all units
pH	>6.0 (at the end of permissible storage period)	4 units per month/ 1% of all units
RBC contamination	Traces to 0.5 ml	4 units per month/ 1% of all units
WBC contamination	<5.5 x 10^{7}	4 units per month/ 1% of all units
Sterility	By culture	Periodically (1% of all units)

Table 23: Quality requirements of buffy coat derived leucocyte depleted pooled (Pooling of 6 units) platelet concentrate

Parameter	Quality requirement	Frequency of QC
Appearance	Swirling present, No visual RBC contamination, No discolouration	All units
Volume	> 200 ml	All units

Platelet count	> 2 x 10^{11}	4 units per month/ 1% of all units
pH	>6.0 (at the end of permissible storage period)	4 units per month/ 1% of all units
Residual red cells	Traces to 0.5 ml	4 units per month/ 1% of all units
Residual leucocytes	< 5.0 x 10^{6}	4 units per month/ 1% of all units
Sterility	By culture	Periodically (1% of all units)

Table 24: Quality requirement of single donor apheresis platelet concentrate

Parameter	Quality requirement	Frequency of QC
Appearance	Swirling present, No visual RBC contamination, No discolouration	All units
Volume	>200 ml	All units
Platelet count	≥3 x 10^{11}	4 units per month/ 1% of all units
pH	>6.0 (at the end of permissible storage period)	4 units per month/ 1% of all units
Residual red cells	Traces to 0.5 ml	4 units per month/ 1% of all units
Residual leucocytes	< 5.0 x 10^{6}	4 units per month/ 1% of all units
Sterility	By culture	Periodically (1% of all units)

Table 25: Quality requirement of single donor apheresis platelet, suspended in platelet additive solution

Parameter	Quality requirement	Frequency of QC
Appearance	Swirling present, No visual RBC contamination, No discolouration	All units
Volume	> 200 ml	All units
Platelet count	> 3 x 10^{11}	4 units per month/ 1% of all units
pH	>6.0 (at the end of permissible storage period)	4 units per month/ 1% of all units
Residual red cells	Traces to 0.5 ml	4 units per month/ 1% of all units
Residual leucocytes	< 5.0 x 10^{6}	4 units per month/ 1% of all units
Sterility	By culture	Periodically (1% of all units)

4.4.4. Quality control of plasma components:

Different plasma components like fresh frozen plasma, cryoprecipitate and cryo poor plasma prepared in blood centres. The quality control criteria for these plasma components are mentioned in Table 26 to Table 28.

Table 26: Quality requirements of fresh frozen plasma (FFP) prepared from 350- and 450-ml whole blood

Parameter	Quality requirement	Frequency of QC
Appearance	No icteric, lipaemia, discolouration present	All units
Volume	180-220 ml from 350 ml bag 220-300 ml from 450 ml bag	4 units per month/ 1% of all units
Stable coagulation factors	200 units for each factor	Occasionally
Factor VIII	70 IU/bag	4 units per month/ 1% of all units
Fibrinogen	200 – 400 mg	4 units per month/ 1% of all units

Table 27: Quality requirements of cryoprecipitate (Factor VIII concentrate)

Parameter	Quality requirement	Frequency of QC
Appearance	Slushy consistency	All units
Volume	15-20 ml	4 units per month/ 1% of all units
Fibronectin	150 mg/bag	Occasionally
Factor VIII	At least 80 IU/bag	4 units per month/ 1% of all units
von-Willebrand factor	40-70% of the original	Occasionally
Factor XIII	20-30% of the original	Occasionally
Fibrinogen	At least 150 mg/bag	4 units per month/ 1% of all units

Table 28: Quality requirements of cryo-poor plasma/single donor plasma

Parameter	Quality requirement	Frequency of QC
Appearance	No icteric, lipaemia, discolouration present	All units
Volume	200 - 220 ml	4 units per month or 1% of all units
Stable coagulation factors	200 units for each factor	Occasionally

4.4.5. Quality control of granulocytes components:

Two types of granulocyte components are prepared in blood centres. The quality control requirements are mentioned in Table 29 and 30.

Table 29: Quality requirements granulocyte concentrate prepared from apheresis method

Parameter	Quality requirement
Volume	200 - 400 ml
Granulocytes	1×10^{10}
Other Leucocytes	$0.1 - 0.7 \times 10^{9}$
Platelets	$2-10 \times 10^{11}$
Red Cells	5-50 ml

Table 30: Quality requirements granulocyte concentrate prepared by pooling of whole blood derived buffycoats

Parameter	Quality requirement
Volume, Plasma	200 - 250 ml
Granulocytes	$0.5-1 \times 10^{9}$

5. Quality control of equipment

5.1. Selection and evaluation

- All equipment used in blood centres should meet mandatory technical, electrical and safety standards.
- Preferably those equipment should be purchased for which expertise for maintenance and repair is available locally with the manufacturer/ supplier.
- The blood centre shall have a policy for selection, procurement, and installation of the equipment. It shall adhere to the following: a) Installation qualification b) Operational qualification c) Performance qualification
- Installation should be formalized with the assistance of commercial installation staff in conjunction with hospital engineering departments, to ensure compliance with electrical safety standards.
- Once the equipment has been installed and calibrated according to the supplier's specifications, it should be confirmed that its performance meets the required standards. It may then be introduced into routine work.
- Each equipment should be checked and assessed for its proper working after repair, and its record should be maintained.
- An annual maintenance contract (AMC) for all the equipment is mandatory.
- Proper disposal and condemnation of non-functional and obsolete equipment.
- Assessment, selection, procurement, installation, calibration/validation, maintenance, troubleshooting, service and repair, and retiring equipment/disposition are the hallmarks of an effective equipment management programme.

5.2. General quality control of equipment

- The performance of laboratory equipment must be monitored periodically; the result must be recorded, and adjustments need to be made if necessary.
- Programme of preventive maintenance including cleaning and recalibration is mandatory. In conjunction with a hospital maintenance engineer or manual maintenance contract specialists, this programme should be planned to minimize disruption of services.
- Laboratory staff should do the cleaning of the equipment and periodic verification of speed by tachometer and temperature by the thermometer.

5.3. Quality control and maintenance of equipment:

5.3.1. Refrigerator for storage of blood:

- Read the recording temperature chart and digital temperature frequently, at least three times a day; the proper temperature range is between 2-6°C.
- The clockwise mechanism driving the temperature recorder should be wound regularly, and the chart should be changed weekly.
- Periodically, the temperatures inside the cabinet should be counter checked with the help of a precision thermometer.
- The alarm system should be battery operated and independent of the main electric supply. The alarm system should be set in such a manner as to make a sound when the temperature is outside the required range of 2^0- 6^0C. The system should be checked once a week by immersing the sensor in ice water (for low temperature) and in water at 15^0-20^0C (for higher temperature). If the alarm system is not working properly, corrective measures should be taken.
- The blood refrigerator must be clean and well lit.

5.3.2. Deep freezers:

- Quality control of deep freezers is similar to blood bank refrigerators. Check the temperature chart and digital temperature frequently at least three times a day.
- Periodically check the temperature of the digital system with precision thermometer kept inside the cabinet periodically.
- If there is no automatic defrosting system, it should be defrosted whenever needed.

5.3.3. Refrigerated centrifuge:

- This should be checked by the service engineer every 3-4 months.
- The accuracy of speed and time should be checked with a precision rpm meter (tachometer) and stopwatch.
- The temperature inside the centrifuge bowl should be recorded by a temperature tester with the lid closed and the rotor stationary.

5.3.4. Laboratory centrifuge (benchtop):

- This should be checked every 3-4 months for accuracy of the speed and time with a precision rpm meter (tachometer) and stopwatch.
- It should be cleaned regularly.

5.3.5. Water bath and incubator:

- They should be kept clean.
- The temperature should be checked daily.
- The accuracy of the thermometer should also be checked periodically
- Water should be changed frequently.

5.3.6. Microscope:

- Keep the microscope covered when not in use
- Lubricate coarse adjustment of rack and condenser every six months.
- Keep the stage clean, clean the condenser, lenses, moistened lens paper, and clean lenses immediately if they accidentally become dirty.

5.3.7. Automatic cell washer:

- The automatic cell washer is a centrifuge programmed to add saline automatically to the test tubes, then centrifuge them and decant the saline. They can be set to perform this function 1-4 times.
- The cell washer should be checked periodically to determine whether the tubes get correctly filled with saline, emptied and proper cell button is formed.
- A kink in the saline entry tubing, blockage of the saline port due to salt crystals or bent or misaligned saline port may give uneven fills and inadequate washes.
- Plastic tubing should be checked monthly for any deterioration.
- Perform duplicate titration of standard antisera, e.g., Anti-D (IgM + IgG) against D positive cells in saline. Wash one set of titres with a cell washer and another set manually, and test both sets by antiglobulin serum. The score should not differ significantly.

5.3.8. Automated equipment for shaking and weighing blood bags :

- It should be checked daily for its performance.
- Check its weighing system with a known weight in grams.

5.4. Frequency of calibration:

Calibration of the equipment depends upon the type of equipment based on its frequency of use in a blood centre. However, the calibration should be done every 6 months to 1 year.

5.5. Breakdown of equipment:

Whenever equipment is found to be defective, it shall be taken out of service, clearly labelled and appropriately stored until it is repaired and shown to be calibrated to meet specified acceptance criteria. The blood centre shall have a policy and procedure for appropriate alternate storage where the blood/blood components shall be shifted in the event of breakdown of storage equipment.

6. Quality control of techniques:

The objective of quality control of techniques is to ensure a consistently high standard of performance of the most commonly used techniques such as ABO and Rh D typing, anti-human globulin test, detection, and identification of irregular antibodies of clinical significance and the compatibility test. Techniques that have been validated for accuracy, reliability, and sensitivity are subject to quality monitoring by using positive, negative, and auto- controls.

- Technique errors may be due to:
  - Lack of proper reagents
  - Lack of attention to incubation time or temperature, or to centrifugation speed and time,
  - Insufficient washing of cells.
  - Too strong or too weak cells suspension, haemolysis read as a negative result
  - Failure to confirm negative results under the microscope.

Proficiency testing of all technical staff and nurses should be done, and their performance evaluated periodically. The blood centre should enrol and actively participate in the EQAS programme. The EQAS sample should be run along with the routine samples, and every technical staff should be allowed to test and interpret the given samples.

Key points

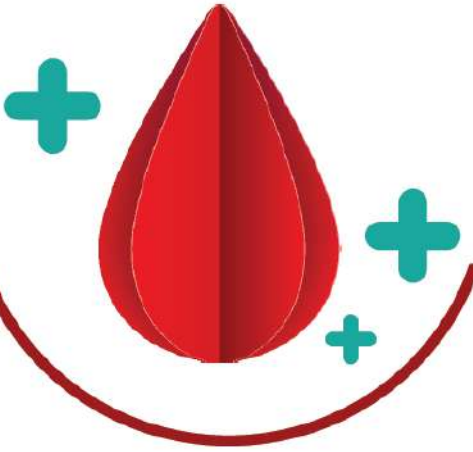
- Quality management is an integrated system of quality assurance covering all matters that individually or collectively influence the components to guarantee their quality.

- Good manufacturing practice (GMP), quality control and audit programme are all closely linked together with the management of errors and accidents.
- Internal quality control and proficiency testing are aspects of a quality system concerned with the examination of material component and the proficiency of the staff.
- Internal quality control should be complemented by participation in the External Quality Assurance Programme.
- Quality assurance describes all the steps taken both in and outside the blood centre to achieve the safest possible blood for the recipient.

References:

1. Drug and Cosmetic Acts and Rules. CDSCO, Ministry of Health and Family Welfare, New Delhi, 2016
2. Quality Management System: Theory and Practice. Technical Manual. American Association of Blood Banks, 18th Edition, 2014;1-40
3. Guidelines for quality assurance programmes for blood transfusion services. World Health Organization, Geneva 1993.
4. Guide to the preparation, use and quality assurance of blood components. European Directorate for the Quality of Medicines & Healthcare.18th Edition; 2015

Section 19



Plasma Derived Medicinal Products

1. Introduction

Plasma-derived medicinal products (PDMPs) form a vital component of contemporary therapeutic modalities for several life-threatening disease conditions. These products are manufactured industrially from human plasma by a specialized technique known as "fractionation". The clinical utility of plasma is limited, predominantly to managing coagulopathies and as a replacement fluid for therapeutic plasma exchange.[1] Nevertheless, it provides the valuable raw material for the production of PDMPs which comprise albumin, coagulation factors (such as factor VIII, factor IX, prothrombin complex, fibrinogen), immunoglobulins (for example, anti-Rho, anti-tetanus, anti-hepatitis B, intravenous immunoglobulins), anticoagulants (antithrombin), protease inhibitors (C1-esterase inhibitor, alpha 1-antitrypsin).[2] Some proteins such as factor VIII and IX can be produced by recombinant technology. However, the other proteins are required to be manufactured from human plasma only. Some of the PDMPs have been included in the World Health Organization Essential Medicines Lists.[3,4]

2. Fractionation of plasma

2.1. Definition:

Blood plasma fractionation refers to the process of separating plasma to isolate its constituents for characterization of the nature and functions of these constituents and as an aid in the diagnosis and therapy in the field of clinical medicine.

2.2. History of fractionation:

The process of plasma fractionation first began at Harvard University, where Edwin Cohn developed a method for purification of albumin using ethanol. By the summer of 1941, he could prepare pure human albumin in his laboratory, which was a remarkable breakthrough in the backdrop of World War II. The stockpile of human albumin was then flown to Honolulu by the government in December 1941 and was used for infusion into naval personnel who had sustained severe burns during the attack on Pearl Harbour. Albumin proved to be lifesaving in several cases.[5] It was substantiated to be a very good substitute for human plasma, which helped expand the blood volume of the wounded soldiers and resulted in their rapid recovery. The product was safe due to virus inactivation. It occupied much less space, did not need reconstitution, and its functionality was preserved even at higher temperatures. During the preparation of albumin, other protein fractions of potential clinical significance were also separated. This stimulated the manufacture of plasma derivatives as a part of industrial pharmaceutics.[6]

Table 1: Chief protein components of human plasma [7,8]

Name of protein component	Concentration in g/dL
Albumin	3.5 -5.5
Immunoglobulin G (IgG)	0.6-1.9

Name of protein component	Concentration in g/dL
Fibrinogen	0.15-0.4
Transferrin	0.2-0.4
Alpha-1-antitrypsin	0.1-0.3
Immunoglobulin A (IgA)	0.1-0.4
Complement component 3 (C3)	0.1-0.18
Immunoglobulin M (IgM)	0.005-0.2

2.3. Basic Steps of Plasma Fractionation:

a. **Procurement of plasma:** Collection of plasma for fractionation should be regarded as an integral part of the entire process. The efficiency and quality of the final product depend on the starting material, which in turn depends upon the methods of plasma collection.[2,7] The plasma for fractionation can be obtained as:

   i. **Recovered plasma**: Plasma retrieved after separation of whole blood into plasma and cellular components. The patterns of red cell utilization drive the availability of recovered plasma.

   ii. **Source plasma**: Plasma collected by plasmapheresis procedure. The availability of automated instruments has facilitated the comfortable and faster collection of a large volume of plasma.

Plasma obtained from both these sources is appropriate for fractionation as long as the collection process conforms with the national blood/plasma policy and Good Manufacturing Practices (GMP) requirement. It needs to be ensured that the qualification criteria for donation are as per those laid down by the regulatory authorities. However, there are inherent differences between recovered plasma and source plasma (Table 2).

Table 2: Comparison between recovered plasma and source plasma used for plasma fractionation [2,7]

Parameter	Recovered plasma	Source plasma
The volume of plasma obtained in one donation	100-250 mL	450-880 mL (WHO recommendation)
Citrate: Plasma ratio	1:7	1:16
pH	Immediate decrease in pH	No significant decrease in pH
Freezing of the plasma	Freezing is delayed due to the time needed for processing	It can be frozen immediately
Factor VIII concentration	66-84.5 IU/dL	10-30% higher than that in recovered plasma. In source plasma, 100 IU/dL(Factor VIII levels are not lowered by serial plasmapheresis)
IgG concentration	9.4 g/L	10-20% lower than that in recovered plasma 8.2 g/L
Resource contribution for plasma fractionation	Major contributor in developing countries	Major contributor in developed countries
Cost-effectiveness	More cost-effective	Less cost-effective if the donors are paid remuneration.

b. **Storage and transportation of plasma:** The overall efficiency and cost-effectiveness of PDMPs manufacturing can be ensured if maximum products (including labile coagulation factors) are prepared from each litre of plasma.[7] Hence, plasma that is stored frozen at less than minus 30°C is the most appropriate starting material. Freezing also lessens the risk of microbial growth in the plasma. In addition, the plasma that is stored at refrigerated temperature can be used for the preparation of albumin and globulin.

The transportation to the manufacturing plant begins after all the tests have been completed and all the information and documentation have been verified. The manufacturing process begins in the manufacturing

plant after a holding period of 60 days after plasma collection to ensure the safety of the product. The plasma is processed typically in a batch of 2000 to 4000 L.[7]

3. Fractionation process:

Plasma proteins can be prepared as purified isolates. While isolating proteins, it is vital to ensure their maximum yield and functionality. The conditions to which the proteins are subjected during processing will affect the functionality of the recovered proteins.[9]

Hence, only those methods of fractionation that do not cause denaturation of proteins need to be used.[7]

Principles of plasma fractionation:

Several methods have been described for plasma fractionation and can be classified in many ways[7,9]:

a. Differential solubility where a precipitant is added decreases the attraction between protein surface and solvent, resulting in increased protein-protein interactions and ultimately precipitation; examples include alcohol fractionation, polyethylene glycol (PEG) fractionation, or salting-out.
b. Differential interaction with solid media or chromatographic methods
    - Ion exchange chromatography
    - Immune affinity chromatography
    - Hydrophobic interaction chromatography
    - Ultrafiltration
    - Gel filtration
c. Differential interaction with physical parameters: (not commonly used)
    - Centrifugal techniques
    - Differential thermal denaturation
    - Electrophoretic techniques

Some of the techniques mentioned above are mainly of research interest. The relevant methods currently in practice are discussed below. For each, the plasma is first taken out of the containers and thawed at 4°C to obtain cryoprecipitate. Then further processing is done.

3.1. Differential solubility:

a. **Ethanol fractionation:** Methods for plasma fractionation were originally based on the principle of differential solubility of plasma proteins.[7] Cohn and colleagues carried out fractionation of plasma at low temperatures by the addition of ethanol in increasing concentrations from 8% to 40% volume/volume. Hence, the process is called cold ethanol fractionation. Organic solvents such as ethanol interact with a polar protein group, displacing water from the protein surface. This leads to protein aggregation and a decrease in solubility, leading to the precipitation of proteins. The concentration of any solvent required to precipitate a protein depends on the basic properties of that particular protein. When successive processing steps are carried out at specific pH, temperature, ethanol concentration, ionic strength, and protein concentration, it results in selective precipitation of proteins due to induced changes in the solubility of albumin and other plasma proteins. Thus, the target protein(s) is/are obtained in the precipitate (called fraction) and the remaining proteins in the supernatant.[9] The supernatant is further treated with the next concentration of ethanol to obtain the subsequent fraction. For obtaining these fractions, it is very important to adjust the above-mentioned physical parameters.

    These precipitated fractions are subsequently separated by centrifugation and/or filtration.[8] Albumin is the final product to be precipitated because of its highest solubility and the lowest isoelectric point of all the major plasma proteins. Therefore, the separations should be carried out at temperatures as close to 0°C as possible to minimize the risk of denaturation.

    The parameters of cold ethanol fractionation and the constituents of each fraction (fractions I to V) are outlined in Table 3.

Table 3: Overview of cold ethanol fractionation [7, 10]

S. No.	Original substance	pH	Temp	% Ethanol	Ionic strength	Precipitate	Constituent proteins of the precipitate	Remainder to be
1.	Plasma	7.2	-3°C	8 %	0.14	Fraction I	Fibrinogen, factor XIII,	Supernatant I (Antithrombin III, Prothrombin complex, Factor IX)
2.	Supernatant I	6.9	-5°C	25%	0.09	Fraction II+III	Immunoglobulins, Plasminogen (plasmin)	Supernatant II+III (Albumin)
3.	Supernatant II+III	5.2	-5°C	18 %	0.09	Fraction IV-1	α-1-proteinase Inhibitor, Apolipoprotein A	Supernatant IV-1
4.	Supernatant IV-1	5.8	-5°C	40 %	0.09	Fraction IV-4	Transferrin, Haptoglobin, Ceruloplasmin, Apolipoprotein A, α-1-proteinase inhibitor	Supernatant IV-4
5.	Supernatant IV-4	4.8	-5°C	40 %	0.11	Fraction V	Albumin, Haptoglobin	Supernatant V

At the end of step V, a rework needs to be done on precipitate V using 10% ethanol (at temperature minus 3°C, pH 4.5, ionic strength 0.1) so that impurities like lipoproteins and globulins get precipitated and are thus removed. The resultant supernatant is further treated with 40% ethanol (at a temperature of minus 3°C, pH 5.2, ionic strength 0.01) to obtain pure albumin. Removal of ethanol is done by freeze-drying or by heat inactivation.

There are several advantages of using cold ethanol fractionation. Ethanol is easily available. It is inexpensive, and can be easily removed and reused in the process. It is bacteriologically safe due to the inhibition of bacterial growth during the process. It is very suitable for large-scale production. However, alcohol is inflammable; there is a stringent requirement for temperature and pH control. It also causes loss and denaturation of some of the proteins. Therefore, ethanol fractionation needs to be combined with newer chromatographic techniques for better yields and functionality of the products (see below).[7, 8]

b. **Polyethylene glycol fractionation:** Polyethylene glycol (PEG) is a non-toxic, water-soluble polymer that can be used as a protein precipitant at an average molecular weight of 4000 to 6000 Da. PEG is less expensive than ethanol. It does not require stringent temperature requirements like alcohol. It causes less foaming and is not flammable. By using PEG 9.06% with NaCl 1.4 M at pH 5.5, 91% of fibrinogen can be obtained in the precipitate. By further adding PEG 12.6% and NaCl 0.35 M to the resultant supernatant at pH 8.22, 88% of immunoglobulins can be precipitated.[9,10] The disadvantage of using this method is the difficulty in removing PEG from the supernatant fraction. Ultrafiltration, gel permeation, or electrodialysis can be employed for this purpose.

c. **Salting-out:** In the salting-out method, salt solutions of high ionic strength are used. The salt pulls water molecules away from protein surfaces which makes the proteins interact and coagulate. Proteins significantly differ in their solubility with high ionic strength solutions. Salting-out can be used to separate and isolate proteins from complex solutions such as plasma. The larger the molecule, the more readily it is precipitated. The aggregated protein is then separated by centrifugation, leaving the remaining proteins in the supernatant. Ammonium sulphate is one of the salts that have been used for protein separation. Fibrinogen can be separated out from human plasma by repeated precipitation with ammonium sulphate (concentration 58% to 73%).[9,10]

Ammonium sulphate precipitation technique can be combined with other methods such as ion exchange or affinity chromatography for better separation of proteins from plasma.

3.2. Differential interactions with solid media:

a. **Chromatographic methods:** Chromatographic methods were introduced in the 1960s. The technique was initially used for isolation of cryoprecipitate and also for better recovery of immunoglobulin G. Since then, large-scale chromatography has become a major technological advancement for improving the purity of the products and achieving better recovery of extracted proteins, including the labile coagulation factors.[7] Consequently, chromatographic techniques have been able to widen the spectrum of therapeutic products obtained from plasma, thus contributing to the cost-effectiveness of the process of plasma fractionation.[2,11] Ethanol fractionation is still the common mode for albumin extraction, while chromatographic procedures are being increasingly used to extract coagulation factors, protease inhibitors, and immunoglobulins (especially anti-D).[2,7]

   Anion exchange chromatography and affinity chromatography using either monoclonal antibodies (immunoaffinity chromatography) or immobilized heparin are the main two techniques used for plasma fractionation.[7] Of these, affinity chromatography is the more commonly employed method based on specific molecular recognition.

   The main advantages of chromatographic techniques are selectivity, functional adaptability, and high separation efficiency. The process does not use denaturing substances like ethanol, and the yield of protein fractions obtained is higher. The technique can also be used for removing viruses in the product. The main disadvantage is that column degradation and leaching (lost ligands from the column).[7] Bacterial growths during processing is another hazard. Additionally, not all chromatographic media are suitable for large-scale usage. Newer combinations based on agarose beads have been established, which facilitate the scaling-up of the process. The performance of agarose beads is not affected by high internal pressure or high flow rate. These beads can be used as a part of either ion-exchange or size-exclusion chromatography. [9]

4. Quality and safety of plasma products (“Five-layer safety”)

The quality of the plasma used for fractionation forms the basis of the safety and efficacy of the pooled industrial plasma products.[2,12] It is required that the starting plasma material conforms to the quality and safety requirements and specifications. This can be achieved by the following means:

- Donor screening and deferral criteria
- Individual donor testing
- Quarantine procedures
- Storage and transportation of plasma
- Process monitoring and viral inactivation
- Traceability and documentation
- Epidemiological surveillance of the donor population
- Lookback policy
- Regular audits of the blood collection facilities
- Post-donation information to the donors

All the above procedures should comply with Good Manufacturing Practices (GMP). The national regulatory authority should license the blood establishments producing plasma (recovered and source plasma) for fractionation.[2] Readers are requested to refer to WHO recommendations for production, quality, control and regulation of plasma for fractionation.[13]

The further quality aspects that may affect the quality and recovery of fractionated products include factors such as rate of freezing, storage temperature, etc. The industrial plasma fractionation process should also be carried out under conditions that comply with GMP. The regulatory authorities should also license these fractionation facilities.[2]

After the final refined products are obtained, they are subjected to processes that inactivate and/or remove enveloped and non-enveloped viruses present in the starting plasma. This step makes the PDMPs safer than the blood components.

5. Virus inactivation/removal process:

The virus inactivation/removal processes should be such that they do not cause deterioration of the therapeutic proteins. The methods also should not give rise to immunogenicity by forming new antigens. [2, 7]

5.1. Virus inactivation methods: Pasteurization:

It is used for albumin preparation. The albumin solutions are heated at 60°C for 10 to 11 hours. Smaller concentrations of sodium caprylate with or without N-acetyl are added to prevent albumin denaturation. All parts of the container must be heated equally so as to achieve a constant temperature throughout the solution. The addition of high concentrations of glycine and sucrose enables pasteurization of labile proteins such as Factor VIII preparations by preventing denaturation. Pasteurization is effective against both enveloped and non-enveloped viruses.

a. **Dry heat**: The freeze-dried or lyophilized proteins are subjected to heating at 100°C for 30 minutes or 80°C for 72 hours. The residual moisture in each vial needs to be controlled because it may interfere with the success of the method of virus inactivation.
b. **Vapour heat**: Lyophilized products are heated at 60°C for 10 hours, followed by 80°C for 1 hour. There is relatively high defined residual moisture.
c. **Solvent/Detergent method**: When the protein product is treated with organic solvents/detergents, the lipid membrane of the enveloped viruses is disrupted. These viruses are then unable to infect the cells. A solvent, e.g., 0.3% tri(n-butyl) phosphate (TNBP) and 1% ionic detergent (Tween 80 or Triton X-100) at 24°C for 4 hours is an example of conditions used for viral inactivation. The intermediate product is then filtered to remove trapped viruses. The solvent-detergent mixture is removed using adsorption chromatography. The procedure inactivates enveloped viruses such as Human Immunodeficiency Virus (HIV), Hepatitis B, and Hepatitis C. However, it is ineffective against non-enveloped viruses (Hepatitis A, Parvovirus B)
d. **Low pH (as an adjunct):** Preparations of immunoglobulins at pH as low as 4 causes inactivation of many enveloped viruses.

5.2. Virus removal methods:

The viruses can be removed by precipitation methods in which either the viruses or the proteins of interest are precipitated. In ethanol precipitation, the virus is separated from protein, causing partitioning. This is especially useful for ensuring the safety of immunoglobulins. Chromatographic techniques (ion-exchange chromatography, affinity chromatography, and hydrophobic interactions) can also be used for the precipitation of viruses. Specially designed steps can also remove viruses during manufacturing; an example is a nanofiltration.

When various viral inactivation/removal techniques are implemented, the sensitivity of each of these methods should be taken into account. While HIV is sensitive to heat, hepatitis viruses require treatment at a higher temperature for a longer duration to be inactivated. As discussed above, non-enveloped viruses are not inactivated by the action of solvent/detergent. Thus, to minimize the risk of virus transmission and to achieve the best possible safety level, two methods of inactivation may be combined. For example, solvent/detergent method with heat treatment.[14] All the processes used must be validated before they are used.[7]

6. Plasma-derived medicinal products

Various plasma-derived medicinal products are available nowadays (Table 4). The most commonly used products are discussed here. [2, 7]

Table 4: Plasma-derived medicinal products

S.No.	Name of Plasma Product	Preparation	Indications for use (Refer to individual product for detailed indications)
1.	Human albumin	5% or 20% for intravenous use	Severe acute hypoproteinaemia Therapeutic plasmapheresis
2.	Immunoglobulins a. Normal serum immunoglobulin b. Immunoglobulin (intravenous) c. Specific OR hyperimmune globulin	 Freeze-dried preparation or Intravenous (16%) solutions Freeze-dried preparation or intravenous (6%) solution	 Hypo/agammaglobulinaemia Autoimmune diseases Hypo-/agammaglobulinaemia Idiopathic thrombocytopenia Prevention of the specific infections Rh immunoglobulin is used to prevent Rh sensitization due to pregnancy/ blood transfusion/ transplantation
3.	Coagulation Proteins a. Factor VIII –Factor IX complex b. Factor IX c. von Willebrand Factor d. Factor VII e. Factor XIII f. Fibrin sealant a. Prothrombin complex	 Freeze-dried for intravenous use Freeze-dried for intravenous use Freeze-dried for intravenous use Freeze-dried for intravenous use Freeze-dried for intravenous use Freeze-dried for topical use Freeze-dried for intravenous use	 Haemophilia A, Haemophilia B, Haemophilia B von Willebrand disease Factor VII deficiency Factor XIII deficiency Wound healing Warfarin overdose, Factor II-, Factor VII-, Factor X-deficiency
4.	Anticoagulants a. Antithrombin	 Freeze-dried for intravenous use	 Antithrombin deficiency
5.	Protease inhibitor a. C1 esteraseinhibitor b. Alpha-1-proteinase inhibitor	 Freeze-dried for intravenous use Freeze-dried for intravenous use	 Hereditary angio-neuroticoedema Alpha-1- proteinase inhibitor deficiency

5.1. Human albumin:

Albumin is the largest component of human plasma proteins (3.5 to 5.5 g/dL). It is a negatively charged molecule synthesized almost exclusively in the liver. The two most important physiological functions of albumin are[15, 16]:

- **Volume effect (colloid oncotic pressure):** Although albumin constitutes only 50 to 60 % of the total protein content of plasma, it is responsible for 80% of intravascular colloid osmotic pressure.
- **Transport function**: Due to the high net negative charge, albumin is an important transport protein for fatty acids, bilirubin, hormones, and many drugs.

a. **Preparation of human albumin:** Human albumin is prepared from pooled human plasma by ethanol precipitation. Albumin is pasteurized for at least 10 hours at 60°C for pathogen inactivation.

b. **Active constituents:** Human albumin preparations are manufactured as hyper-oncotic (20-25%) and iso-oncotic (5%) infusion solutions. The active constituent is human albumin, made up of a known sequence of 584 amino acids. The molecular weight is around 66kDa. The preparation may contain monomers, dimers, and small amounts of polymers of albumin.

c. **Quality criteria:** Human albumin solutions must contain 95% albumin as per the European Pharmacopoeia. The maximum limit of polymers and aggregates permitted is 10%. Sodium octanoate (up to 3.2 g/L) and acetyl tryptophan (4.29 g/L) are added as stabilizers. Additionally, the currently available preparations contain sodium (87-160 mmol/L) and potassium (less than 2 mmol/L). Albumin solutions do not contain any blood group substances. Hence, blood grouping or compatibility testing is not required while administering albumin.

   Albumin solutions should preferably contain only trace quantities of aluminium to ensure safe use in renal dialysis patients, premature infants, and burn patients (British Pharmacopoeia). The solution should be sterile, non-pyrogenic and the endotoxin levels not be more than 0.5 EU/ml (EP/USP recommendations).

d. **Storage:** Human albumin preparations are available as 5% and 20% solutions. They can be stored at room temperature not exceeding 25°C and should be kept protected from light.

e. **Clinical indications for the use of albumin:** Human albumin preparations are costly and have limited availability. Hence, the recommendations for its appropriate use must be properly defined. The indications for albumin use are divided into three categories and are discussed here.

   i. **Appropriate use (based on most common consensus):**
      - Spontaneous bacterial peritonitis: Used in combination with antibiotics.
      - Therapeutic plasmapheresis: When plasma removal is more than 20 ml/kg/week
      - Paracentesis: Albumin 5 g/L of ascitic fluid removed after paracentesis of volumes more than 5 L

   ii. **Occasionally appropriate use:**
      - Cardiac surgery: Last choice of treatment after crystalloids and non-protein colloids
      - Major surgery: Not in the immediate postoperative period. The only indication for use is albumin, less than 2 g/dL after normalization of circulatory volume
      - Cirrhosis of the liver with refractory ascites
      - Contraindications for the use of non-protein colloids: Acute liver failure, intracranial haemorrhage, pregnancy and breastfeeding, perinatal period and early infancy, moderate to severe renal failure, dialysis with severe haemostatic abnormalities and serum albumin <2.5 g/dL
      - Hepatorenal syndrome: Human albumin to be used in association with vasoconstrictive drugs
      - Haemorrhagic shock: Only in case of lack of response to crystalloids or colloids and when the use of non-protein colloids is contraindicated.
      - Nephrotic syndrome: Only in patients with albumin less than 2 g/L (and hypovolemia and/or pulmonary oedema)
      - Organ transplantation: Post-operative period after liver transplant to prevent ascites

   iii. **Inappropriate use**
      - Malnutrition
      - Wound healing
      - Ascites responsive to diuretics
      - Burns in the first 24 hours
      - Malabsorption
      - Non-haemorrhagic shock

f. **Dose:** The dose needed to achieve serum albumin ≥ 2.5 g/dL is calculated using the following formula
Dose (g) = [desired albumin concentration (2.5 g/dL) − actual albumin concentration (g/dL)] x plasma volume (0.8 x kg)

g. **Contraindications:**
- Allergy against albumin
- Hypervolemic state
- Congestive heart failure
- Pulmonary oedema
- Dilutional coagulopathy

h. **Adverse effects of human albumin:** Immediate-allergic reactions with fever, shivers, nausea, salivation. It can precipitate congestive cardiac failure in those who are at risk.

5.2. Human immunoglobulins:

Immunoglobulins are protein molecules that have the following two functions:

- **Antigen binding**: Each immunoglobulin actually binds to a specific antigenic determinant. Antigen binding by antibodies is the primary function of antibodies and can result in the protection of the host.
- **Effector functions**: Immunoglobulins may fix complement resulting in lysis of cells and release of biologically active molecules. The binding of immunoglobulins to receptors on certain cells can activate the cells to perform certain functions.

a. **Preparation of human immunoglobulins:** Hyperimmune globulins are special immunoglobulins directed against infectious organisms like rabies, hepatitis B, hepatitis C, and tetanus. They are liquid preparations for intramuscular use. They contain a standardized amount of antibody to a specific infectious antigen and are prepared from source plasma. The donors may be screened for specific antibodies, and their plasma is stored for special fractionation. The plasma may also be collected from paid donors who have been immunized with specific commercially available antigens (vaccines), a process called hyper immunization.

The collected plasma is subjected to 25% ethanol fractionation to obtain human immunoglobulin preparations. The preparations for subcutaneous (SCIg) or intramuscular(IM-Ig) and intravenous (IVIg) applications differ with respect to their protein content. Total chromatographic procedures are used for specific immunoglobulin preparations such as Rh Immune Globulin (RhIG).

b. **Active constituents of immunoglobulin preparations:** The active components of human immunoglobulin preparations are specific antibodies. These preparations may be used for prophylactic or therapeutic indications. Immunoglobulins in solution contain stabilizers like albumin, amino acids (glycine, proline, isoleucine), and sugars (glucose, sucrose, sorbitol, maltose).

Normal immunoglobulins for subcutaneous/intramuscular injection or for intravenous injection contain more than 90% monomeric IgG1-4 and very small amounts of IgM and IgA. The specific immunoglobulins (hyper immunoglobulin) have concentrations of the specific antibody which is many times higher than normal Immunoglobulin preparations.

c. **Quality criteria for immunoglobulin preparation:** The quality criteria are laid down by European Pharmacopoeia. The product must, at a protein concentration of 50–120 g/l (IVIg) or 160 g/l and 165 g/l (SCIg), contain defined antiviral and antibacterial antibodies at a concentration of at least three-fold (IVIg) or ten-fold (SCIg) above that of the starting material. The preparation must not transmit infections.

High volume infusions may be required in certain clinical conditions to attain the optimal dosage in some patients. Hence, the maximum allowable anti-A and anti-B haemagglutinin titre is 1:64 (EP) to avoid haemolytic transfusion reactions. Antibody titre to hepatitis B should be atleast 0.5IU/g IgG (EP).

WHO has laid down standards for lyophilized preparations after they are dissolved according to instructions. A therapeutic dose of IVIg causes a sharp rise in plasma concentration, followed by a decrease to about half the peak concentration within the next 6-12 hours (due to distribution into ex-

travascular space). Plasma levels decrease slowly over 2 to 4 weeks to their original levels. Antibodies appear in circulation about 20 minutes after administration of IM-Ig and SC-Ig; maximum antibody titres are reached after approximately 4 days.

d. Storage, shelf life, and package sizes: Immunoglobulin preparations are available in lyophilized form or in stabilized solution. They are available in different pack sizes so that the required dose can be adjusted according to individual indications. Shelf life and storage temperature must be followed as per the manufacturer's instructions

e. Indications for the use of immunoglobulins: [17, 18]

I. Intravenous injection of normal immunoglobulins[17]

i. Primary immunodeficiency diseases: In primary immunodeficiency diseases, accompanied by antibody deficiencies and increased susceptibility to infections (Bruton's X-linked agammaglobulinaemia (XLA), severe combined immunodeficiency (SCID and variants), common variable immunodeficiency (CVID), and various forms of hyper-IgM syndromes, continuous therapy with IVIg or SCIg shall be performed. In other rare immunodeficiency diseases (e.g., Wiskott-Aldrich syndrome), IVIg substitution therapy is indicated only when there are severe recurrent infections and insufficient antibodies are formed after vaccination. It is given in the dose of IVIg 0.4–0.8 g/kg body weight initially. A maintenance dose is 0.4–0.6 g/kg body weight at 2- to 10-week intervals depending on the clinical situation. Subcutaneous Ig is an alternative to continuous IVIg treatment.

i. Secondary immunodeficiency diseases:

- Substitution therapy in patients with chronic lymphocytic leukaemia (CLL) and multiple myeloma with a secondary antibody deficiency and susceptibility to infections
- Substitution therapy in patients who are chronically immunosuppressed, patients after stem cell transplantation, and patients with malignancies who develop a secondary antibody deficiency with increased susceptibility to infections.
- HIV-infected infants and small children who have an increased susceptibility to bacterial infections despite highly active antiretroviral therapy.

ii. Autoimmune diseases: High dose IVIg: Accepted indications for IVIG in treating immune-mediated disease are the following[18]:

- Replacement in primary and secondary immunodeficiency diseases (such as in B-cell lymphoproliferative disorder)
- Allogeneic marrow transplantation
- Kawasaki disease
- Guillain-Barre syndrome
- Chronic inflammatory demyelinating polyneuropathy (CIDP).
- Myasthenia gravis
- Dermatomyositis
- Immune thrombocytopenic purpura (ITP), pure red cell aplasia, and alloimmune mediated thrombocytopenia

II. Indications for specific (enriched) immunoglobulins

i. Anti-D (RhIG): Prophylactic application of specific immunoglobulins for RhD[17] has been outlined in Table 5.

Table 5: Use of specific immunoglobulins (anti-D)

Indication	Type	Explanation
Rh(D)-negative (dd) women		
After delivery of an Rh-positive child	Anti-D IMIg	Post-partum prophylaxis

Indication	Type	Explanation
During pregnancy	Anti-D IMIg	Antepartum prophylaxis
After the abortion, placenta praevia,ectopic pregnancy, amniocentesis, chorionic biopsy or cord puncture, after forced inversion, after removal of a hydatid mole	Anti-D IMIg	Prophylaxis
Rh(D)-incompatible transfusions		
Prophylaxis for immunization against D in Rh-negative (dd) recipients of Rh-positive (D+) RBC or granulocyte concentrates or platelets	Anti-D IV Ig	For prevention of anti-D formation, especially for women of reproductive age; not applicable in emergency transfusion
Immune thrombocytopenia	Anti-D IVIg	Second-line therapy after IVIg
	Anti-D SCIg	After splenectomy

ii. Specific immunoglobulins (hyperimmune) against infectious diseases:

They are used in the treatment of rabies, tetanus, hepatitis B, hepatitis C, varicella, and cytomegalovirus, i.e., after the patient has actually contracted the disease but does not have sufficient antibodies against the infectious agent. It can also be used prophylactically for CMV infection.

f. Absolute and relative contraindications: [17]

- Selective IgA deficiency: IVIg and IMIg are contraindicated.
- Antibodies to IgA: SCIg can be used safely.
- Transient hypogammaglobulinaemia during childhood unless there is a failure to develop normal amounts of antibodies following vaccination.
- A minimum interval of 2 weeks between parenteral administration of specific immunoglobulins and attenuated live vaccines (measles, rubella, mumps, chickenpox, yellow fever) is required.

g. Adverse reactions to immunoglobulins: The adverse effects are more common with IVIg than with SCIg and include aseptic meningitis, flu-like symptoms, epidermolysis, headache, arrhythmia, hypotension, hyperviscosity, embolic events, immediate anaphylaxis.[17] There is also the risk of transmission of variant Creutzfeldt-Jakob disease (vCJD)- a prion protein.

5.3. Factor VIII concentrate

Factor VIII is a labile coagulation factor and an acute phase reactant synthesized in the liver. It is the cofactor in the tenase complex in the intrinsic pathway of coagulation that activates factor X to Xa. Haemophilia A is an X-linked recessive disorder in which factor VIII activity is reduced in the plasma of the affected patients with haemophilia A.

a. Preparation of factor VIII concentrate: In 1964, Judith Pool discovered that the fraction cryoprecipitate from plasma contained large amounts of factor VIII. This led to the production of lyophilized plasma concentrates manufactured using Cohn-Oncley fractionation. The technological advancement made home replacement therapy possible in patients with haemophilia A with early control of haemorrhages. For the production of factor VIII by plasma fractionation, cryoprecipitate is subjected to precipitation using glycine or aluminium hydroxide adsorption and precipitation to remove fibrinogen. Further purifications are done using immunoaffinity and monoclonal antibodies to obtain highly purified factor VIII concentrate with a specific activity of 100 to 250 IU/mg. Now factor VIII can be produced by recombinant DNA technology as well.[7,14]

b. Active constituents: Factor VIII concentrates contain high concentrations of highly purified clotting factor VIII (factor VIII: C, i.e., factor VIII clotting activity).[19]

c. Quality criteria: The quality of Factor VIII concentrate depends on the starting plasma, the production process, the degree of purity of the concentrate, and its immunogenicity.[19]

The specific activity should not be less than 1 IU of factor VIII per milligram of total protein before the addition of any protein stabilizer.

d. Storage: Generally, factor concentrates must be stored protected from light. The standard storage temperature for concentrates is 2–8°C. Some factor concentrates can be stored temporarily or over their entire shelf life at up to 25–30°C. The manufacturer's instructions should be followed.

e. Indications for the use of Factor VIII concentrate: Factor VIII concentrate is used in the treatment of haemophilia A (prophylactic and on-demand use of factor VIII).[14,19] The therapy for haemophilia aims at the following primary goals:

- Prevention of bleeding (prophylaxis)
- Treatment of bleeding and its complications
- Maintaining the joints and/or reinstating the function of affected joints,
- Ensuring generalized psychological and social well-being of haemophiliacs.

Further criteria depend on:

➢ **Patient variables**

- Age (small children and infants usually require higher doses of factor VIII per kg body weight because of higher relative plasma volume)
- Degree of severity
- Inhibitor formation
- Individual variations in recovery and half-life
- Adverse reactions to the therapy

➢ **Clinical variables**: Frequency and site of bleeding, state of the particular joints and other co-morbid conditions.

➢ **Factor VIII replacement therapy:** Factor replacement on demand shall be performed during spontaneous or traumatic bleeding episodes if it exceeds a minimum degree.

f. Dose:

- Bleeding into joints and muscles (30-40 IU/kg), life-threatening (80-100 IU/kg), surgery (major bleeding 80-100 IU/kg), minor wounds (50-100 IU/kg).
- Full-time prophylactic replacement therapy shall be carried out mostly in children and adolescents with severe haemophilia, which is self-administered physician-controlled treatment with the primary aim of preventing haemophilic arthropathy. (Mean dose: 20–30 IU/kg body weight at least 3 times per week).
- Full-time prophylactic replacement therapy in adults: prevention of development of late-consequence arthropathy. (Mean dose: 20–30 IU/kg body weight at least 3× weekly).
- Prophylactic therapy to prevent bleeding shall be provided before and after surgical interventions.
- Temporary prophylactic therapy to prevent bleeding should be provided during physical or psychological stress.

The activity of clotting factors is expressed in units. One unit of a clotting factor corresponds to '100% factor activity' and is defined as the activity in 1 ml of pooled plasma from healthy donors.

Patients with severe or moderate haemophilia A usually require exclusively factor VIII concentrates. In contrast, most patients with mild haemophilia A or von Willebrand syndrome type 1 can be treated with desmopressin(dose of 0.3 μ g/kg body weight or as nasal spray) with the exception of severe bleedings or during major surgery.

g. Use of Factor VIII in patients who have developed inhibitors: Development of neutralizing alloantibodies (inhibitors) against the infused factor (Factor VIII or IX) is a challenging complication of haemophilia treatment. Approximately 30% of patients with severe haemophilia A will develop inhibitors, while up to 5% of patients with mild and moderate haemophilia A and 3% of patients with haemophilia B develop inhibitors. The development of inhibitors renders replacement treatment partially or completely ineffective and poses an increased risk of morbidity and mortality in patients.

The level of inhibitor development is measured in Bethesda Units (BU)

- Children and adults who are low responders (<5 BU) and have the possibility of acute bleeding may be treated with high-dose factor VIII infusions to achieve haemostatically effective factor

VIII levels. Other treatment modalities are Activated Prothrombin Complex Concentrate or Recombinant Factor VIIa. (See below).

- Children and adults who are high responders (>5 BU) cannot be treated with factor VIII therapy. They require treatment with Activated Prothrombin Complex Concentrate or Recombinant Factor VIIa, or immunoadsorption apheresis to remove the antibodies.
- Factor VIII is also used for inhibitor elimination by inducing immune tolerance.

➢ **Children:**

- Low Responders (<5 BU): Factor VIII concentrates 50-100 IU/kg body weight 3 times per week until normal recovery and half-life are achieved. Weekly monitoring for inhibitors needs to be done.
- High Responders (>5 BU): Factor VIII concentrate at a dose of 100–200 IU/kg body weight twice daily until normal recovery and half-life over several months are achieved.

➢ **Adults:**

- Low Responders (<5 BU): Elimination therapy is not recommended during continuous therapy with factor VIII concentrate.
- High responders (>5 BU): Factor VIII concentrate at a dose of 100–200 IU/kg body twice daily until normal recovery and half-life are achieved.

The immune tolerance therapy should be followed by individualized continuous therapy with Factor VIII.

Table 6: Recommended dosages of factor VIII in the management of Haemophilia A [14]

S. No	Type of Haemorrhage	Dose of Factor VIII(IU/kg)
1.	Mild or moderate haemarthroses or haematomas	20-30
2.	Severe haemarthroses of haematomas External bleeding with anaemia Moderate posttraumatic bleeding	30-50
3.	Cranial trauma Cerebral haemorrhage	50–100
4.	Surgery prophylaxis	50-100*
5.	Primary prophylaxis	25-30 (thrice-weekly)

*For surgical prophylaxis, factor VIII levels should be maintained above 50% for 7-15 days after surgery

h. Use of factor VIII concentrate in von Willebrand disease: Plasma-derived FVIII concentrates may also contain variable amounts of von Willebrand factor (vWF). Hence, some Factor VIII products are used in the management of those patients with severe von Willebrand disease in whom response to desmopressin is impossible or insufficient.[14,19]

i. Adverse effects and contraindications for the use of factor VIII concentrate: The development of inhibitors is the major adverse effect of the use of Factor VIII.[19] There are no contraindications for the use of Factor VIII.

6.4. Factor IX concentrate

Factor IX is the proenzyme that activates factor X in the presence of cofactor VIII in the intrinsic pathway of coagulation. It is synthesized in liver cells. It is dependent on vitamin K for synthesis. Factor IX formation is encoded by a gene on the X chromosome. Haemophilia B is an X-linked recessive disorder in which Factor IX activity is reduced.

a. Preparation of Factor IX concentrates: Factor IX concentrate is prepared by plasma fractionation. It is prepared from the prothrombin complex concentrates (PCC) remaining in the supernatant. Factor IX is isolated by affinity chromatography or by ion-exchange chromatography. [19]

b. Active constituents of Factor IX concentrate: Factor IX concentrates contain high concentrations of factor IX. The biological half-life of factor IX is 20–24 h. [19]

c. **Quality criteria:** The quality of Factor IX concentrate depends on the starting plasma, the production process, the degree of purity of the concentrate, and its immunogenicity

d. **Indications for the use of factor IX concentrate:** Factor IX concentrates are used in the treatment of haemophilia B. Table 7 shows the indications and dosages of Factor IX.[19,20] The indications for the use in children and adults for prophylaxis and on-demand treatment in haemophilia B are similar to those of Factor VIII used in haemophilia A.

Table 7: Recommended dosages of factor IX in the management of Haemophilia B [20]

Type of Haemorrhage	Dose of Factor IX (IU/kg)
Mild or moderate haemarthroses or haematomas	20-40
Severe haemarthroses of haematomas External bleeding with anaemia Moderate posttraumatic bleeding	40-60
Cranial trauma Cerebral haemorrhage	50–100
Surgery prophylaxis	50–100*
Primary prophylaxis	30-40 (twice weekly)

*For surgical prophylaxis, factor IX levels should be maintained above 50% for 7-15 days after surgery

e. **Adverse effects and contraindications for the use of factor IX concentrate:** The development of inhibitors is the only adverse effect of the use of Factor IX. There are no contraindications for the use of Factor IX.

6.5. Fibrinogen concentrate

Fibrinogen is a glycoprotein produced in the liver and stored in both endothelial tissue and platelets. It's biological half-life is 96–120 h. The normal fibrinogen concentration in plasma is 1.5–4 g/L.

Fibrinogen plays a critical role in haemostasis as it acts as an endogenous substrate for fibrin formation. It also induces clot formation and platelet aggregation. Fibrinogen deficiency may be inherited or acquired. The deficiency may be treated using fresh-frozen plasma, cryoprecipitate, or plasma-derived fibrinogen concentrate. Benefits of fibrinogen concentrate over FFP and cryoprecipitate include viral inactivation, rapid reconstitution, accurate dosing, and a lower volume of administration for equivalent fibrinogen supplementation (Table 8).

a. **Preparation of fibrinogen concentrate**: Fibrinogen concentrate is produced from pooled human plasma using the Cohn/Oncley cryoprecipitation procedure. [21]

b. **Active constituents:** The concentration of fibrinogen per vial of the product is standardized and mentioned on the vial.[21] Some preparations contain albumin as a stabilizer. The storage conditions will be as prescribed by the manufacturer.

c. **Indications for the use of fibrinogen:** [21,22]

   i. **Congenital fibrinogen deficiency:** Congenital hypofibrinogenaemia (fibrinogen levels between 0.5 and 1.5 g/L), congenital, haemorrhagic dysfibrinogenemia.

   Generally, no fibrinogen replacement therapy is required. However, replacement therapy will be required if fibrinogen levels are <1 g/L and surgical intervention is planned. Fibrinogen levels of at least 1 g/l must be aimed for in congenital afibrinogenaemia (no functional fibrinogen is formed)., The plasma fibrinogen levels of at least 1 g/L should be aimed from beginning prior to surgery and continued till wound healing. In some cases, continuous infusion may be required.

   ii. **Acquired fibrinogen deficiency:** Fibrinogen can be substituted in the following situations:

   - Confirmed fibrinogen deficiency with risk of acute bleeding (massive transfusion, dilutional coagulopathy)
   - Liver damage with fibrinogen deficiency, haemorrhagic dysfibrinogenemia as prophylaxis
   - Fibrinogen deficiency due to leukaemia, obstetric complications with haemorrhage

Table 8 Differences between cryoprecipitate and fibrinogen concentrate for fibrinogen replacement [22]

Cryoprecipitate	Fibrinogen concentrate
No viral inactivation, potential risk of pathogen transmission	Viral inactivation, minimal risk of pathogen transmission
Variable fibrinogen levels, accurate dosing not possible	Standardized fibrinogen content, accurate and consistent dosing
Infusion volume lower than fresh-frozen plasma but higher than fibrinogen concentrate	Low infusion volume

iii. Dose: The required fibrinogen dose is estimated as follows[21]:

Fibrinogen dose (g) = desired increase (g/l) × plasma volume (l)

When the half-life is shortened, fibrinogen levels should be monitored more frequently.

iv. Absolute and relative contraindications: Overt thrombosis and myocardial infarction are contraindications except in cases of life-threatening haemorrhage.[21]

5.6. Prothrombin complex concentrate (Factor II, Factor VII, Factor IX, and Factor X)

The clotting factors II, VII, IX, and X (prothrombin complex) stimulate coagulation. These factors are dependent on vitamin K for their synthesis. Congenital factor II, VII, IX, and X deficiencies predispose the patient to bleed depending on whether it is a heterozygous or homozygous state.

a. Preparation of prothrombin complex concentrate (PCC): The factors constituting the PCC are isolated from large cryoprecipitate-poor plasma pools, using ion-exchange chromatography in combination with various precipitation and adsorption methods.[7, 21]

b. Active constituents: Prothrombin complex concentrate contains factors of the prothrombin complex, namely the human clotting factors II (prothrombin), VII (proconvertin), X (Stuart-Prower factor), IX (antihaemophilic globulin B), which are proenzymes in the coagulation cascade.[21]

c. Storage, shelf life, and package sizes: PCC currently commercially available are to be stored at a maximum temperature of 25°C or at 2–8°C. They should be used immediately.[21]

d. Clinical indications for the use of PCC: Prothrombin complex concentrates (PCCs) were initially developed as a source of factor IX for the treatment of patients with haemophilia B.[21,23] Later on, high purity factor IX could be separated out as a purified product from PCC. Hence, the clinical use of PCCs consequently shifted to the treatment of bleeding in haemophilia patients who had developed inhibitors. Now PCC has been superseded in this clinical setting by recombinant factor IX and bypassing agents (activated PCC and recombinant activated factor VII).

The main indications for the use of PCC are:

- Replacement therapy in cases of congenital and acquired deficiencies of vitamin K dependent coagulation factors and when the individual factor concentrates are not available (factor II and factor X).
- Urgent reversal of over-anticoagulation with warfarin: The primary complication of oral anticoagulant therapy with warfarin (coumarin) is bleeding. The goal of urgent reversal is to raise the levels of vitamin-K dependent clotting factors and stop bleeding. The general agreement is that any major bleeding demands rapid and complete warfarin reversal. Either PCC or Fresh Frozen Plasma can be used for this purpose. However, PCC has several advantages over FFP. PCC is more effective in shortening the time required for INR correction. Smaller volumes of PCC are required, so lesser risk of fluid overload. In addition, PCC undergoes viral inactivation. PCC does not need thawing. Blood group matching is not required.

  When used at appropriate doses (25–50 units/kg), the international normalized ratio (INR) should begin to decline within 10 minutes of PCC injection, and the duration of effect is equivalent to the duration of endogenous clotting factors.

  FFP should only be used in cases where PCC is unavailable or contraindicated (e.g., in known heparin-induced thrombocytopenia type II)

e. **Adverse effects of PCC administration:** The most important safety concern associated with the use of PCC is thrombogenic complications manifested as stroke, myocardial infarction, deep vein thrombosis, and pulmonary embolism.[23] The incidence of thromboembolic complications has reduced now due to the inclusion of coagulation inhibitors (antithrombin, protein C, protein S) in the product. Other adverse events that may be associated with PCC are immediate allergic reactions and heparin-induced thrombocytopenia (for the products containing heparin).

5.7. Factor VII concentrate

Clotting factor VII is a procoagulant that is synthesized in the liver. It is a vitamin-K dependent coagulation factor. [21]

a. Preparation of factor VII concentrate: Factor VII is prepared from cryoprecipitate-poor plasma pools using ion-exchange chromatography and adsorption to aluminium hydroxide. It is for intravenous use. The mean half-life of factor VII following substitution is 5 h.

b. Indications for the use of factor VII concentrate:

Congenital factor VII deficiency:

- Treatment of bleeding disorders that were caused by isolated congenital factor VII deficiency.
- Prophylaxis of bleeding disorders that could be caused by isolated congenital factor VII deficiency.

c. **Absolute and relative contraindications:**

- Factor VII concentrate must be applied with caution in patients with known intolerance to components in the product.
- Factor VII concentrate should be applied only following careful consideration during pregnancy and lactation.

5.8. Alpha-1-proteinase inhibitor

This product (also known as alpha1-antitrypsin inhibitor) can be isolated from Fraction IV by polyethylene glycol (PEG) precipitation and ion-exchange chromatography. The product is used for patients with an inherited deficiency of the inhibitor resulting in emphysema and liver disease.[7]

5.9. Topical products

Fibrin glue is a combination of thrombin and fibrinogen concentrate. Fibrin glue is prepared from Cohn Fraction I or from cryoprecipitate. It is formulated to contain fibronectin, vWF, plasminogen, and/ or factor XIII. They are used topically to induce haemostasis and healing.[7,21]

5.10. Recombinant factors

Recombinant coagulation factors produced through genetic engineering and mammalian cell culture techniques have led to the improvement in purity and safety of coagulation factors. It has also reduced dependence on human plasma as a source of therapeutic proteins.

The products obtained by recombinant deoxyribonucleic acid (rDNA) technology that are in clinical use include Factor VIII, Factor IX, and Factor VIIa (activated factor VII).[7,14,20] The main problem associated with these products is their high cost, and hence affordability, especially in developing countries.

6. Plasma fractionation in India

The plasma-derived medicinal products are not available in adequate quantities in India. Unfortunately, plasma fractionation has not received enough consideration in the country due to demanding challenges and priorities. The main products currently being manufactured in India are albumin and intravenous immunoglobulin (IVIG) using plasma collected in India. The other products required for many other clinical conditions are largely imported. There are also issues regarding the affordability of these products. [25]

Considering these facts, the Department of AIDS Control, Ministry of Health & Family Welfare, Government of India has issued 'National Policy for Access to Plasma Derived Medicinal Products from Human Plasma for Clinical/Therapeutic Use, in 2014 (as an addendum to National Blood Policy 2003). The policy reiterates the endeavour of the government to facilitate the supply of affordable products to the needy, regardless of their economic status.[2]

To achieve the overall goal of facilitating national access to Plasma Derived Medical Products (PDMPs) for therapeutic use, the policy has put forth the following objectives:

1. To reiterate that Government will facilitate the availability and utilization of a safe and adequate quantity of plasma-derived products for clinical/ therapeutic use.,
2. To make available adequate resources to develop and organize the plasma/ PDMPs mobilization throughout the country.
3. To take adequate Regulatory and Legislative steps for monitoring of activities related to plasma-derived products.
4. To encourage Research & Development in the field of blood components, plasma fractionation, and plasma-derived products.
5. To strengthen Quality Systems in Blood Transfusion Services for plasma collection, transportation, processing, production, and distribution of PDMPs.

The policy also details the strategies to be adopted by the government to achieve each of the above objectives.

Summary

Plasma-derived medicinal products constitute an important therapeutic modality. Their clinical utility comprises substitutive and/or augmentative therapy to treat haemostatic, immunological, and metabolic disorders. Blood plasma fractionation refers to the process of separating plasma to isolate its constituents. Plasma fractionation first began at Harvard University at the time of World War II when Edwin Cohn was successful in purifying albumin by using ethanol. Albumin proved to be lifesaving for war victims. It was realized that other plasma proteins of therapeutic importance could also be separated. Though Cohn's ethanol fractionation remains the backbone of the plasma industry, many newer techniques such as chromatography have been developed for plasma fractionation. The plasma procured for the purpose of fractionation is either obtained by apheresis (called source plasma) or from whole blood donation (recovered plasma). The criteria for whole blood and plasma donation are as per those laid down by the regulatory authority. The product then is made safer by viral inactivation/virus removal techniques, which are pre-validated.Therapeutic proteins fractionated from plasma are albumin, immunoglobulins, factor VIII, factor IX, fibrinogen, factor VII, prothrombin complex concentrate, alpha-1-antitrypsin inhibitor, which have been described above. Topical agents such as fibrin sealant can be obtained from cryoprecipitate. The associated adverse effects and contraindications for use have been described above, if applicable. Recombinant factors are now available for factor VIII, factor IX, and factor VIIa.

The plasma fractionation industry in India has not received enough consideration. As a result, there is a huge gap between demand and supply. The Government of India has developed a plasma policy with the overall objective of facilitating the supply of affordable products to the needy, regardless of their economic status. Though the situation of supply of plasma products has improved in the last decade, more concentrated efforts are required in the direction of strategies laid down by the policy to achieve self-sufficiency in the field of plasma-derived medicinal products.

Key points

- Plasma Derived Medicinal Products (PDMPs) are manufactured industrially from human plasma by a specialized technique known as fractionation.

- Blood plasma fractionation refers to the process of separating plasma to isolate its constituents for characterizing the nature and functions of these constituents and as an aid in the diagnosis and therapy in clinical medicine.
- PDMPs include human albumin, immunoglobulins, coagulation proteins, anticoagulants, and protease inhibitors.
- Procurement, storage, transportation, and fractionation of plasma are the key steps involved in the manufacturing of PDMPs.
- Each of the above steps should follow Good Manufacturing Practice and meet the qualification criteria laid down by the regulatory authority to ensure the safety and efficacy of the final product.
- Plasma for fractionation can be procured as recovered plasma (from whole blood donation) or as source plasma (by apheresis).
- Plasma stored below minus 30°C is the most appropriate starting material for fractionation.
- Various methods of plasma fractionation are classified as differential solubility (such as cold ethanol fractionation), differential interaction with solid media (chromatography), and differential interaction with physical parameters.
- The most important advantage of PDMPs over blood components is their safety as they undergo viral inactivation /or removal process.
- PDMPs differ in their concentrations, dosages, storage conditions, and routes of administration. The manufacturer's instructions should be followed for the same.
- Human albumin is used as a replacement fluid in therapeutic plasmapheresis and in the treatment of spontaneous bacterial peritonitis.
- Normal immunoglobulins are used in immunodeficiency and autoimmune disorders. Hyperimmune immunoglobulins are used to treat specific infectious diseases, while anti-D is used to prevent sensitization to D antigen in Rh-negative individuals.
- Factor VIII, factor IX, and fibrinogen concentrate are used in the treatment of haemophilia A, haemophilia B, and fibrinogen deficiency, respectively. Prothrombin complex concentrate (PCC) is used for urgent reversal of the warfarin effect.
- In India, there is a huge gap between the demand and supply of PDMPs since plasma fractionation has not received enough consideration.
- The Government of India has devised a plasma policy as an addendum to the National Blood Policy (2003) with the overall objective of facilitating the supply of affordable PDMPs to the needy regardless of their economic status.

References:

1. National Policy for Access to Plasma Derived Medicinal Products from Human Plasma for Clinical/ Therapeutic Use: Addendum to National Blood Policy 2003, published byDepartment of AIDS Control, Ministry of Health & Family Welfare, Government of India Plasma policy, NACO,2014.
2. Burnouf T. An overview of plasma fractionation. Ann Blood 2018;3:33.
3. WHO Model List of Essential Medicines. 21st list (June 2019). World Health Organization.http://www.who.int/medicines/publications/essentialmedicines/21st_EML_Final_web_l13.pdf. Accessed on 14/10/2019.
4. WHO Model List of Essential Medicines for Children. 7th list (June 2019). World Health Organization. Available: http://www.who.int/medicines/publications/essentialmedicines/4th_EMLc_FINAL_web_8Jul13.pdf. Accessed on 14/10/2019.
5. Rosen FS. Edwin J. Cohn and the Development of Protein Chemistry. N Engl J Med 2003; 349:511-2.
6. Farrugia A, Robert P. Plasma protein therapies: current and future perspectives. Best Pract Res ClinHaematol 2006;19:243-58.

7. Simon TL, Seidel K, Gröner A. Preparation of Plasma Derivatives. In: Simon TL, Snyder EL, Solheim BG, Stowell CP, Strauss RG, Petrides M, editors. Rossi's Principlesof Transfusion Medicine.4th ed. Bethesda: Blackwell Publishing;2009.p.273-97.
8. Denizli A, Hacettepe J. Plasma fractionation: conventional and chromatographic methods for albumin purification. J BioChem 2011; 39: 315–41.
9. Lynch SA, Mullen AM, O'Neill EE, Garc CA. Harnessing the Potential of Blood Proteins as Functional Ingredients: A Review of the State of the Art in Blood Processing. Comprehensive Reviews in Food Science and Food Safety 2017;16: 330-44.
10. Hosseini KM, Ghasemzadeh M.Implementation of Plasma Fractionation in Biological Medicines Production. Iran J Biotechnol 2016 ; 14: 213–20.
11. Burnouf T. Chromatography in plasma fractionation: benefits and future trends.J Chromatogr B Biomed Appl 1995;664:3-15.
12. Burnouf T. Quality of plasma and its fractionation. In: Pieter van der Meer, editor. ISBT Science Series 2008;3:148-51.
13. WHO. Recommendations for the production, quality control and regulation of plasma for fractionation. Available online: http://www.who.int/bloodproducts. Geneva: World Health Organization, 2005. Accessed on 14/10/2019.
14. Morfini M, Coppola A, Franchini M, Minno GD. Clinical use of factor VIII and factor IX concentrates .Blood Transfus 2013; 11(Suppl 4): s55–s63.
15. Liumbruno G, Bennardello F, Lattanzio A,Piccoli P,Rossettias G, as Italian Society of Transfusion Medicine and Immunohaematology (SIMTI) Working Party. Recommendations for the use of albumin and immunoglobulins. Blood Transfus 2009 l; 7: 216–34.
16. Human Albumin. Transfus Med Hemother 2009 ; 36: 399–407.
17. Human Immunoglobulins. Transfus Med Hemother2009;36:449–59.
18. Nydegger UE. Immunoglobulins.Preparation of Plasma Derivatives. In: Simon TL, Snyder EL, Solheim BG, Stowell CP, Strauss RG, Petrides M, editors. Rossi's Principles of Transfusion Medicine.4th ed. Bethesda,MD: Blackwell Publishing;2009.p.260-72.
19. Factor VIII Concentrates, Factor VIII/von Willebrand Factor Concentrates, Factor IX Concentrates, Activated Prothrombin Complex Concentrates. Transfus Med Hemother 2009; 36:409-18.
20. Franchini M, Fraltini F, Silvia C, Sissa C, Bonfanti C. Treatment of haemophilia B: focus on recombinant factor IX.Biologics. 2013; 7: 33–8.
21. Procoagulators.Transfus Med Hemother 2009; 36: 419–36.
22. Franchini M, Lippi G. Fibrinogen replacement therapy: a critical review of the literature. Blood Transfus 2012 ; 10: 23–7.
23. Franchini M, Lippi G. Prothrombin complex concentrates: an update. Blood Transfus 2010 ; 8: 149–54.
24. Franchini M.Plasma-derived versus recombinant Factor VIII concentrates for the treatment of haemophilia A: recombinant is better. Blood Transfus 2010; 8: 292–6.
25. Ajmani RS. Indian plasma fractionation industry: challenges and opportunities. Ann Blood 2018;3:30.

Section 20

Potential Use of Convalescent Plasma in Infectious Disorders

1. Introduction

Immunity is the body's ability to recognize non-self-antigens and to mount a response to those antigens to prevent them from causing illness or damaging the body.

An immune system may contain innate and adaptive components. The innate system is essentially made up of barriers that aim to keep viruses, bacteria, parasites, and other foreign particles out of the body, while the adaptive system is composed of more advanced lymphatic cells that are programmed to recognise self-substances and foreign substances (antigens such as infectious pathogens) and to react appropriately. The adaptive immune system, also referred to as the acquired immunity, is a subsystem of this immune system that is composed of specialized, systemic cells (B-cells and T-cells)and processes that eliminate foreign antigens and any toxic molecules they may produce.

There are two types of adaptive immunity: active and passive.

- **Active immunity** - Antibodies that develop in a person's own immune system after the body is exposed to an antigen through a disease or when the person gets an immunization (i.e., a flu shot). This type of immunity lasts for a long time.
- **Passive immunity (PI)** - Antibodies given to a person to prevent disease or to treat disease after the body is exposed to an antigen. This type of immunity is fast-acting but lasts only a few weeks or months. Passive immunity is given from mother to child through the placenta before birth and through breast milk after birth. It can also be given medically through blood products that contain antibodies, such as immunoglobulins (IG).

Artificially acquired passive immunity is a short-term immunization achieved by the transfer of antibodies, which can be administered in several forms; as human plasma, as pooled human immunoglobulin (IG) for intravenous immunoglobulin (IVIG) or as high-titre human IG from the plasma of immunized donors or from donors recovered from the disease, something referred to as convalescent plasma (CP) or Convalescent blood products (CBP).

2. History of convalescent plasma therapy

Human blood has been identified as a source of antibodies for a very long time now. The concept of passive immunotherapy emerged in the late 19th century, following the set up of experimental medicine.[1] It derived from the earliest works of Ehrlich in Germany and, though less directly, of Pasteur in France and Bordet in Belgium, who identified the key factor that "complements" the neutralizing action of antibodies. The use of PI, is the transfer of ready-made antibodies (humoral immunity) from the blood of one individual to another, for the management of infectious diseases, can be traced back to about 140 years, when specific antibodies were taken from the serum of animals exposed to a particular infection (e.g., rabbits, horses or other animals).[2] From the 1880s, CBP were used to treat many infections in humans and in animal models. In 1890, the first

rational approach exploited by the physiologists Emil Adolph von Behring and Kitasato Shibasaburō to treat diphtheria was blood serum.[3] Shibasaburo and von Behring immunized guinea pigs with the blood products from animals that had recovered from diphtheria and realized that the same process of heat-treating blood products of other animals could treat humans with diphtheria.[3]

Initially, it was produced from immunized animals, but soon whole blood or serum from recovered donors with a specific humoral immunity were identified as a possible source of specific antibodies of human origin.

Neutralizing antibodies (nAb's) are crucial in virus and bacterial clearance and have been considered essential in protecting against or preventing the spread of many infectious diseases. Passive immunity driven by CBP can provide these nAb's that restrain the infection. The efficacy of this therapy has been associated with the concentration of nAb's in plasma from recovered donors. In addition to nAb's, there are other protective antibodies, including immunoglobulin G (IgG) and immunoglobulin M (IgM). Non-nAb's that bind to the pathogen but do not affect its capacity to replicate, might contribute to prophylaxis and/or recovery improvement. Moreover, other antibody-mediated pathways such as complement activation, antibody-dependent cellular cytotoxicity, and/or phagocytosis may also promote the therapeutic effect of CBP.

In 1890 antibody therapy was used to treat tetanus when serum from immunized horses was injected into patients with severe tetanus in an attempt to neutralize the tetanus toxin and prevent the dissemination of the disease.

Antibody therapy is also used to treat viral infections. Studies conducted during the Spanish influenza pandemic of 1918 to 1920 suggested that the use of CBP might be helpful. Once plasma fractionation into plasma derived-therapeutic factors was made available around the 1940s-50s, immunoglobulins (Ig's) were purified and concentrated from highly immunized donors having fully recovered from past clinical or asymptomatic infection constituting the so-called hyperimmune immunoglobulin fractions.

In the 1940s, hepatitis A infections were managed by immunoglobulin treatment. Similarly, hepatitis B immune globulin (HBIG) effectively prevents hepatitis B infection. Antibody prophylaxis of both hepatitis A and B has largely been supplanted by the introduction of vaccines; however, it is still indicated following exposure and prior to travel to areas of endemic infection.

In 1953, human vaccinia immunoglobulin (VIG), made from the pooled blood of individuals who have been inoculated with the smallpox vaccine, was used to prevent the spread of smallpox during an outbreak in Chennai, India and continues to be used to treat complications arising from smallpox vaccination.

There are several examples of the use of CBP for the prophylaxis or treatment of bacterial infectious diseases such as scarlet fever in the 1920–40s and pertussis until the 1970s.

Several groups on both sides of the Atlantic attempted to infuse heated plasma collected from HIV-1 seropositive symptom-less individuals to AIDS patients. The French group led by Jean-Jacques Lefrère was indeed a pioneer in such compassionate protocol.[4] The outcome was the maintenance over weeks of anti-p24 Abs in transfused patients and a delay in the appearance of further AIDS symptoms.

Besides all the above, possible therapeutic efficacy of CBP has been claimed for the management of measles, Argentine haemorrhagic fever, influenza, chickenpox, infections by cytomegalovirus, parvovirus B19 and, more recently, Middle East respiratory syndrome coronavirus (MERS-CoV), H1N1 and H5N1 avian flu, and severe acute respiratory infections (SARI) viruses.

WHO has also stated that in the absence of a proven treatment available for Ebola virus disease (EVD), whole blood collected from patients in the convalescent phase of infection has been used as an empirical treatment with promising results in a group of EVD cases.[5]

In the last two decades, the human SARS-CoV outbreak has been active and, to a large extent, successfully managed by convalescent therapy initiatives, with reports being made available quite early after the episodes. All released publications claimed that this therapy was followed by a drop of viral load and an improvement of patient's symptoms.

More recently, in SARS-CoV-2 associated COVID-19, given the lack of evidence for treatment and vaccines, classical and historical interventions such as CBP have re-emerged as options for the control

of the disease. Given its rapid availability, CP has been considered an emergency intervention. With CP, in addition to neutralizing antibodies, other proteins such as anti-inflammatory cytokines, clotting factors, natural antibodies, defensins, pentraxins and other undefined proteins are obtained from donors. In this sense, transfusion of CP to infected patients may provide further benefits such as immunomodulation via amelioration of severe inflammatory response seen in some infections such as COVID-19.

3. Convalescent plasma therapy in SARS-CoV-2 infection (COVID-19 Convalescent Plasma [CCP]):

At the time of writing, the Indian Council of Medical Research (ICMR) and the U.S. Food and Drug Administration (FDA) had issued advisories on the emergency use of COVID-19 convalescent plasma (CCP).[6, 7]

3.1 Potential recipients:

The CCP therapy is advisable in the early stage of COVID-19 disease within 3-7 days of symptoms (not later than 10 days) in hospitalised patients or in patients with impaired humoral immunity who do not have IgG antibody against virus.[6, 7] Early in the course of disease generally means before respiratory failure requiring intubation and mechanical ventilation. Limited clinical evidence suggests the potential therapeutic window following symptom onset may be longer in patients with suppressed or deficient humoral immunity.

3.2 Potential donors:

The CCP donors must undergo a standard pre-donation assessment to ensure compliance with current regulations regarding plasma donation. In addition to laboratory tests, it is critical to recognize the emotional situation, explore susceptibilities, and guarantee that there is no exploitation of donors.

The following criteria should be met for potential donors[6]:

a. 18 years to 60 years of age
b. Males or nulliparous female donors of weight >50Kg
c. Diagnosis of COVID-19 by RT-PCR positive *OR* Rapid antigen test positive.
d. The recovered patient (CCP donor) should preferably have had symptoms (fever, cold, cough, etc.) since there is a greater probability of the presence of anti-SARS-CoV-2 IgG antibodies as compared to an asymptomatic patient.
e. Asymptomatic donors may be accepted if anti-SARS-CoV-2 IgG antibodies are present and verified by two different approved tests.
f. Evidence of recovery from COVID -19 as anti-SARS-CoV-2 IgG antibodies must be demonstrated.
g. Complete resolution of symptoms at least 14 days prior to donation, RT-PCR negative report is not mandated in this situation
h. In addition, donor eligibility criteria for whole blood donation will be followed in accordance with Drugs and Cosmetics (Second Amendment) Rules, 2020.[8]

Screening of eligible donor should be done as follows:

a. A donor should be screened, followed by a brief physical examination.
b. Donors who have had a transfusion of blood products in the last 12 months should be excluded.
c. Donors who have had COVID-19 diagnosis for more than 4 months should be excluded from donation.
d. The following pre-donation tests are required for convalescent plasmapheresis.
    i. Blood group (ABO grouping and Rh phenotyping) and antibody screening for clinically significant antibodies (Extended Rh, Kell, Duffy, Kidd, MNS) – Antibody screen positive donors will be deferred.
    ii. Complete blood count including Haemoglobin (Hb), Haematocrit (HCT), Platelet count, Total leucocyte count (TLC) and differential leucocyte count (DLC). Donors with Hb>12.5g/dl, platelet count >1,50,000 per microliter of blood and TLC within normal limits will be accepted.

iii. Screening for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis and malaria should be done at least by serology. Negative donors will be included.

iv. Donors with total serum protein > 6gm/dl should be accepted

e. Titration of anti-SARS-CoV-2 neutralizing antibodies may be done depending on the availability of facilities at the time of testing. Unavailability of antibody titres will not preclude convalescent plasma transfusion. Desired titres for IgG antibodies are 1:640 by Enzyme-linked immunosorbent assay (ELISA) method OR 13 arbitrary unit (AU) by chemiluminescence method OR for neutralizing antibodies titre of 1:80 (Plaque reduction neutralization test (PRNT) / micro neutralisation test (MNT).[6]

3.3. Collection of CCP:

Apheresis is the recommended procedure to obtain CCP. However, the centres that lack the facility for plasmapheresis can also use whole blood-derived plasma after the donor fulfils the National Blood transfusion Council (NBTC) Guidelines for donor eligibility.

CCP collection through plasmapheresis is based on continuous centrifugation using any of the apheresis equipment available at the facility. The efficiency of this technique is around 500 mL from a single apheresis donation. This amount of plasma could be stored in units of 200 or 250 ml. The procedure shall be done following the standard operating procedure (SOP) for plasmapheresis and in accordance with the Drugs and Cosmetics (Second Amendment) Rules, 2020.[8]

Throughout the procedure, the extracorporeal volume of blood should never exceed >15% of the total blood volume of the donor. Pooling of plasma is not recommended due to variability of SARS-CoV-2 IgG antibody level in different donors.

Repeat donations can be scheduled after at least 2 weeks from the first plasma donation, maximum of 24 times a year. If there was a loss of red cells at the time of the first donation owing to any procedural problems or otherwise, the donor will be deferred for a period of 3 or 4 months for male or female donors, respectively. All the donor selection guidelines described above will apply to repeat donation, as well.

3.4. Storage of CCP:

It may be stored frozen at –30°C or colder and has an expiration date one year from the date of collection. Collected plasma can be divided into smaller aliquots of 200 ml for easy storage. Once thawed, it can be stored at 4°C for up to 5 days prior to patient transfusion.

3.5. Dosage:

Clinical dosing may start with one high titer CCP unit ranging from 4 to 13 ml/kg (about 200 mL). Transfusion of additional units is based on the clinician's decision and the patient's clinical response. Patients with risk of circulatory overload may require a smaller volume or more prolonged transfusion times.

3.6. Transfusion of CCP:

It can be transfused through a peripheral or central venous catheter. For transfusion of CCP, SOP for transfusion of plasma should be followed with special care to monitor these patients during and post-24 hours of transfusion. All such transfusions must be done using blood transfusion sets. The clinician will send a request for a plasma component specifically mentioning the diagnosis, and that convalescent plasma is required. An ABO compatible plasma bag of approximately 200mL will be issued, maintaining all the blood bank records after thawing at 37^{0}C.

3.7. Expected adverse events (AEs):

3.7.1. Adverse events in CP donor:

They are divided into local reactions and systemic reactions. AEs are classified according to severity into mild, moderate, and severe and according to aetiology in a donor, into hypotensive reactions, citrate reactions, haematomas, vaso-vagal reaction, seizures, and allergic reactions.

3.7.2. Kit/ Equipment-related adverse events:

These are secondary to improper disposable sets. These are haemolysis, thrombus formation, air embolism, leakage, infection, improper mounting of the kits etc.

3.7.3. Adverse transfusion reaction in CP recipient:

A transfusion-related reaction is a response or effect in a patient temporally associated with the administration of blood or blood components. The majority of these are non-lethal and medically treatable. Adverse effects commonly associated with transfusion of plasma include transfusion-related acute lung injury (TRALI); transfusion-associated circulatory overload (TACO); allergic/anaphylactic reactions; transfusion-related transmission of infections (TTI); febrile non-haemolytic transfusion reactions (FNHTR); haemolytic transfusion reactions (HTR); and rarely RBC allo-immunisation. The transfusion reactions, if encountered, should be managed as per the institutional protocol.

There is one more theoretical risk of CCP transfusion, that is, the antibody-dependent enhancement of infection (ADE) phenomenon. It involves an enhancement of disease in the presence of certain antibodies and has also been described in other viral infections, such as dengue. For coronaviruses, several mechanisms of ADE have been proposed, including the theoretical concern that antibodies to one type of coronavirus could increase infection to another strain. Preparations with high titres of antibody against the same virus strain are thought to be less likely to cause ADE. Another theoretical risk is that antibody administration may attenuate the immune response and make patients more susceptible to re-infection.

3.8. Safety and Efficacy of CCP:

The potential benefits of CCP therapy could include improvement in symptoms, reduced need for supplemental oxygen and mechanical ventilation, and reduced mortality. Available evidence suggests that CCP with high antibody titre may be effective in reducing disease progression and reducing mortality in hospitalized patients with COVID-19 when administered early in the course of the disease, and those hospitalized with impaired humoral immunity.

A meta-analysis of the effectives of CP therapy for SARS showed that early treatment with plasma might be beneficial.[8] Besides, a total of 84 Ebola virus disease patients who were treated with CP therapy showed no significant improvement in survival. A case reports that an influenza A (H5N1) virus-infected male after receiving CP from a patient who had recovered from H5N1 infection, the patient's viral load was reduced during the first 8 hours and was undetectable within 32 hours.[11]

The efficacy of CCP therapy for COVID-19 is still under investigation, and there have been multiple ongoing randomised controlled trials to assess it. A systematic review and meta-analysis by the MAYO clinic highlight, that the mortality rate of transfused COVID-19 patients was lower than that of non-transfused patients and suggests that early transfusion of high-titer plasma represents the optimal use scenario to reduce the risk of mortality among patients with COVID-19.[11] These results favour the efficacy of convalescent plasma as a COVID-19 therapeutic agent. Another systematic review also found that CCP has some curative effect in the early time of disease after symptom onset and also offers a promising rescue option for severe COVID-19 cases.[12] Cochrane review with nearly 20 studies and 5443 patients has been uncertain whether convalescent plasma has any effect on either all-cause mortality at hospital discharge, prolonging time to death or improvement of clinical symptoms at seven days.[13]

ICMR conducted an open-label phase II multicentre randomised controlled trial on the use of CP therapy in the management of 464 moderate COVID-19 patients in India (PLACID Trial). It was concluded that CP therapy did not lead to a reduction in progression to severe COVID-19 or all-cause mortality in the group that received CP therapy as compared to the control group.[14]

The use of CCP therapy in elderly patients was found safe and efficacious in some studies. In the RESCUE trial by an Italian group of researchers, it was concluded that early administration of high titre CCP to elderly, symptomatic patients in a long-term care facility helped in improving patients' survival by virus elimination, restoration of patients' immunity, and blocking disease progression.[15] The adverse event rates in elderly patients have not been provided yet.

The safety and effectiveness of CCP in pregnant and lactating female patients has not been evaluated still, so the decision to treat these kinds of patients should be based on an individualized assessment of risk and benefit. A few case reports published on the usage of CCP in pregnancy, and they found it safe and effective.[16,17] Pregnancy is associated with haemodynamic changes, including increased cardiac output and blood volume, the rate of transfusion & CCP dose should be adjusted carefully to avoid circulatory overload. Similarly, for paediatric patients, the use of CCP therapy is still under evaluation. Only a few case reports are available, which found it safe.[18,19]

In conclusion, CP therapy is a potential treatment for many infections, especially the ones which cause sudden large-scale epidemics and for which we lack a specific anti-pathogen therapy. Yet, there are still some challenges to be dealt with.

4. Challenges of convalescent plasma therapy:

In order to take full advantage of this promising treatment, there are still several critical problems that need to be clarified. Apart from other factors, the lack of high-quality studies (i.e., randomised clinical trials) remains a concern. Other challenges of CP therapy are as follows:

a. Finding a suitable donor is the biggest challenge for CP collection. The correct time at which to collect CP is crucial as the donor should be free from the pathogen itself yet should have enough titres of neutralizing antibodies in his/her body.
b. There are various limitations of acquiring CP such as age, weight, state of health, informed consent, the amount of CP required, the ratio of recovered patients to those who need plasma, causing the shortage of CP.
c. CP therapy's most common adverse reaction is transfusion-related events, allergic or anaphylactic reactions, transfusion-related acute lung injury, circulatory overload, and haemolysis.
d. Meanwhile, the risk of transfusion-transmitted infections, such as HIV, HBV, HCV, and syphilis, remains the same as with any other transfusion.
e. Immunotherapy using monoclonal antibodies could be more effective.
f. Antiviral and antibacterial drugs, as and when developed, may be a ready and safer source of pathogen neutralizing agents.
g. Another challenge for blood transfusion services is to provide dedicated space, equipment consumables and training staff for the collection of CP.

Key points:

- Convalescent plasma (CP) or Convalescent blood products (CBP) provides artificial passive immunity as a short-term immunization by the transfer of antibodies, which are collected from recovered patients.
- Convalescent plasma therapy was used in many infectious diseases previously, like measles, Argentine haemorrhagic fever, influenza, chickenpox, infections with cytomegalovirus, parvovirus B19, Middle East respiratory syndrome coronavirus (MERS-CoV), H1N1 and H5N1 avian flu, and severe acute respiratory infections (SARI) viruses, etc.
- COVID-19 convalescent plasma (CCP) therapy is advised in the early stage of COVID-19 disease within 3-7 days of symptoms in hospitalised patients.
- The CCP donors must undergo a standard pre-donation assessment to ensure compliance with current regulations regarding plasma donation.
- CCP can be collected through apheresis or whole blood, but apheresis is the recommended procedure.
- CCP may be stored frozen at -30° or colder and has an expiration date of one year from the date of collection.
- Clinical dosing of CCP may start with one high titer plasma unit of about 200 mL volume.
- Other than common adverse events of blood component transfusion, there is a theoretical risk of CCP transfusion which is the antibody-dependent enhancement of infection (ADE).

- The potential benefits of CCP therapy include improved symptoms, reduced need for supplemental oxygen and mechanical ventilation, and reduced mortality.

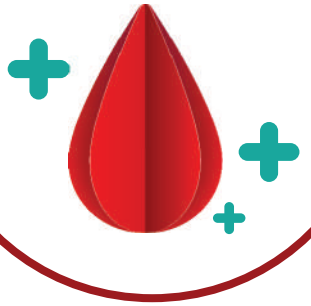
References

1. Casadevall A. Passive antibody administration (immediate immunity) as a specific defence against biological weapons. Emerging infectious diseases. 2002 Aug;8 (8):833.
2. Marano G, Vaglio S, Pupella S, Facco G, Catalano L, Liumbruno GM, Grazzini G. Convalescent plasma: new evidence for an old therapeutic tool?. Blood Transfusion. 2016 Mar;14(2):152.
3. Haas LF. Emil Adolph von Behring (1854–1917) and Shibasaburo Kitasato (1852–1931). Journal of Neurology, Neurosurgery & Psychiatry. 2001 Jul 1;71(1):62
4. Garraud O, Heshmati F, Pozzetto B, Lefrere F, Girot R, Saillol A, Laperche S. Plasma therapy against infectious pathogens, as of yesterday, today and tomorrow. Transfusion Clinique et Biologique. 2016 Feb 1;23(1):39-44.
5. World Health Organization, 2014. Use of convalescent whole blood or plasma collected from patients recovered from Ebola virus disease for transfusion, as an empirical treatment during outbreaks: interim guidance for national health authorities and blood transfusion services (No. WHO/HIS/SDS/2014.8). World Health Organization.
6. Evidence Based Advisory to address Inappropriate Use of Convalescent Plasma in COVID-19 Patients. (accessed 15/12/2020) https://www.icmr.gov.in/pdf/covid/techdoc/ICMR_ADVISORY_Convalescent_plasma_17112020_v1.pdf
7. "COVID-19-Related Guidance Documents for Industry, FDA Staff, and Other Stakeholder," available at https://www.fda.gov/emergency-preparedness-and-response/mcm-issues/covid-19-relatedguidance-documents-industry-fda-staff-and-other-stakeholders.
8. Government of India. Drugs and Cosmetics Act 1940 and Rules 1945 amended.2020. https://cdsco.gov.in/opencms/opencms/system/modules/CDSCO.WEB/elements/download_file_division.jsp?num_id=NTc2MQ== (accessed 10 Apr 2020).)
9. Van Griensven J, Edwards T, de Lamballerie X, Semple MG, Gallian P, Baize S, Horby PW, Raoul H, Magassouba NF, Antierens A, Lomas C. Evaluation of convalescent plasma for Ebola virus disease in Guinea. New England Journal of Medicine. 2016 Jan 7;374(1):33-42.
10. Zhou B, Zhong N, Guan Y. Treatment with convalescent plasma for influenza A (H5N1) infection. N Engl J Med. 2007; 357: 1450-1451.
11. Klassen S A, Senefeld J W, Johnson P W, et. al. The Effect of Convalescent Plasma Therapy on COVID-19 Patient Mortality: Systematic Review and Meta-analysis. medRxiv 2020.07.29.20162917; doi: https://doi.org/10.1101/2020.07.29.20162917
12. Mengyao Sun, Yinghui Xu, Hua He, Li Zhang, Xu Wang, Qing Qiu, Chao Sun, Ye Guo, Shi Qiu, Kewei Ma. A potentially effective treatment for COVID-19: A systematic review and meta-analysis of convalescent plasma therapy in treating severe infectious disease. International Journal of Infectious Diseases, 98 (2020); 334-346, https://doi.org/10.1016/j.ijid.2020.06.107.
13. Davey Jr RT, Fernández-Cruz E, Markowitz N, Pett S, Babiker AG, Wentworth D, Khurana S, Engen N, Gordin F, Jain MK, Kan V. Anti-influenza hyperimmune intravenous immunoglobulin for adults with influenza A or B infection (FLU-IVIG): a double-blind, randomised, placebo-controlled trial. The Lancet Respiratory Medicine. 2019 Nov 1;7(11):951-63.
14. Agarwal A, Mukherjee A, Kumar G, Chatterjee P, Bhatnagar T, Malhotra P et. al. Convalescent plasma in the management of moderate covid-19 in adults in India: open label phase II multicentre randomised controlled trial (PLACID Trial) BMJ 2020; 371 :m3939
15. Franchini M, Glingani C, Morandi M, et al. Safety and efficacy of convalescent plasma in elderly COVID-19 patients: the RESCUE trial. Mayo Clin Proc Innov Qual Outcomes. 2021 Feb 8. doi: 10.1016/j.mayocpiqo.2021.01.010. Epub ahead of print.

16. Jafari R, Jonaidi-Jafari N, Dehghanpoor F, Saburi A. Convalescent plasma therapy in a pregnant COVID-19 patient with a dramatic clinical and imaging response: A case report. World J Radiol. 2020;12(7):137-141. doi:10.4329/wjr.v12.i7.137
17. Grisolia G, Franchini M, Glingani C, et al. Convalescent plasma for coronavirus disease 2019 in pregnancy: a case report and review. Am J Obstet Gynecol MFM. 2020;2(3):100174. doi:10.1016/j.ajogmf.2020.100174
18. Schwartz S P, Thompson P, Smith Melissa et al. Convalescent Plasma Therapy in Four Critically Ill Pediatric Patients with Coronavirus Disease 2019: A Case Series, Critical Care Explorations. 2020;2(10) e0237 doi: 10.1097/CCE.0000000000000237.
19. Balashov D, Trakhtman P, Livshits A, et al. SARS-CoV-2 convalescent plasma therapy in pediatric patient after haematopoietic stem cell transplantation [published online ahead of print, 2020 Nov 1]. Transfus Apher Sci. 2020;102983. doi:10.1016/j.transci.2020.102983

APPENDIX 1

Methods



1. Blood Collection

1.1 Haemoglobin estimation methods

1.1.1 Copper sulphate specific gravity method

a. **Principle:** Based on the principle that the specific gravity of blood, with Hb of 12.5 g/dL and normal protein levels, is 1.053. When a drop of blood is allowed to fall on the copper sulphate solution with a specific gravity of 1.053, it becomes encased in a sac of copper proteinate, preventing dispersion of fluid for 15 sec. It is an indirect qualitative measure of the Hb value.

b. **Preparation of copper sulphate solution:** There are two methods of preparation as follows:

i. **Method-1:**

- **Prepare stock solution (specific gravity 1.100)**: Dissolve 159.63g of pure air-dried crystals of copper sulphate ($CuSO_4$. 5H2O) in distilled water and makeup to exactly 1000ml at 25°C. The specific gravity of the solution must be 1.100.
- **Preparation of working solution (1.052-1.055):**

S. No.	Sp.gr	Stock solution	Distilled water	Hb equivalent
1	1.052	51ml	100 ml	12.0 g
2	1.053	52ml	100 ml	12.5 g
3	1.054	53ml	100 ml	13.0 g
4	1.055	54ml	100 ml	13.4 g

ii. **Method-2:** Dissolve 8.33 g of pure air-dried crystals of copper sulphate ($CuSO_4$.5H2O) in 100 ml of distilled water. The specific gravity of the solution must be 1.053.

c. **Quality control:** Quality control of the working solution of $CuSO_4$ can be done by visual inspection, check for specific gravity and functional validation before it is released for use.

- **Visual inspection:** Hold the bottle of $CuSO_4$ against the light and check for cloudiness or the presence of precipitates. If it is not completely clear, it must not be used.
- **Check for specific gravity:** The specific gravity of the $CuSO_4$ can be measured directly with a calibrated hydrometer; a specific gravity 1.053 + 0.0003 g/ml renders the $CuSO_4$ solution acceptable for use in donor screening.
- **Validation of $CuSO_4$ solution:**
  - Every batch of $CuSO_4$ should be checked with blood samples of known haemoglobin in a range around 12.5 g/dl, (e.g., 12 g/dl; 12.5 g/dl; 13.0 g/dl and 13.5 g/dl)
  - Gently place a drop of each blood sample into $CuSO_4$ solution of sp. gr. 1.053.
  - Drops of all blood samples with haemoglobin of 12.5g/dl or above will sink, and those with Hb level below 12.5g/dl will float.

d. **Storage:** Both the working and stock solutions should be stored at room temperature (22-24^{0}C) in tightly capped containers to prevent evaporation.

f. Method to use $CuSO_4$ solution for donor Hb testing:

A $CuSO_4$ solution of specific gravity 1.053 is used for determining a haemoglobin level of 12.5g/dl.

- Dispense 30 ml of $CuSO_4$ solution into an appropriately labelled clean and dry beaker. Change the solution daily or after 25 tests, and be sure that the solution is properly mixed before use.
- The site of skin puncture, i.e., the fingertip, is cleaned with an antiseptic solution and allowed to dry.
- Sterile disposable lancet is used for prick, and there should be free flow of blood.
- The drop of blood is collected either in a capillary tube or pipette and allowed to fall gently from a height of l cm above the surface of the copper sulphate solution.
- *Interpretation:* Observe the blood drop till 15 sec in $CuSO_4$ solution.

S. No.	The action of the blood drop within 15 sec	Haemoglobin of blood	Donor selection or Deferral
1.	Drop remains at the surface	Hb <12.5g/dl	Defer the donor
2.	Drop rises from the bottom of the solution	Hb <12.5g/dl	Defer the donor
3.	Drop sinks	Hb ≥ 12.5 g/dl + 0.19	Select the donor

NOTE: If the plasma protein level of donors is at the lower limit of normal, it is possible a donor may be rejected, though he/she may have the required Hb.

g. Remedial source of errors in Hb estimation by $CuSO_4$ method:

- Taking the first drop of blood from a finger prick.
- Squeezing the finger because the blood is not flowing freely.
- Dirty pipette (pipette not flushed out properly every time).
- Chip at the delivering end of the pipette.

1.1.2 Portable haemoglobinometer:

These are portable, simple to use, precise, accurate method for measuring haemoglobin.

a. Principle: Based on the principle that when a drop of blood is placed on a cuvette containing sodium deoxycholate, the RBCs are haemolysed and release Hb. Sodium nitrite converts this free Hb to met-Hb, which together with sodium azide produces azide-met-Hb. The absorbance of which is measured at two wavelengths (570 and 880 nm).

The newer modified devices consist of reagent free cuvettes and measure the absorbance of whole blood at 506 nm isosbestic point (where the absorbance of two main Hb derivatives, HbO_2 and deoxy-Hb, is the same) and at 880 nm to compensate for turbidity.

b. Method of testing:

- Cleanse the donor's ring or middle finger with an alcohol wipe or isopropyl alcohol. Allow the air to dry or wipe off excess alcohol.
- Using your thumb, lightly press the finger from the top of the knuckle towards the tip. This stimulates the blood flow towards the sampling point.
- For best blood flow and least pain, sample at the side of the fingertip, not the centre. Applying light pressure towards the fingertip, puncture the fingertip using a sterile, disposable lancet.
- Wipe away the first drops of blood with sterile gauze.
- Re-apply pressure towards the fingertip until another drop of blood appears.
- When the drop is large enough, fill the cuvette in one continuous process.
- Wipe off excess blood from the outer surface of the cuvette with lint-free tissue, being careful not to touch the open end of the cuvette.
- Lay the cuvette in the cuvette holder. Wait 2 minutes before reading.
- Note down the value on the screen.

- The range of the instrument is 0-25.6 g/dL.
- *Reference Values:*

Males	13.0-17.0 g/dL
Females	12.0-15.0 g/dL
Infants	11.0-14.0 g/dL

NOTE: The principle of equipment and method of testing might vary according to the manufacturer. So read the manufacturer's instructions before using the equipment.

c. Limitations of portable haemoglobinometers:

- Moisture can collect in the cuvettes, so the cuvette container needs to be kept closed when not in use.
- A large drop of blood on the finger is required to fill the cuvette.
- The cuvette must fill in one continuous draw.
- The cuvette must not be taken away from the drop of blood until it is full.

d. Advantages:

- Quick, safe and hygienic handling.
- Accuracy is ± 1.5 %.
- Microcuvette automatically draws a precise volume of blood.
- Automatically zeroes itself after measurements.
- Automatically checks the intensity of light and operation of photo cells.
- No blood dispensing, pippetting or mixing of blood with the reagent.
- Could be used in both clinical as well as mobile settings.

e. Disadvantage:

- It is costlier in the blood centre setting when compared with other methods for donor screening.

1.1.3 Automated haematology analyzer:

a. Principle: It is based on either Cyanmethaemoglobin (HiCN) or the oxyhaemoglobin method.

- The cyanmethemoglobin method is based on the principle of conversion of Hb to HiCN by the addition of potassium cyanide and ferricyanide, whose absorbance is measured at 540 nm in a photoelectric calorimeter against a standard solution.
- The oxyhaemoglobin method includes dilution with aqueous solution of tetrasodium salt of ethylene di-amine tetra acetic acid (EDTA) and mixing with air to convert Hb to oxyhaemoglobin and then measuring of absorbance of oxyhaemoglobin (HbO_2) at 540 nm wavelength.

Note: This method is not recommended for whole blood donor Hb estimation due to the requirement of venous sample; it is commonly used for complete blood counts for apheresis donors.

b. Method of testing:

- Turn ON the power switch. Self-check, auto rinse, and background check will be automatically performed, and the "Ready" (ready for analysis) will appear.
- Input from the panel keyboard.
- Press [SAMPLE No.] key in the Ready status.
- Entering patient ID, sample ID, patient name, etc
- Press the [ENTER] key; this will fix the sample No. and the status becomes ready for analysis.
- Take a 2-3 ml venous sample in an EDTA vial.
- Mix the sample properly.
- Remove the plug while taking care not to allow blood to scatter.
- Set the tube to the sample probe, and in that condition, press the start switch.

- When the LCD screen displays "analyzing", you remove the tube.
- After that, the unit executes automatic analysis and displays the result on the LCD screen.

c. Quality control: Quality control of automated analysers can be done by functional validation before using the equipment for routine use every six months and if equipment shows any kind of error.

- Three known value samples are required for quality control: Abnormal Low, Normal, Abnormal High.
- Controls are stored at 2-8^{0}C and brought to room temperature on a roller mixer before use.
- From the RUN screen, press [SPECIMEN TYPE].
- Use the arrow key on the keyboard to move the cursor to the appropriate QC file (i.e., low, normal or high) and press the [QC SPECIMEN] key.
- Control values must be within three standard deviations; otherwise, the measurement has to be repeated if the control is still out of range:
  - o Check the operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
  - o Check reagents for expiration dates and lot numbers. Ensure that all machine lines are in an appropriate receptacle where applicable,
- All control data is managed using software that provides graphical reports (Levey Jennings graphs and monthly cumulative histograms).
- A typical analyser has a CV of 1.2% with venous blood.

NOTE: The principle of equipment and method of testing might vary according to the manufacturer. So read the manufacturer's instructions before using the equipment.

d. Limitations: Automated analysers may give false results of various parameters in the following situations:

- A low sample volume of <1 mL may dilute patient samples with EDTA in the collection tube, giving falsely low results. If a low sample volume is expected, use a paediatric EDTA tube.
- Haemoglobin (Hb): Lipemia, abnormal proteins in blood plasma, severe leucocytosis (above 100,000/μl).
- Haematocrit (Hct): Cold agglutinins, leucocytosis (above 100,000/μl), abnormal red cell fragility.
- Red blood cells (RBC): Cold agglutinins, severe microcytosis, fragmented RBCs, large numbers of giant platelets, in vitro haemolysis.
- White blood cells (WBC): Platelet aggregation, giant platelets, nucleated RBCs, cryoglobulins, lyse-resistant RBCs in patients with haemoglobinopathies, severe liver disease or neonates.
- Platelets (PLT): Pseudo thrombocytopenia, platelet aggregation, increased microcytosis, megalocytic platelets.
- Dilute the sample if white blood cell counts ≥100,000 /mm^3 and platelet counts ≥1,000,000 /mm^3 are outside the linearity specifications of the instrument.

Advantages:

- These analyzers provide high throughput of samples with high precision.

Disadvantages:

- Requirement of venous sample
- Requirement of control and calibrators
- Requirement for trained manpower
- Requirement for regular maintenance
- Requirement of specific lab conditions
- Requirement of high cost of consumables

1.2. Preparation of donor's arm for blood collection

Two common procedures can be followed to disinfect the phlebotomy site, as described below. If the site is visibly dirty, then ask the donor to wash with soap and water and then dry using a single use towel before proceeding with the disinfection procedure.

1. **One step procedure** – This is the recommended procedure and takes about 1 minute to complete.
   a. This method is to be used when a product combining 2% chlorhexidine gluconate with 70% isopropyl alcohol is available.
   b. Select the vein and the site to be disinfected.
   c. Observe hand antisepsis and wear appropriate gloves.
   d. Cover the selected area with a cotton swab dipped in antiseptic solution (step a).
   e. Wipe the area to be cleaned in such a way that the antiseptic soaked cotton swab should move from the central area (area over the selected vein) to peripheral areas in a circular manner covering an area of 5 cm.
   f. Ensure that the skin is in contact with the solution for at least 30 seconds.
   g. Allow to dry completely (30 seconds).
2. **Two-step procedure** - If chlorhexidine gluconate in 70% isopropyl alcohol disinfectant is not available, use this procedure. This procedure takes about two minutes.
   a. Select the vein and the site to be disinfected
   b. Observe hand antisepsis and wear appropriate gloves

   Step 1- Use 70% isopropyl alcohol
   c. Cover the selected area with a cotton swab dipped in 70% isopropyl alcohol.
   d. Wipe the area using the alcohol-soaked swab from the central area (area over the selected vein) to peripheral areas in a circular manner.
   e. Ensure that the skin is in contact with alcohol for at least 30 seconds.
   f. Allow to dry completely (30 seconds).

   Step 2- Use a tincture of iodine or chlorhexidine (2%).
   g. Now cover the selected area with a cotton swab dipped in a tincture of iodine or chlorhexidine (2%).
   h. Wipe the area from the central area (area over the selected vein) to peripheral areas in a circular manner.
   i. Ensure that the skin is in contact with the solution for at least 30 seconds.
   j. Allow to dry completely (30 seconds).

   DO NOT touch the site after disinfection in both the procedures.

2. Component preparation

2.1. Preparation of packed red blood cells from whole blood

Principle

Packed red blood cells (PRBC) are prepared by removing the supernatant plasma from a centrifuged whole blood unit. Red cells having higher specific gravity than plasma settle in the lower portion of the bag during centrifugation. Supernatant plasma is then transferred into a satellite bag. The volume of plasma removed determines the haematocrit of the component.

Procedure

- Collect the appropriate volume (350/450 ml) of donor blood in a double/triple blood bag.
- Store at an appropriate temperature depending on what component is intended to be made till processed (if platelets are to be prepared, store the collected blood unit at room temperature. Otherwise, the temperature of the collected blood bag should not exceed 10°C).

- After tapping the port of the primary bag, place the bag in the bucket of a refrigerated centrifuge with the label of the primary bag facing the flat surface of the bucket and the secondary bag/bags folded on the other side.
- Balance the two buckets on the weighing balance accurately.
- Centrifuge the whole blood unit in a refrigerated centrifuge, selecting appropriate parameters. Each individual laboratory must establish its own parameters. However, the general rule followed is as:
  a. For the harvest of PRBC and platelet-rich plasma (PRP), centrifuge whole blood using light spin (2000 × g for 3 minutes plus deceleration time)
  b. For the harvest of PRBC and fresh frozen plasma (FFP),centrifuge whole blood using a heavy spin (5000 × g for 5 or 7 minutes plus deceleration time at a temperature of 4°C)

Relative centrifugation force (RCF) can be expressed in units of gravity (g force) by using the following formula:

$$\mathbf{RCF = 28.38 \times R \times (RPM/1000)^2}$$

Where, RCF = Relative centrifugal force (×g), R = Radius in inches, RPM = Revolutions per minute

- Once the refrigerated centrifuge stops, lift the buckets carefully without disturbing the interface to avoid mixing of PRP/FFP with PRBC.
- Place the primary bag containing centrifuged blood on a plasma expressor. Release the spring allowing the plate of the expressor to come in contact with the bag.
- If two or more satellite bags are attached, apply the haemostat (clamp) to allow plasma to flow into only one of the satellite bags.
- Remove the appropriate amount of plasma to obtain the desired haematocrit. A haematocrit of 70% or lower should be maintained in CPDA-1 RBC units to ensure the presence of adequate glucose for red cell metabolism for up to 35 days of storage.
- Seal the tubing between the primary bag and the satellite bag.
- Check that the satellite bag has the same registration number as that on the primary bag.
- Cut the tubing between the two seals.
- Store the PRBC unit at the appropriate temperature.

2.2 Preparation of buffy coat reduced packed red blood cells suspended in additive solution

Principle

Buffy coat reduced packed red cells suspended in additive solution are usually prepared from quadruple top and top or top and bottom blood bags. The quadruple blood bag system has an additional bag containing the red cell additive solution and is usually used in automated systems to separate red cell concentrate (RCC), from which the white blood cells have been removed, and an additive solution has been added.

Procedure

- Collect the appropriate volume of donor blood in a primary bag of additive system, consisting of a primary bag containing anticoagulant solution CPD or CP2D attached with at least two satellite bags, one of which is empty, and another contains 100 ml of additive solution, e.g., Adsol or SAGM.
- Whole blood should be stored at 20°C to 24°C before centrifugation if platelets are to be prepared.
- After tapping the port of the primary bag, place the bag in the bucket of a refrigerated centrifuge with the label of the primary bag facing the flat surface of the bucket and the secondary bag/bags folded on the other side.
- Balance the two buckets on the weighing balance accurately.
- Centrifuge whole blood at “heavy spin” protocol.
- Carefully take out the buckets containing blood bags without disturbing the centrifuged unit containing three layers – upper platelet-poor plasma (PPP) layer, middle buffy coat layer of white cells and platelets and the lower layer of packed red cells.

- Components can be separated manually using plasma expressors or using automated cell extractors.
- Top and top blood bags can be expressed either manually or by automated expressors. The top and bottom are expressed using automated expressors.

Manual expression of top and top blood bags

- Carefully place the centrifuged unit in the plasma expresser and release the spring handle so that the plate presses firmly against the blood bag.
- Place a clamp on the tubing of the satellite bag intended for the transfer of the buffy coat layer.
- Break the seal and allow the free flow of platelet-poor plasma in the satellite bag labelled as FFP, leaving nearly 50-80 ml of plasma with the buffy coat layer. Seal the FFP bag using the dielectric tube sealer and store at -30°C or colder.
- Remove the clamp, and the buffy coat layer along with the remaining plasma is drawn into the second satellite bag, along with some red cells. Again, the clamp is placed on the tubing of the bag containing the buffy coat layer.
- Hang a satellite bag containing the additive solution in an inverted position and break the seal to allow the additive solution to pass into the mother bag. This additive solution is mixed with the red cells by gently rotating movements.
- Seal and detach the mother blood bag containing additive solution buffy coat reduced packed red blood cells from the satellite bags. Weigh the blood bag and store it in the blood bank refrigerator at 2°C – 6°C.
- Buffy coat can be discarded or used to produce platelet concentrates. Details of this process are described under “Preparation of platelet concentrates”.

Automated component extraction

- The centrifuged unit is put in the automatic component extractor, and press a button to activate the pressure plate.
- Open the tube connection between the primary bag and the plasma transfer pack to allow the flow of plasma from the top to one of the satellite bags. Then open the tube connection between the primary bag and the bag with SAG-M to allow the flow of blood. Red cells are pushed out of the bottom, leaving the buffy coat in the primary collection bag.
- When the process is complete, both top and bottom tubing automatically clamp, and the flow of red cells and plasma stop.
- The bags containing red cells and plasma are sealed with a di-electric sealer and separated.
- The unit of Packed Red Blood cells in additive solution is kept at 2-6°C and plasma at -30°C or below.

2.3 Preparation of pre-storage leucocyte reduced packed red blood cells from whole blood

Principle

The general principle and materials of preparation of RBCs apply, except that the red cells are filtered using a special leucocyte reduction filter.

Procedure

- Centrifuge the anticoagulated whole blood with the in-line filter attached and follow the steps in method 2.2 for the preparation of RBCs.
- After centrifugation, the plasma is expressed. The AS is added, and the red cells in the AS are filtered by gravity through the in-line filter.

2.4 Preparation of fresh frozen plasma from whole blood

Principle

Plasma is separated from cellular blood elements and is frozen to preserve the activity of labile coagulation factors. Plasma must be placed in the deep freezer within 6 hours of the blood collection.

Procedure

- Centrifuge blood soon after collection, using a "heavy" spin (see Method for preparation of PRBCs).
- Use a refrigerated centrifuge at 4^0C unless preparing platelets (see Method 6-13 for preparation of platelets from whole blood).
- Place the primary bag containing centrifuged blood on a plasma expressor and express the plasma into the satellite bag.
- Seal the transfer tubing with a dielectric sealer in such a manner so as not to obliterate the segment numbers of the tubing.
- Label the transfer bag with the unit number before it is separated from the primary bag.
- Affix a Fresh Frozen Plasma (FFP) component label and record the volume of plasma on the label.
- Store the plasma at –30°C or colder within 6 hours of phlebotomy.

2.5 Preparation of cryo-precipitated anti haemophilic factor from fresh frozen plasma

Principle

Coagulation Factor VIII (antihaemophilic factor or AHF) can be concentrated from freshly collected plasma by cryoprecipitation. Cryoprecipitation is accomplished by slow thawing of Fresh Frozen Plasma (FFP) at 1°C to 6°C.

Procedure

- Allow FFP to thaw at 1°C to 6°C by placing the bag in a 1°C to 6°C water bath or in a refrigerator. If thawed in a water bath, use a plastic overwrap to keep the bag ports dry.
- When the plasma has a slushy consistency, centrifuge the plasma at 1°C to 6°C using a "heavy" spin. (See Method for preparation of PRBCs)
- Place the thawing plasma in a plasma expressor when approximately one-tenth of the contents are still frozen.
- With the bag in an upright position, allowing the supernatant plasma to flow slowly into the transfer bag, using the ice crystals at the top as a filter.
- The cryoprecipitate paste will adhere to the sides of the bag or to the ice. Seal the bag when 90% of cryoprecipitate reduced plasma has been removed.
- Refreeze the cryoprecipitate immediately.
- The cryoprecipitate should be refrozen within 1 hour of thawing, store it preferably at –30°C or colder, for up to 12 months from collection.

2.6 Preparation of platelets from the whole blood

Principle

Platelets can be prepared by the platelet-rich plasma (PRP) method or by the buffy-coat method. In the PRP method, PRP is first separated from the whole blood by "light-spin" centrifugation, the platelets are then concentrated by "heavy spin" centrifugation, and the supernatant plasma is subsequently removed. In the buffy coat method, whole blood is centrifuged at "heavy spin" with subsequent collection of the buffy coat. The buffy coat is then centrifuged at "light-spin" to concentrate platelets and to remove red cells and white cells. Both the methods are described below.

Procedure

Preparation of PRP platelets

Do not chill the blood at any time before or during platelet separation. Set the temperature control of the refrigerated centrifuge at 20°C - 24°C and allow the temperature to rise to approximately 20°C - 24°C. Centrifuge the blood using a "light" spin (see Method for preparation of PRBCs).

- Express the PRP into the transfer bag intended for platelet storage. Seal the tubing twice between the primary bag and Y connector of the two satellite bags and cut between the two seals. Place the red cells at 1°C to 6°C.

- Centrifuge the PRP at 20°C - 22°C using a "heavy" spin (see Method for preparation of PRBCs).
- Express the platelet-poor plasma into the second transfer bag and seal the tubing.
- The platelet concentrate bags should be left stationary, with the label side down, at room temperature for approximately 1 hour.
- Place the platelet concentrate bags subsequently in a platelet agitator-cum-incubator.
- Platelets should be inspected before issuing to ensure that no platelet aggregates are visible.

Preparation of buffy-coat platelets

- Whole blood should be stored at 20°C to 24°C before centrifugation.
- Centrifuge whole blood at "heavy spin."
- Remove supernatant plasma and red cells manually or by using an automated instrument. Approximately 50 – 80 mL of buffy coat remains in the bag (see Method for preparation of buffy coat reduced additive solution packed red blood cells).
- Hang the two satellite bag pairs (one containing a buffy coat layer and another empty) buffy coats for approximately an hour for resuspension of platelets.
- Centrifuge two satellite bag pairs (one containing buffy coat layer and another empty) at "light spin".
- Carefully take out the buckets containing satellite bags without disturbing the centrifuged unit containing an upper layer of platelet-rich plasma (PRP) and a lower layer of buffy coat mixed with red cells.
- Transfer the supernatant PRP manually or by an automated instrument into a platelet storage bag and discard the satellite bag containing the buffy coat.

2.7 Preparation of Frozen/Cryopreserved red cells using high concentration glycerol -Merryman method

Principle

Cryoprotective agents make possible the long term (10 or more years) preservation of red cells in the frozen state. High-concentration glycerol is particularly suitable for this purpose.

A practical method for red blood cells (RBCs) collected in a 450-mL bag is described below.

Procedure

Preparing RBCs for glycerolization

- Prepare RBCs from Whole Blood units by removal of supernatant anticoagulant preservative or additive solution. Weigh the RBC unit to be frozen and obtain the net weight of the red cells. The combined weight of the cells and the collection bag should be between 260 g and 400 g.
- Underweight units can be adjusted to approximately 300 g either by the addition of 0.9% NaCl or by the removal of less plasma than usual. Record the weight; if applicable, document the amount of NaCl added.
- Record the Whole Blood number, the ABO group and Rh type, the anticoagulant, the date of collection, the date frozen, the expiration time, and the identity of the person performing the procedure. If applicable, document the lot number of the transfer bag.
- Warm the red cells and the glycerol to at least 25°C by placing them in a dry warming chamber for 10 to 15 minutes or by allowing them to remain at room temperature for 1 to 2 hours. The temperature must not exceed 42°C.
- Apply a "Frozen Red Blood Cells" label to the freezing bag in which the unit will be frozen. The label must include the name of the facility freezing the unit, Whole Blood number, ABO group and Rh type, and the expiration date. The label must also include tracking for the date collected, the date frozen, and the cryoprotective agent used.

Glycerolization

- Document the lot numbers of the glycerol, the freezing bags, and, if used, the 0.9% NaCl.
- Place the container of red cells on a shaker and add approximately 100 mL of glycerol as the red cells are gently agitated.

- Turn off the shaker and allow the cells to equilibrate, without agitation, for 5 to 30 minutes.
- Allow the partially glycerolized cells to flow by gravity into the freezing bag.
- Add the remaining 300 mL of glycerol slowly in a stepwise fashion, with gentle mixing. Add smaller volumes of glycerol for smaller volumes of red cells. The final glycerol concentration is 40% w/v. Remove any air from the bag.
- Allow some glycerolized cells to flow back into the tubing so that segments can be prepared. Preferably, two segments should be prepared so that the unit may be crossmatched and/or phenotype before a decision to thaw.
- Maintain the glycerolized cells at temperatures between 25°C and 32°C until freezing. The recommended interval between removing the RBC unit from refrigeration and placing the glycerolized cells in the freezer should not exceed 4 hours.

Freezing and storage

- Place the glycerolized unit in a cardboard or metal canister and place it flat in a freezer at –65°C or colder.
- Label the top edge of the canister with freezer tape marked with the whole blood number, the ABO group and Rh type, and the expiration date.
- Do not bump or handle the frozen cells roughly.
- The freezing rate should be less than 10°C /minute.
- Store the frozen RBCs at –65°C or colder for up to 10 years. For the blood of rare phenotypes, a facility's medical director may wish to extend the storage period. The unusual nature of such units and the reason for retaining them past the routine 10-year storage period must be documented.

Thawing and deglycerolizing

- Put an overwrap on the protective canister containing the frozen cells and place it in either a 37°C water bath or 37°C dry warmer.
- Agitate it gently to speed thawing. The thawing process takes at least 10 minutes. The temperature of the thawed cells should be 37°C. After the cells have thawed, use a commercial instrument for batch or continuous-flow washing to deglycerolize the cells. Follow the manufacturer's instructions.
- Record the lot numbers and manufacturers of all the solutions and software used. Apply a "Deglycerolized Red Blood Cells" label to the transfer pack; be sure that the label includes identification of the collecting facility, the facility preparing the deglycerolized cells, the ABO group and Rh type of the cells, the whole blood number, and the expiration date and time.
- Dilute the unit with a quantity of hypertonic (12%) NaCl solution appropriate for the size of the unit. Allow it to equilibrate for approximately 5 minutes.
- Wash the cells with 1.6% NaCl until deglycerolization is complete. Approximately 2 litres of wash solution are required.
- Suspend the deglycerolized cells in isotonic (0.9%) NaCl with 0.2% dextrose.
- Fill the integrally attached tubing with an aliquot of cells sealed in such a manner that it will be available for subsequent compatibility testing.
- Deglycerolized RBCs must be stored at 1°C to 6°C for no longer than 24 hours. (A closed system has been licensed that allows storage of deglycerolized RBCs at 1°C to 6°C for 2 weeks. The closed system deglycerolization requires that the glycerolization step also be performed by a closed system in accordance with the manufacturer's instructions).

3. Immunohaematology

3.1 Preparation of red cell suspension (2-5%)

Human red blood cells carry more than 300 blood group antigens expressed either in the homo- or hetero-zygous state. However, not every individual's red cell carries all the antigens. Therefore, in order

to get an equal representation of all clinically significant red cell antigens on the reagent red cells, a pool of red cells from at least 3 or more than 3 ABO matched individuals is required so as not to miss any important antibody.

The concentration of erythrocytes in saline suspension is also important as the antigen-antibody reaction takes place more readily at an optimum concentration of both antigen and antibody. A 2% to 5% red cell suspension is generally used in the conventional test tube method for performing various immunohaematological investigations such as ABO and Rh typing, antiglobulin test, antigen typing and compatibility testing.

Materials required

- 5 ml anticoagulated blood each from at least 3 ABO matched blood donors
- Antisera (anti-A, anti-B)
- Normal Saline
- Test tubes
- Tabletop centrifuge
- Pasteur pipette

Procedure

3.1.1 Preparation of packed pooled reagent red cells

1. Confirm the ABO group of red cells to be used by performing forward grouping only (see method 3.3)
2. Identify three large test tubes or falcon tubes as “A”, “B”, and “O” using a marker pen.
3. Add 5 ml anticoagulated blood from at least 3 “A” group donors in a tube marked A. Similarly, add “B” and “O” group blood from donors in tubes marked as B and O, respectively.
4. Fill these three test tubes 2/3rd with normal saline.
5. Centrifuge all three test tubes at 3000 rpm for 3 min.
6. Aspirate & discard the clear supernatant carefully, not disturbing the red cells at the bottom of the test tube.
7. Repeat steps 4-6 at least 3 times or until the supernatant is clear.
8. Aspirate and discard clear supernatant from the last wash to obtain washed packed red cells to be used for preparing a suspension of different concentrations. (please see 3.1.3)
9. Test the prepared cells using known antisera (anti-A, anti-B).

3.1.2 Preparation of packed red cells (un-pooled) of patient/donor

1. Identify the test tube with the patient or donor ID using a marker pen.
2. Add 1 drop of either anticoagulated or clotted blood of the patient or donor, depending upon the investigation to be performed.
3. Follow steps 4-8 of method 3.1.1.

3.1.3 Preparation of suspensions of different concentrations of washed pooled or un-pooled cells.

1. The volume of normal saline and packed red cells (prepared in 3.1.1 and 3.1.2) can be adjusted to obtain red cell suspension of the desired concentration as under.

Preparation of red cell suspensions using saline

% suspension	PRBC	Saline
1%	0.1 ml (or 1 volume)	9.9 ml (or 99 volume)
2%	0.2 ml (or 2 volume)	9.8 ml (or 98 volume)
5%	0.5 ml (or 5 volume)	9.5 ml (or 95 volume)

2. A 2 to 5% suspension of washed packed red cells will produce a cherry red colour, and 1% suspension will impart a pink colour.

Note: Red cell suspensions to be used for different method of testing is as under:

• *Test tube method*	*2 to 5 %*
• *Column Agglutination card*	*0.8 to 1%*
• *Microplate*	*0.8 to 1%*

3.2 Preparation of sensitized / check cells

IgG sensitized red cells/check cells are added to all the negative antiglobulin test results to ensure that the results are not false negative due to neutralization/poor quality of AHG reagent. The free AHG present in a negative test will cause agglutination of check cells, thus validating the result.

Materials required

- 75 x 12 mm tubes
- Pasteur pipettes
- Group O Rh-positive red cells
- Polyclonal Anti-D (IgG) reagent
- A serum sample from a known patient with anti-D if a commercial Anti-D (IgG) reagent is unavailable
- Normal Saline (0.9%)
- Antiglobulin (AHG) reagent
- Tabletop centrifuge
- Serologic Incubator.

Procedure

1. The first step involves selecting a dilution of an available anti-D (IgG) reagent, which will show a 2+ agglutination with the selected O cell.
   a. Prepare and label 12 test tubes as neat, 2, 4, 8……1024.
   b. Add 100 μl to all the test tubes except the tube labelled as neat.
   c. Add 100 μl anti-D (IgG) in test tube labelled 'neat' and '2'.
   d. Mix the contents of the test tube labelled '2' gently and transfer 100 μl of diluted reagent to tube marked 4 without touching the pipette tip with the contents of the tube. Mix the contents of the tube labelled '4' with a new tip gently and transfer 100μl of mixed contents to the tube labelled '8'. Again, mix the contents of tube '8' with a new tip. Repeat the transfer of mixed, diluted anti-D (IgG) till you have reached dilution '1024'.
   e. Once dilutions have been prepared, add 50 μl of 5% O Rh D positive red cells to all the tubes from neat to 512.
   f. Perform IAT on each of these tubes following steps described in the procedure of IAT (M5b).
   g. Note the dilution where a reactivity of 2+ is detected.
2. Prepare a diluted anti-D (IgG) reagent of the dilution noted in step 1g.
3. Add one volume of selected Group O Rh D positive red cells with one volume of diluted reagent prepared in step 2. Mix them gently and incubate them for 30-45 min at 37˚C.
4. Wash these incubated cells 3 times with normal saline.
5. To check the validity of cells, add 1 drop of AHG to 1 drop of 5% cell suspension of sensitized cells obtained in step 4. Mix and centrifuge at 1000 rpm x 1 min. Cells should give +2 agglutination. If there is no agglutination, the whole procedure is repeated by taking a less diluted anti-D (For example- the initial dilution was 1:32, a repeat test can be performed with a dilution of 1:16).

Note – In case commercial anti-D (IgG) is unavailable, the same procedure could be done with sample from a patient with anti-D. It is advisable to choose a patient sample with good titre (preferably >32).

3.3 Procedure for ABO and Rh D typing including Weak D (D^u)

Agglutination of red cells with known antisera (antibody) indicates the presence of the corresponding antigen on the red cells. Serum can also be tested with known red cells (antigen) to determine the presence or absence of specific antibodies. ABO blood groups are classified as A, B, AB, or O depending on the presence or absence of the A or B antigens on the red cells and the presence or absence of the corresponding anti-A or anti-B antibodies. However, individuals are classified as Rh (D) positive or negative depending on the presence or absence of Rh (D) antigen only.

Material required

- Blood sample in EDTA anticoagulant
- Commercial blood grouping antisera (Anti-A, -B, -D)
- AHG reagent
- Check cells
- Reagent (pooled) red cells (O-cell, A-cell, B-cell) prepared in-house.
- Test tubes 10 X 75 mm
- Normal saline (0.9 % NaCl)
- Test tube holder rack
- Pasteur pipette
- Tabletop centrifuge
- 37°C dry incubator
- High-intensity lamp / view box

Procedure

3.3.1 Procedure for forward grouping (cell grouping)

1. Confirm the identification of the blood sample of the patient/donor.
2. Prepare 5% suspension of the EDTA red cell in normal saline (see method 3.1) and label it.
3. Label three test tubes with patient/donor name or number and tube contents (-A, -B and –D).
4. To the test tube labelled A, add 1 drop of Anti-A antiserum. To tube B, add 1 drop of Anti-B, and to the tube labelled D, add 1 drop of anti-D antiserum.
5. Add 1 drop of the 5 % suspension of red cells to each of the test tubes.
6. Mix well and centrifuge the test tubes for 1 min at 1000 rpm.
7. Re-suspend the cells with gentle agitation and examine macroscopically for agglutination.

3.3.2 Procedure for reverse grouping (serum grouping)

1. Confirm the identification of the blood sample.
2. Separate the plasma from the EDTA blood sample by centrifuging it at 3000rpm for 3 min.
3. Label three test tubes with patient/donor name or number and tube contents (Ac, Bc, Oc).
4. To each test, tube adds 2 drops of the patient's plasma to be tested.
5. To the tube labelled Ac, add 1 drop of 5 % suspension of reagent A red cells.
6. To the tube labelled Bc add 1 drop of 5 % suspension of reagent B red cells.
7. To the tube labelled Oc, add 1 drop of 5 % suspension of reagent O red cells.
8. Mix well and centrifuge all the test tubes for 1 min at 1000 rpm.
9. Re-suspend the red cell button by gentle agitation and examine macroscopically for agglutination.

3.3.3 Procedure for determination of weak D status

This procedure should be performed on all donor samples giving a negative reaction for Rh (D) antigen on forward grouping.

1. Label one test tube with the donor number and name.
2. Add 1 drop of 5% suspension of patient's, red cells to the test tube.
3. Add 2 drops of preferably polyclonal anti-D (IgG), in the absence of which a blend of monoclonal IgG & IgM can also be used to the test tube.
4. Incubate at 37˚C for at least 30 min.
5. Wash the red cells 3 times using normal saline & decant the last wash completely.
6. Add 1 drop of Polyspecific AHG to the washed red cell button.
7. Centrifuge at 1000 rpm for 1 min
8. Re-suspend the red cell button by gentle agitation and examine macroscopically for agglutination.
9. Add 1 drop of check cells to the tube, giving no agglutination after adding AHG.
10. Record the results.

Interpretation

***ABO grouping**:* Interpret the test using the following Table.

Forward grouping				Reverse grouping				Final group
Anti-A	Anti-B	Anti-D	Group	Ac	Bc	Oc	Group	
+	0	+	**A+**	0	+	0	**A**	A
0	+	+	**B+**	+	0	0	**B**	B
+	+	0	**AB neg**	0	0	0	**AB**	AB
0	0	+	**O+**	+	+	0	**O**	O
0	0	+	**O+**	+	+	+	**? Oh**	? Oh

If cell and serum interpretation do not match, repeat the test to rule out identification errors & reagent/sample dispensing errors. If the discrepancy persists, follow method 3.4.

Rh (D) typing: Interpret the Rh typing as per table below.

Initial Rh (D) test	Weak D test	Check cells	Interpretation
Positive	Not applicable	Not applicable	Positive
Negative	Positive	Not applicable	Positive
Negative	Negative	Positive	Negative
Negative	Negative	Negative	Repeat test

Note:

- All the test tubes must be properly labeled.
- It is important to follow the manufacturer's instruction for the specific antisera in use.
- Do not perform the test at the temperature higher than room temperature (22-24° C) as testing at 37°C weakens reactions.
- Observe agglutination against a well-lighted background.
- Remember that contaminated blood specimens, reagents or supplies may interfere with the test results
- In case of neonates (< 4 months) only forward grouping is to be performed as serum will have passively acquired maternal antibodies.

- Weak D testing after Rh (D) negative results on initial testing is not required for patients requiring blood transfusion.
- In cases where blood is urgently required or already released, typing results that do not match historical reports should be immediately reported to the lab supervisor.
- Routine ABO grouping must include both cell and serum testing as each test serves as a check on the other.
- Serum should always be added before adding cells and examine each tube after serum has been added to ensure that none has been missed
- Controls should always be run with respect to ABO grouping. Most laboratories have quality control of antisera once a day in order to eliminate the need to run individual controls every time the reagents are used.

3.3.4 Grading & scoring of agglutination reaction

Grading of agglutination reactions (w+ to 4+) should be uniform & standardized to obtain reproducibility of test results. A numerical value is assigned to each grade of reaction to obtain a "score" for an agglutination reaction. Both the grade and the score of reaction are important for the proper characterization of a reaction. The score is generally reflective of the avidity of antigen/antibody reaction.

Reaction grade	Description	Titration score
4 +	The cell button remains in one clump macroscopically visible	12
3 +	The cell button dislodges into numerous large clumps, macroscopically visible	10
2 +	The cell button dislodges into many small clumps, macroscopically visible	8
1 +	The cell button dislodges into finely granular but definite, small clumps, macroscopically visible	5
weak +	The cell button dislodges into fine granules, only microscopically visible	3
Negative result	All cells are free and evenly distributed	0

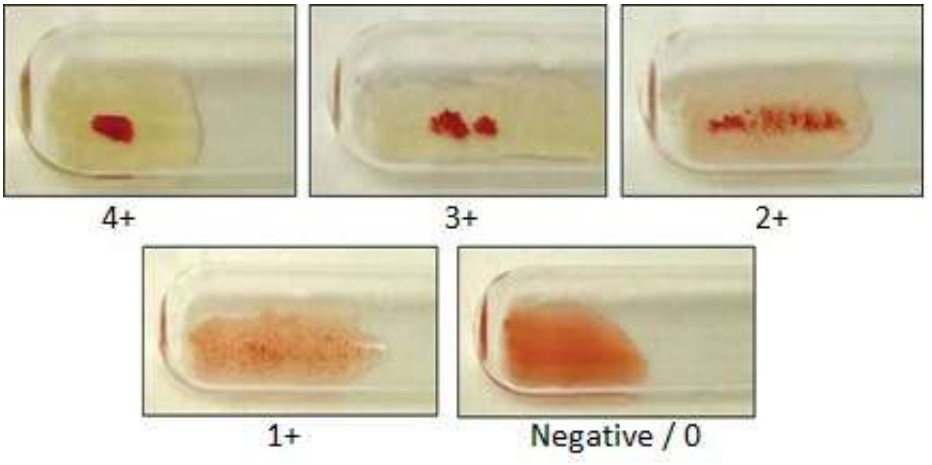


Figure showing grading Ag-Ab reaction in a conventional test tube

- Serum overlying the centrifuged cell button must be inspected for haemolysis, which is a positive sign of an antigen and antibody reaction, provided the pre-test serum was not haemolyzed, and no haemolytic agent was added to the test.

- Loose, stringy, or retractile agglutinates should be noted as they provide valuable clues in the investigation of reaction.
- Two separate populations of red cells observed microscopically, one as free cells and another as a small aggregate, may provide a valuable clue in the investigation of reactions.

3.4 Resolution of ABO blood group discrepancy

Results of the ABO blood group can be considered to be discrepant if,

1. There is discordance between cell and serum group (i.e., Landsteiner's Law is violated)
2. There are more than or less than two positive and two negative test results from among the cell and serum grouping results. More reactions suggest extra reactivity, and fewer reactions suggest missing reaction.

A valid positive reaction is considered to be a reaction ≥2+; when weaker reactions are observed, additional testing is necessary.

For a detailed description of ABO, discrepancies see section 6.

- Before commencing to resolve the discrepancy, technical factors that may contribute to discrepant ABO typing results should be ruled out. These include improper storage of reagents, use of the incorrect technique; use of the wrong reagent; omission of antisera; use of antisera contaminated with bacteria, foreign matter, or the contents of other reagent vials; and incorrect centrifugation of tests.

  The following may be followed depending on the type of discrepant result:

  Apparent weak or missing antigen

  1. Wash the RBCs and repeat the tests with extended incubation.
  2. Test with anti-AB and lectins (A_1 and H-lectin). Adsorb/elute with anti-A and/or anti-B.
  3. Determine transfusion history (especially O RBC transfusions).
  4. Determine diagnosis (especially leukaemia and pregnancy).
  5. Determine if the patient is a marrow transplant recipient.
  6. Determine if there is evidence for twinning at birth.
  7. Perform ABH secretor studies.

  Apparent absent or weak agglutinin

  1. Incubate the tests.
  2. Test the serum at room temperature and 4°C with two examples, each of A_1, B, and O RBCs, and the autologous RBCs.
  3. Determine the age and diagnosis of the patient (e.g., newborn, impaired immunity).
  4. Determine the transfusion history of the patient (e.g., whether non-ABO-type-specific plasma infusions were given).

  Apparent extra antigens

  1. Wash the RBCs and repeat the tests. If the tests are nonreactive, consider an antibody in the patient or donor serum to an ingredient of the ABO typing reagent.
  2. Test the patient or donor serum at room temperature with two examples of group O RBCs and the autologous RBCs. Then, treat the patient or donor RBCs with 2-ME or DTT and repeat the tests.
  3. Test the washed RBCs with a different manufacturer's reagents.
  4. Determine the diagnosis of the patient (e.g., septicaemia, gastrointestinal lesion).
  5. If acquired B is suspected from Steps 3 and 4 above, repeat the tests with acidified anti-B.
  6. Perform ABH secretor studies.

Apparent extra agglutinin(s)

1. Test the serum at room temperature and 4°C with 2 examples each of A_1, A_2, B, O, and O I– (cord and/or adult) RBCs as well as the autologous RBCs. Evaluate reactions for the presence of cold reactive

auto- or alloantibodies (e.g., anti-I, anti-M). If a specific alloagglutinin is present, repeat the tests with A_1 and B RBCs lacking the relevant antigen(s).

2. Wash the reagent RBCs and repeat the tests. If the discrepancy is resolved with washed RBCs, consider antibody to an ingredient in the reagent RBC preservative.
3. Test the serum with fresh A_1 and B RBCs. If the discrepancy is resolved, consider antibodies to stored RBCs.

Some of the common procedures used to resolve ABO discrepancies are described below.

3.4.1. *Cold temperature enhancement of ABO reactions by prolonged incubation at 4˚C*

Principle

ABO antibodies are predominantly IgM type which reacts strongly as the temperature is lowered, and these reactions get enhanced when prolonged incubation at low temperatures is provided can enhance antibody binding and detection of weak ABO antigens and antibodies. Both the cell and serum grouping should be incubated for prolonged periods irrespective of the type of discrepancy.

Materials required

- Blood sample in EDTA anticoagulant
- Commercial blood grouping antisera (Anti-A, -B, -D)
- Reagent (pooled) red cells (O-cell, A-cell, B-cell) prepared in-house
- Test tubes 10 X 75 mm
- Normal saline (0.9 % NaCl)
- Test tube holder rack
- Pasteur pipette
- Tabletop centrifuge
- High-intensity lamp/view box

Procedure

1. Perform steps 1-5 of cell grouping and steps 1-7 of serum grouping described in method 3.3.
2. Mix well and incubate all these test tubes at room temperature for 30 minutes.
3. Centrifuge the tubes at 1000rpm for 1 min or follow the manufacturers instructions.
4. Gently resuspend cell buttons and examine for agglutination.
5. If no agglutination is observed, incubate tubes for 15 to 30 minutes at 4˚C.
6. Repeat steps 3 and 4.

Interpretation:

Interpret the blood group after steps 7 and 9 as described in method 3.3.

If O cells show agglutination in the reverse group, then the result cannot be interpreted, and it will indicate the presence of a cold auto- or allo-antibody.

3.4.2 Adsorption elution technique for determination of A or B subgroup

Principle

Red cells having weak A or B antigen may not show visible agglutination with anti-A or anti-B but may adsorb the specific antibody. Removing the adsorbed antibody by elution and testing with appropriate cells makes it possible to identify the presence of antigenically active material capable of reacting with the antibody of known specificity.

Materials required

- Blood sample in EDTA anticoagulant
- Human anti-A and/or anti-B. Because some monoclonal ABO typing reagents are sensitive to changes in pH and osmolarity, the reagents may not be suitable for use in adsorption or elution tests.
- Unpooled group O, A_1 and B red cell samples (three examples) as appropriate
- Test tubes 10 X 75 mm
- Normal saline (0.9 % NaCl)
- Test tube holder rack
- Pasteur pipette
- Tabletop centrifuge
- High-intensity lamp / view box
- Water bath (56˚C)

Procedure

1. Wash 1 ml of cells to be tested at least 3-4 times with saline. Remove the supernatant completely after the last wash and discard.
2. To the washed red cells, add 1 ml of reagent anti-A if a weak variant of A is suspected or 1 ml of anti-B if a weak variant of B is suspected.
3. Mix the red cells with antiserum gently and incubate the mixture at room temperature (22˚C-24˚C) for one hour.
4. Centrifuge the mixture to pack the red cells (3000 rpm for 3 minutes). Remove the supernatant antiserum.
5. Wash the packed red cells obtained in step 4 at least five times with large volumes of saline (10 ml or more). Save the last 1-1.5ml of the fifth wash to test for free antibody (control).
6. Add an equal volume of saline to the washed packed red cells. Mix well.
7. Elute the adsorbed antibody by placing the tube in a 56˚C water bath for ten minutes. Mix the red cell-saline mixture 3-4 times during this period. (*for details, see heat elution method 3.6.1*)
8. Centrifuge the tube at 3000 rpm for 3 minutes to pack the red cells.
9. Transfer the cherry coloured supernatant eluate to another glass test tube. Discard the cells.

Testing the eluate and last wash

10. If anti-A was used, test the eluate against three different samples of A_1 cells and three different samples of group O cells at room temperature and 4˚C.
11. If anti B was used, test the eluate against three different samples of group B and three samples of group O cells at room temperature & 4˚C.
12. Test the last saline wash (step 5, above) in parallel to serve as a control.
13. Make three sets of test tubes for testing of eluate with A-Cells, B-Cells and O-Cells and label them as follows:

    EA1, EA2, EA3

    EB1, EB2, EB3

    EO1, EO2, EO3
14. Make three sets of test tubes for testing of last wash with A-Cells, B-Cells and O-Cells and label them as follows.

    LWA1, LWA2, LWA3

    LWB1, LWB2, LWB3

    LWO1, LWO2, LWO3
15. Add two drops of 'Eluate' to the tubes labelled in step 13.

16. Add two drops of 'Last Wash' to the tubes labelled in step 14.
17. Add one drop of A1c, A2c, A3c; B1c, B2c, B3c and O1c, O2c, O3c to all the corresponding test tubes of 'Eluate' and 'Last wash.'
18. Centrifuge all the tubes at 1000 rpm for 1 minute.
19. Gently resuspend cell buttons and examine for agglutination.

Interpretation

1. A positive reaction, i.e., agglutination of the eluate with A1c, A2c, A3c cell but no agglutination with O1c, O2c, O3c cells, indicates the presence of A receptors on the cells being tested.
2. A positive reaction, i.e., agglutination of the eluate with B1c, B2c, B3c cell but no agglutination with O1c, O2c, O3c cells, indicates the presence of B receptors on the cells being tested.
3. If agglutination of O cells also occurs, it indicates a non-specific reaction.
4. If the last saline wash is reactive with A or B cells, the results of the test are invalid because it indicates the presence of free antibody in the RBC suspension prior to elution.

3.4.3 Determination of ABH secretor status (Saliva testing)

Principle

Individuals who have inherited at least one Se (secretor) gene secrete A, B or H antigen in the saliva depending on the person's blood group. This is capable of neutralizing the corresponding antibody, i.e., A, B and H. When appropriate red cells are added to the saliva treated antiserum, reduced or absence agglutination is seen (agglutination inhibition).

Determining the secretor status is useful in resolving ABO grouping discrepancies and is based on the presence of ABO blood group substances in the saliva.

Materials required

- Saliva to be tested
- Test tubes 10 X 75 mm
- Normal saline (0.9 % NaCl)
- Anti - A, Anti-B, Anti-H
- 5% suspension of reagent cells, i.e. A cells, B cells and O cells
- Pasteur pipette
- Tabletop centrifuge
- High-intensity lamp / view box
- Boiling water bath

Procedure

1. Collect 3-4 ml of saliva to be tested in a small beaker or 15 ml glass test tube. The mouth should be rinsed before collecting saliva. A few grains of salt may be sprinkled on the tongue to induce salivation.
2. Place the tube containing saliva in a boiling water bath for 10 minutes to inactivate any enzymes present.
3. Centrifuge the boiled saliva at 3000 rpm for 10 minutes. Transfer the clear supernatant to another tube.
4. Prepare one set of test tubes for 'TEST' for Anti-A and label them T1, T2, T4, and T8 with marking Anti-A on each of tubes and one set for CONTROL for Anti-A and label them C1, C2, C4, and C8 with marking Anti-A on each of the tubes.
5. Prepare similar sets of tubes as in step 4 for Anti-B and Anti-H but in these sets, mark the test tubes Anti-B and Anti-H, respectively.
6. Make a 20-fold dilution of Anti-A and Anti-B and use them as stock. Do not dilute Anti H.

7. Pour 100 μl of normal saline into all the tubes labelled as CONTROL except C1 for all three sets of tubes for Anti-A, Anti-B and Anti-H.
8. Add 100 μl of Anti-A (1:20 diluted) to tube C1 of Anti-A of CONTROL and mix. Again add 100 μl Anti-A (1:20 diluted) to tube no C2 of Anti-A of CONTROL and mix with saline already present. Subsequently, prepare serial doubling dilutions by transferring 100 μl to the diluted solution from C2 to C4 and mix with saline which is already present. Transfer 100 μl of this diluted solution in C4 to C8, mix with saline which is already present, and discard 100 μl.
9. Transfer 50 μl of Anti-A from C1 to T1, C2 to T2, C4 toT4 and C8 to T8.
10. Repeat step 8 and 9 for Anti-B using stock Anti B (1:20 diluted).
11. Repeat step 8 and 9 for Anti-H using stock Anti H(Undiluted).
12. Add 50 μl of prepared saliva in each of the tubes labelled as TEST and mix.
13. Add 50 ul of normal saline in each of the tubes labelled CONTROL and mix.
14. Incubate both TEST & CONTROL tubes at room temperature for 30 minutes.
15. Add one drop of 5% A cells to all the tubes of Anti A; B cells to all the tubes of Anti B & O cells to all the tubes of Anti H.
16. Incubate all the tubes at room temperature for 30 minutes.
17. Post-incubation centrifuge at 1000 rpm for 1 minute.
18. Grade and record results obtained with each tube in each set of dilutions.

Interpretation

1. Agglutination of RBC in the CONTROL and no agglutination or weak agglutination in the corresponding TEST indicates that the individual is a secretor for that antigen i.e. A, B and H antigen in the saliva which has neutralized the corresponding antibody.
2. Agglutination of the same grade in the CONTROL and TEST of each pair of dilutions indicates that soluble antigen is not present in the saliva and the individual is a non-secretor.

3.4.4. Treatment of auto-agglutinable red cells with sulfhydryl reagents dithiothreitol (DTT) or 2-mercaptoethanol (2-ME)

Principle

Red cells heavily coated with IgM autoantibodies can spontaneously agglutinate during centrifugation, leading to false-positive reactions in red cell typing. DTT or 2-ME can break the disulfide bonds of IgM molecules, decreasing their ability to directly agglutinate red cells and thus resolve the discrepancy in red cell typing.

Materials required

- Blood sample in EDTA anticoagulant
- 0.01 M DTT - 0.154 g of DTT dissolved in 100 mL of phosphate-buffered saline (PBS) at pH 7.3; store at 4ºC
- 0.1 M stock 2-ME - 0.7 mL of a 14 M stock solution of 2-ME diluted in 100 mL of PBS at pH 7.3; 2-ME should be stored in a dark glass container at 4°C
- PBS at pH 7.3
- Antigen-positive control red cells for antigen typing to be treated in parallel
- Anti-A and anti-B
- 6% albumin (control reagent)
- Normal saline (0.9 % NaCl)
- Test tubes 10 X 75 mm
- Pasteur pipette

- Tabletop centrifuge
- High-intensity lamp / view box

Procedure

1. Wash red cells three times with saline and dilute to a 50% concentration in PBS.
2. Add an equal volume of 0.01 M DTT in PBS or 0.1 M 2-ME in PBS to the red cell suspension.
3. Incubate at 37˚C for 15 minutes (DTT) or 10 minutes (2-ME).
4. Wash red cells three times in saline and dilute to 2% to 5% suspension in saline.
5. Test the treated cells with 6% albumin (immediate-spin test) to make sure the cells do not spontaneously agglutinate. If the test result is negative, the red cells are now ready for use in red cell typing tests.

Remarks

- Treated red cells should not agglutinate in 6% albumin.
- Antigen-positive control red cells should react equally strongly with typing reagent before and after treatment.
- This procedure is normally used only for ABO forward typing, Rh determination, and DAT.

3.5 Antiglobulin test

The direct Coombs test is used to demonstrate in vivo coating of red cells with Immunoglobulin G (IgG) &/or complement (C3d). It is used in investigating AIHA, HDFN, alloimmune reactions to recently transfused red cells & drug-induced haemolysis. IAT is used to detect in vitro sensitization of red blood cells.

3.5.1 Direct antiglobulin test

Materials required

- Patient Sample (EDTA Blood)
- AHG reagent-Polyspecific (anti-IgG & C3d)
- Sensitized red cells / Check cells
- Normal saline (0.9%)
- 75 x 12mm glass tubes
- Pasture pipette
- Tabletop centrifuge

Procedure

1. Identify the patient sample by checking and matching patient details from the request form and sample
2. Centrifuge the sample by hard spin (3000 rpm for 2-3 min) to separate red cells from plasma.
3. Take a clean test tube and label with a patient identifier (name or hospital number).
4. Transfer patient's packed red cells to the labelled test tube and washed them at least three times.
5. To wash the red cells, fill two-third of the test tube with 0.9% normal saline, thoroughly mix the red cells with it followed by centrifugation by giving a hard spin and then decant the supernatant saline. Repeat this at least three times.
6. Prepare 3 - 5% red cell suspension of washed red cells by adding 1 drop of red cells to 19 drops of 0.9% normal saline.
7. Take another clean test tube and label it with patient detail and test name.
8. Add 1 volume (1 drop) of 3-5% red cell suspension and 1 volume (1drop) of AHG to the identified test tube and mix.
9. Centrifuge the tubes at 1000 rpm x 1min.

10. Remove the tubes & read over a lightbox, grade & record the reaction.
11. Add one drop of 5% red cell suspension of sensitized red cells to negative results.
12. Repeat steps 9 and 10.

Interpretation of DAT

1. If agglutination is observed at step no 10, DAT is recorded as positive. Report the result as positive with grading in brackets along with the technique of testing. e.g.
   - DAT – Positive (3+) by conventional test tube technique
2. If no agglutination is observed at step no 10 and agglutination is observed with check cells, then report the result as negative along with the technique of testing. e.g.
   - DAT – Negative by conventional test tube technique
3. If no agglutination is observed at both step 10 and with check cells, then the result is invalid and needs to be repeated.

Note:
Please note that some manufacturers recommend different volume of AHG to be added in step no 8 and some recommend room temperature incubation of the test tube after addition of the AHG and before centrifugation in step no 8 and 9. It is thus advisable to read the manufacturer's instructions before use of AHG and change the procedure accordingly.

3.5.2 *Indirect antiglobulin test (IAT):*

Materials required

- Test serum/plasma
- 75 x 12mm glass tubes and Test tube racks
- Pasteur pipettes
- Normal Saline (0.9 %)
- LISS (optional)
- AHG reagent-Polyspecific (anti-IgG & C3d)
- Pooled 'O' cells (minimum 2 'O' cells, preferably 3 'O' cells)
- Sensitized red cells / Check cells
- Tabletop centrifuge
- Serologic Incubator

Procedure

1. Identify the patient sample by checking and matching patient's details from the request form and sample.
2. Centrifuge the sample by hard spin (3000rpm for 2-3 min) to separate red cells from plasma.
3. Take a clean test tube and label with a patient identifier (name or hospital number) and test name (IAT in this case).
4. Add 2 volumes of patient's plasma (2 drops) and 1 volume (1 drop) of 5% red cell suspension of reagent red cells (pooled "O") prepared in normal saline (or LISS) to an already labelled test tube and mix well.
5. Incubate the tube at 37^0C in a serologic incubator for at least 30 min (30-60 min) (In case LISS is used to make 5% cell suspension of pooled cells, the incubation period will reduce to approx. 15 min.).
6. After the incubation time is over, wash the red cells at least 3 times. To wash the red cells, fill two-third of the test tube with 0.9% normal saline, thoroughly mix red cells with it followed by centrifugation at 3000 rpm for 2-3 min and then decant the supernatant saline. Repeat this at least three times to obtain a dry red cell button at the end.

7. Add 1 volume of AHG to the dry cell button and mix well.
8. Centrifuge the tubes at 1000 rpm x 1min.
9. Remove the tubes & read over a lightbox, and grade & record the reaction.
10. Add one drop of 5% red cell suspension of sensitized red cells to negative results.
11. Repeat steps 9 and 10.

Interpretation

1. If agglutination is not observed at step no 9 and agglutination is observed after the addition of check cells, then report the result as negative along with the technique of testing. e.g.
   - IAT – Negative by conventional test tube technique
2. If agglutination is observed at step no 9, it is suggestive of clinically significant IgG antibodies. Report the result as positive along with the technique of testing. e.g.
   - IAT – Positive by conventional test tube technique using a pool of 'O' cells
3. If agglutination is not observed at step no 9 as well as after addition of check cells the test is considered invalid and need to be repeated.

Note:

- IAT / antibody screen of blood donors can be done using a pool of 2 or 3 'O' Rh (D) positive red cells.
- IAT / antibody screen of patients is not recommended to be done with pooled O cells. A panel of 2 or preferably 3 'O' cells either prepared in-house or using commercial cell panels should be used for testing of patient samples. The procedure described above should be followed for individual cells of the panel.

3.6 Procedures relevant to cases with a positive DAT

For cases with positive DAT, it is important to differentiate if it is caused by an autoantibody or an alloantibody (in which case it suggests a delayed haemolytic/serologic transfusion reaction). Elution procedures are helpful in dissociating the bound antibody in the eluate, which can then be screened for a defined specificity. Similarly, in the case of DAT positivity due to AIHA who provides a history of transfusion, it becomes important to detect and identify any alloantibodies underlying the autoantibodies and masked by them so that appropriate blood unit may be provided for them. Allo-adsorption studies help to separate the autoantibodies from the patient's serum by adsorbing them onto either the patient's own red cells or selected red cells. The remaining plasma can then be tested for the presence of allo-antibodies.

3.6.1 Elution procedures

Elution may be defined as the removal of antibody from coated or sensitized RBCs by using a variety of physical and chemical treatments which dissociate antigen-antibody complexes with the primary objective of recovering bound antibody for study by routine serological methods.

Some of the modalities used for elution include heat, ultrasound, freeze-thawing, detergents, or organic solvents or by alteration in pH or salt concentration.

Elution techniques have been useful in

a. Evaluation of blood sample with a positive DAT.
b. Combined adsorption-elution procedures.
c. Preparation of Antibody free intact red cells for antigen typing.

Selecting an ideal elution method will require an idea of the antibody being detected (i.e., warm or cold reacting antibody) and whether the red cells are required to be intact after completion of the procedure. For example, if a warm antibody is suspected to be coating the red cells, then either cold acid elution or ether

elution may be attempted, and for a cold antibody, heat elution is the preferred method. The procedure for two commonly used elution methods, i.e., cold acid elution and heat elution, are being discussed here.

a) ***Heat elution method***

Materials required

- Test tubes
- Normal saline
- Water bath (37°C & 56°C)
- Tabletop centrifuge
- Test cells (DAT positive RBCs)

Procedure

1. Wash the test RBCs six times with normal saline. Keep the supernatant of the last wash. Pack the RBC well.
2. To one volume of these packed RBCs, add one volume of normal saline.
3. Incubate the tube at 56°C for 10 minutes in a serological water bath. Constantly agitate the test tube during incubation.
4. Centrifuge the tube at 3000 rpm for 3 minutes.
5. Decant the supernatant eluate in a test tube.
6. Test the eluate and the last wash in parallel.

Note: Haemolysis of red cells will be observed in the technique resulting in haemolyzed eluate, which is an expected finding. Addition of washed packed cells with 6% bovine serum albumin.

b) ***Cold acid elution method***

Materials required

- Glycine- HCl (0.1 M, pH 3.0) – prepared by adding 3.754 gm Glycine and 2.992 gm NaCl in 500ml of distilled water. Adjust the pH to 3.0 with 12 N HCl. Store at 40°C.
- Phosphate buffer (0.8 M, pH 8.2) - prepared by adding 109.6gm Na_2HPO_4 and 3.8 gm KH_2PO_4 in 600ml distilled water. Store at 4°C.
- Normal saline (0.9%)
- Ice bath
- Serological Water Bath
- Tabletop centrifuge
- Test cells (DAT positive RBCs)

Procedure

1. Wash the test RBCs six times with normal saline. Keep the supernatant of the last wash. Pack the RBC well.
2. Keep the red cells, saline and glycine-HCl solution in an ice water bath for 5 minutes.
3. To one volume of the packed red cells, add 1 volume of chilled saline and 2 volumes of glycine -HCl and keep the mixture in an ice-water bath for 1 minute.
4. Centrifuge the tube immediately at 3000rpm for 3 min and transfer the supernatant into a clean test tube.
5. To 1 volume of the supernatant obtained in step 4, add 0.1 volume of phosphate buffer pH 8.2 and mix well.
6. Centrifuge the tube at 3000rpm for 3 min and transfer the eluate to a clean test tube.
7. Test the eluate obtained in step 6 in parallel with the supernatant of the last wash obtained in step 1 by reagent O cells used for antibody screen.

3.5.2 *Adsorption procedures*

a) **Adsorbing warm-reactive autoantibodies using autologous RBCs**

Principle

Warm reactive autoantibody in serum may mask the concomitant presence of clinically significant alloantibodies. Adsorption of the serum with autologous red cells can remove autoantibody from the serum, permitting detection of underlying alloantibodies. However, autologous red cells in the circulation are coated with autoantibodies. Autologous adsorption of warm reactive autoantibodies can be achieved by dissociating autoantibodies from the red cell membrane, thereby uncovering antigen sites that can bind free autoantibody to remove it from the serum or plasma. Treatment of cells with enzyme enhances the adsorption process by removing membrane structures that otherwise hinder the association between antigen and antibody.

The most effective procedure involves the use of ZZAP reagent, a mixture of proteolytic enzyme and sulfhydryl reagent. Treatment of IgG molecules with sulfhydryl reagent increases their susceptibility to digestion by proteases. When IgG coated red cells are treated with ZZAP reagent, the immunoglobulin molecule loses its integrity and dissociate from the cell surface, and the action of the proteolytic enzyme increases the adsorbing capacity of the treated cells.

Red cells from recently transfused patients should not be used for auto adsorption because transfused red cells present in the circulation are likely to adsorb the alloantibodies that are being sought. It may also be difficult to perform this procedure in patients with low haemoglobin (<7g/dl) as a large amount of the patient's sample is required to obtain sufficient auto red cells required for the procedure.

Materials required

- Patients sample (EDTA) – minimum 5-10ml sample may be required depending on patient haematocrit
- 75 x 12 mm tubes
- Pasteur pipettes
- 1% Cysteine-activated papain
- Phosphate buffered saline (PBS) at pH 7.3 and pH 8.0
- 0.2 M DTT
- 2 ml packed autologous red cells
- Normal Saline (0.9%)
- Tabletop centrifuge
- Serologic Incubator

Procedure

1. Prepare the ZZAP reagent by mixing 0.5 ml of 1% cysteine activated papain with 2.5 ml 0.2 M of DTT in PBS pH 7.3 and 2 ml of PBS pH 7.3.
2. Prepare two tubes containing 1ml of packed autologous red cells each. For this, take 1 ml autologous red cells in each of the test tubes and mix with normal saline. Centrifuge the tube at 3000 rpm for 3 min and remove the saline. Repeat this two more times with the washed cells to get packed autologous red cells.
3. To each of two tubes containing 1 ml of packed autologous red cells, add 2 ml of ZZAP reagent. There is no need to wash the Red Cells before treatment. Mix and incubate at 37°C for 30 minutes with periodic mixing.
4. After 30 min, wash the red cells 3 times in saline at 2000-3000 rpm for 3 min. Centrifuge the last wash for at least 5 minutes. Use a Pasteur pipette to remove as much of the supernatant saline as possible.
5. Add the patient's plasma to an equal volume of ZZAP treated auto red cells, mix and incubate at 37°C for 30-45 minutes.
6. After incubation, centrifuge and carefully remove the adsorbed plasma. Transfer the removed adsorbed plasma to the 2nd tube of ZZAP treated and washed 1ml auto red cells. Mix and incubate at 37°C for 30-45 minutes.

7. If the initial IAT reactivity in LISS was 3+ or less, generally one-two adsorptions remove sufficient autoantibody so that alloantibody reactivity, if present, is readily apparent. More adsorptions will dilute the alloantibody.
8. Test the adsorbed plasma against group O reagent cells.

Interpretation

If the twice auto adsorbed serum reacts with defined specificity, as shown by testing against a small antibody identification panel, then the defined specificity of the antibody is probably an alloantibody. If the serum reacts with all cells on the panel, 1) additional auto adsorptions are necessary, 2) the serum contains an antibody to a high-prevalence antigen or 3) the serum contains an antibody (e.g., anti-Kp^a) that does not react with ZZAP treated cells and thus will not be absorbed by this procedure. To confirm this possibility, test the reactive auto-adsorbed serum against reagent cells that have been pre-treated with the ZZAP reagent.

Note: ZZAP treatment destroys all Kell system antigens and all other antigens that are destroyed by proteases.

b) **Adsorbing warm-reactive autoantibodies using allogenic RBCs**

Principle

Warm-reactive autoantibodies may mask the presence of alloantibodies in the serum of a patient with AIHA with a history of transfusion. When serum from a recently transfused patient (i.e., transfused within the preceding 120 days) is suspected of containing autoantibodies, autologous adsorption cannot be performed reliably because the transfused RBCs may adsorb alloantibodies. Recognition of alloantibodies may be facilitated by the removal of autoantibodies through adsorption with allogeneic RBCs which have a phenotype matching the patient or which have been treated by ZZAP. ZZAP treatment of RBCs destroys most antigens of the major blood groups, with the exception of ABO, Rh, and Kidd antigens. This facilitates the adsorption by using only 3 different sets of RBCs, one each of R_1R_1, R_2R_2 and rr phenotype one of which is preferably homozygous for -Jk^a and one for -Jk^b.

Materials required

- Patients sample (EDTA) – a minimum 5-10ml sample may be required
- 75 x 12 mm tubes
- Pasteur pipettes
- 1% Cysteine-activated papain
- Phosphate buffer saline (PBS) pH 6.5
- 0.2M DTT in PBS pH 7.3
- RBCs matched for the patient's Rh and Jk type if known
- If the phenotype is unknown, use RBCs of the following three Rh phenotypes: R_1R_1, R_2R_2, and rr. At least one sample should be Jk(a+b–), and one, Jk(a–b+)
- Normal Saline (0.9%)
- Tabletop centrifuge
- Serologic Incubator

Procedure (using R_1R_1, R_2R_2 and rr allogeneic cells)

1. Prepare ZZAP reagent by mixing 0.5 ml of 1% cysteine activated papain with 2.5 ml of 0.2M of DTT and 2 ml of PBS pH 7.3.
2. Prepare three test tubes and label them as R_1R_1, R_2R_2 and rr. Take adequate cells depending on the number of adsorptions required. A rough guide to the number of adsorptions required could be taken from the grade of DCT reactivity. If the DCT reactivity is 1+, 2+, 3+ and 4+ the number of adsorptions required will be roughly 2, 3, 4 and 5, respectively. The number of adsorptions required may vary from case to case. For example, for a patient of AIHA showing 3+ DCT reactivity, the number of adsorptions required

to completely remove autoantibodies can be roughly taken as 4, and the volume of each type of RBCs to be enzyme/ZZAP treated should be 4ml.

3. Treat the appropriate volume (depending on the potential number of adsorptions required as explained in step1) of R_1R_1, R_2R_2 and rr cells with ZZAP reagent. To each of the tube containing 1 volume of packed cells, add 2 volumes of ZZAP reagent. Mix and incubate at 37^0C for 20-30 minutes with periodic mixing.
4. Wash the red cells 3 times in saline. Centrifuge the last wash for at least 5 minutes at 900-1000 x g. Use Pasteur pipette to remove as much of the supernatant.
5. Divide each of the treated cells (R_1R_1, R_2R_2 and rr) into the desired number of aliquots (depending on the number of adsorptions required) of 1ml each in appropriately labelled test tubes. If the serum/plasma to be treated is of insufficient volume, less volume of cells and plasma may be taken.
6. Add serum/plasma to an equal volume of ZZAP treated cells, mix, and incubate at 37^0C for 30 minutes.
7. Centrifuge and carefully remove serum/plasma.
8. Step 6 and 7 should be repeated once more using the once adsorbed patient's serum/plasma with the second aliquot of ZZAP treated cells. The adsorption steps should be continued till the DCT of adsorbed cells is negative or crossmatch of adsorbed serum with ZZAP treated cells is negative. This ensures complete adsorption of autoantibodies. A maximum of eight serial adsorptions may be attempted.
9. After the final adsorption, test the adsorbed serum/plasma with an antigen screening panel (3-cell panel).
10. If the antibody screening is positive on the adsorbed plasma, perform antibody identification on the adsorbed plasma.

Interpretation

If the adsorbed serum (after complete adsorption) reacts with determined specificity, as shown by testing against an antibody screening and identification panel, then the defined specificity of the antibody is probably an alloantibody. If the serum reacts with all the cells on the panel, the serum contains an antibody (e.g., Anti-Ge) that does not react with ZZAP treated cells and thus will not be absorbed by this procedure. To confirm this possibility, test the reactive absorbed serum against reagent cells that have been pre-treated with the ZZAP reagent. If the adsorbed serum does not show any specificity on the antibody screening panel after complete adsorption, it suggests that alloantibodies against the minor antigens are absent, and only autoantibodies are present.

Note: ZZAP treatment destroys all Kell system antigens and all other antigens that are destroyed by proteases.

3.7 Procedure for antibody titration

Titration is a semi-quantitative method used to determine the concentration of antibody in a serum sample or to compare the strength of antigen expression on different red cell samples. The usual application of titration studies are as follows:

- Estimating antibody activity in an alloimmunized pregnant woman to determine whether and when to perform a more complex invasive investigation of fetal condition
- Elucidating autoantibody specificity and treatment follow up in the case of AIHA
- Characterizing antibodies as high-titre, low avidity
- Observing the effect of sulfhydryl reagents on antibody behaviour to determine immunoglobulin class (IgG or IgM)
- Quality control testing of various antisera

Materials required

- 75 x 12mm glass tubes
- Tabletop centrifuge
- Dry air incubator

- Micropipette & tips
- Serum / anti-sera to be titrated
- Red cell expressing antigen corresponding to antibody specificity (indicator RBCs 5% suspension)
- Normal saline (0.9%)
- AHG (in case of Rh Ab titration) (anti IgG)

Procedure

1. Label 10 test tubes according to the serum dilution (starting from 1:1, 1:2, 1:4, 1:8 up to 1:512) as shown in Figure below.
2. Deliver 1 volume (2 drops) of saline into each tube except the first.

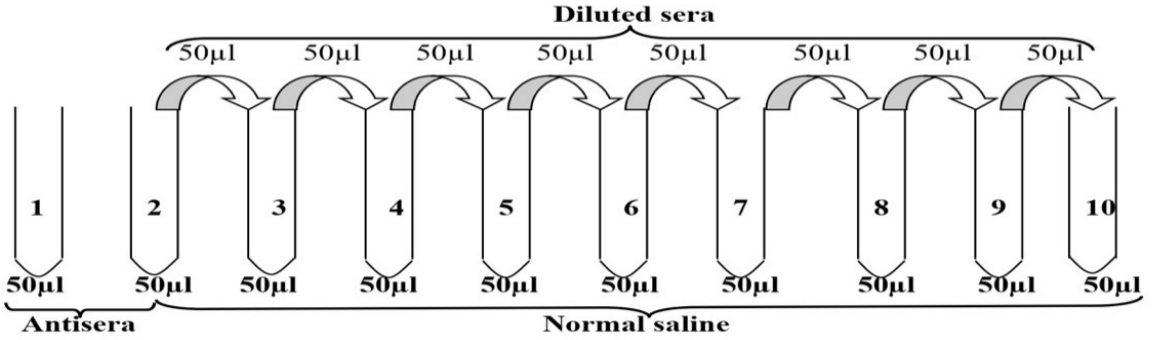


Figure showing method to prepare serial doubling dilution and antibody titration

3. Add 1-volume serum to the first test tube (1:1 dilution) & 1 volume to the second test tube (1:2 dilution).
4. Mix the contents of the second tube (1:2 dilution) & transfer 1 volume into the next tube (1:4 dilution).
5. Continue the same process for all dilutions, using automatic pipettes with disposable tips that are changed for each transfer. Save 1 volume from the last test tube to be used if further titration is necessary.
6. Add 1 drop (0.05 ml) of the red cell suspension to each of the 10 tubes.
7. Mix well & incubate the tubes at 37°C for 1 hr.
8. Wash red cells at least 3 times using normal saline and completely decant the final wash to obtain a dry cell button.
9. Add 1 volume of AHG to the dry cell button and mix well.
10. Centrifuge the tubes at 1000 rpm x 1min.
11. Remove the tubes & read over a lightbox.
12. Grade score & record the reaction.

Procedural notes

- *For titration of cold reacting / IgM antibodies such as ABO antisera, the test tubes are incubated at room temperature for 15 min, and the reaction is performed in saline phase without AHG.*

Interpretation

- Results are reported as the reciprocal of the highest dilution (for example, 32—**NOT** 1 in 32 or 1:32) that produces 1 + macroscopic agglutination, which may differ from the titration endpoint.
- If there is agglutination in the tube containing the most dilute serum, the endpoint has not been reached, and additional dilutions of the saved serum should be prepared and tested.
- The score values can be assigned as per the following table.

Table showing example of titration and scoring

	T1 1:1	T2 1:2	T3 1:4	T4 1:8	T5 1:16	T6 1:32	T7 1:64	T8 1:128	T9 1:256	T10 1:512	Titre	Score
Strength	4+	3+	3+	2+	2+	1+	1+	W+	0	0	64	
Score	12	10	10	8	8	5	5	3	0	0		61

Points to remember

- The titration should be performed upon initial detection of the antibody.
- A difference in titre of at least 3 test tubes or a difference in score of at least 10 is considered a significant difference.
- Comparisons are valid only when specimens are tested concurrently.
- Technical variables greatly affect the results.
- To ensure objectivity, it is preferable to read the test tube containing the most dilute serum & proceed to the most concentrated sample, reading one test tube at a time.

3.8 Differentiation of IgM and IgG antibodies using sulfhydryl reagents

Principle

Sulfhydryl reagents reduce the -S-S- bond and thus abolish the agglutinating activity of IgM antibodies. Thus, treatment of plasma or serum with sulfhydryl reagent will help in distinguishing the class of antibody as well as the presence of underlying IgG antibodies.

Material required

- Plasma sample to be tested
- 0.01 M DTT - 0.154 g of DTT dissolved in 100 mL of phosphate-buffered saline (PBS) at pH 7.3; stored at 4°C
- 0.1 M stock 2-ME - 0.7 mL of a 14 M stock solution of 2-ME diluted in 100 mL of PBS at pH 7.3; 2-ME should be stored in a dark glass container at 4°C
- PBS at pH 7.3
- Test tubes 10 X 75 mm
- Pasteur pipette
- Tabletop centrifuge
- High-intensity lamp/view box

Procedure

1. Dispense 1 mL of serum or plasma into each of two test tubes. Label one of the tubes as DC (for DILUTION Control) and the other tube as TT (for TEST Tube).
2. To the tube labelled as DC, add 1 mL of pH 7.3 PBS and mix it gently with the plasma.
3. To the other tube labelled TT, add 1 mL of 0.01 M DTT (or 0.1M 2-ME) and mix it gently with the plasma.
4. Incubate both the tubes at 37°C for 30 to 60 minutes.
5. Use the DTT-treated and dilution control samples in standard procedures.
6. One can use a sample known to contain IgM antibodies in parallel as a control.

Interpretation

- Reactivity in the dilution control serum and no reactivity in the DTT-treated serum indicate an IgM antibody.

- Reactivity in the dilution control serum and in the DTT-treated serum indicates an IgG antibody or an IgG and IgM mixture.
- No reactivity in the dilution control serum indicates a dilution of weak antibody reactivity and an invalid test.

3.9 Use of enzyme (papain) in immunohaematology

Enzymes such as papain are useful in immunohaematological investigations such as antibody identification when multiple antibodies are present in patient's plasma. For details, see section 6. The methods here describes the preparation of papain enzyme stock solution, a method to standardize the papain working solution and treatment of red cells with papain working solution using a two-stage technique.

3.9.1 Preparation of papain enzyme stock solution 1% w/v

Material required

- L-cysteine hydrochloride (0.5 M), 0.88 g in 10 mL distilled water
- Dry papain powder, 2 g
- Phosphate buffer (0.067 M at pH 5.4), prepared by combining 3.5 mL of Na_2HPO_4 and 96.5 mL of KH_2PO_4

Procedure

1. Add 2 g of powdered papain to 100 mL of phosphate buffer (pH 5.4).
2. Agitate this enzyme solution for 15 minutes at room temperature.
3. Collect clear fluid by filtration or centrifugation.
4. Add L-cysteine hydrochloride and incubate the solution at 37°C for 1 hour.
5. Add phosphate buffer (pH 5.4) to a final volume of 200 mL,—store aliquots at –20°C or colder. Do not refreeze aliquots.

3.9.2 Standardizing working solutions for prepared stock papain enzyme

Each lot of enzyme differs from the other, and the prepared solution may differ in terms of reactivity and incubation time for optimal results. The following method may be adopted to standardize the reactivity and incubation time for papain solution.

Material required.

- 1% stock solution of papain in PBS, pH 7.3
- Antibody negative plasma
- Anti-D that agglutinates the only enzyme-treated D+ red cells and does not agglutinate untreated D+ red cells
- Anti-Fy^a of moderate or strong reactivity
- O group D+ and Fy(a+b–) red cell samples
- Antihuman globulin (AHG) reagent

Procedure

1. Prepare 0.1% papain by diluting one volume of stock papain solution with nine volumes of PBS, pH 7.3.
2. Take three test tubes and label them as 5 minutes, 10 minutes, and 15 minutes.
3. Add equal volumes of washed red cells {O D+ Fy(a+b–)} and 0.1% papain to each tube.
4. Mix and incubate the three test tubes at 37°C for the time designated on the label of each.
5. Immediately wash the red cells three times with large volumes of saline.
6. Prepare 5% suspension of each of these red cells in saline.
7. Make three sets of four tubes labelled as untreated, 5 min, 10 min, and 15 min to be tested with antibody-

negative plasma, anti-D and anti-Fy^a.

8. Add 2 drops of the appropriate plasma/antisera to each of the four tubes.
9. Add 1 drop of the appropriate red cell suspension to each of the labelled tubes.
10. Mix and incubate at 37°C for 15 minutes.
11. Centrifuge and examine for agglutination by gently resuspending the red cell button.
12. Continue with testing in each tube and proceed with the IAT test as described in method 3.5.2.

Interpretation

The following table shows an example of one such standardization exercise of papain solution.

Example of results with D+, Fy(a+b–) Red Cells

Cell type		Antibody negative plasma	Anti-D	Anti-Fya
Untreated cells	37°C incubation	0	0	0
	AHG test	0	1+	3+
Cells treated with papain for 5 min	37°C incubation	0	1+	0
	AHG test	0	2+	1+
Cells treated with papain for 10 min	37°C incubation	0	2+	0
	AHG test	0	2+	0
Cells treated with papain for 15 min	37°C incubation	0	2+	0
	AHG test	w+	2+	w+

Here, the optimal incubation time would be 10 minutes. Incubation for only 5 minutes does not completely abolish Fy^a activity or maximally enhance anti-D reactivity. Incubation for 15 minutes causes false-positive AHG reactivity with inert serum.

3.9.3 Two-stage enzyme procedure

Material required

- Patient serum to be tested
- Reagent red cells
- Papain stock solution 1% w/v

Procedure

1. Prepare a diluted papain solution by adding 9 mL of PBS, pH 7.3, to 1 mL of stock papain solution.
2. Add one volume of diluted papain to one volume of packed, washed reagent red cells.
3. Incubate at 37°C for the time determined to be optimal for the papain solution as determined by method 3.9.2.
4. Wash treated red cells at least three times with large volumes of saline, and prepare a 5% suspension of these cells in normal saline.
5. Add 2 drops of plasma to be tested to an appropriately labelled tube.
6. Add 1 drop of 5% suspension of papain-treated red cells.
7. Mix and incubate for 15 minutes at 37°C.
8. Centrifuge at 1000rpm for 1 min and gently resuspend the red cells and observe for agglutination. Grade and record the results.

Interpretation

One needs to compare the results of antibody screening after treating reagent cells with the enzyme as described above with the result of antibody screen without enzyme-treated reagent cells. There would be the disappearance of reactivity by antibodies that are directed towards antigens susceptible to enzyme treatment. This would help in identifying other antibodies in the case of multiple antibodies. Similarly, the reactivity of some antibodies will be enhanced, thus helping in identifying them. The table below may be helpful in identifying antigens that are susceptible to enzyme treatment or enhanced by enzyme treatment.

Effect of enzyme and DTT on antigens in antibody identification

SN	Papain / Ficin	DTT (200mM)	Possible specificity
1.	Negative	Positive	M, N, S, s, Fy^a, Fy^b, Ch/Rg, Xg^a
2.	Negative	Negative	Indian, JMH
3.	Positive	Weak	Lutheran, Dombrock, Cromer, knops
4.	Variable	Negative	Yt^a
5.	Positive	Negative	Kell
6.	Positive	Positive	A, B, H, P_1, Rh, Lewis, Kidd, Fy3, Diego, Colton
7.	Positive	Enhanced	Kx

4. Compatibility testing

Compatibility testing is a step of pre-transfusion testing where a selected donor unit is cross-matched with the patient's plasma. It is based on the principle that any clinically significant antibodies (both IgG and IgM) present in the patient's plasma and directed against the antigens on the red cell unit being crossmatched will react in-vitro, resulting in incompatibility on testing.

With the preparation of components, only major crossmatching is being done where donor red cells are tested with patient's plasma. The testing could be done in two ways, as described below; f*or details, see section 7.*

4.1 Immediate spin technique

The immediate spin technique will identify ABO compatibility only in addition to the presence of other IgM antibodies. This technique will not detect incompatibility due to clinically significant antibodies of IgG type against minor blood group antigens, including anti-D.

Material required

- Donor red cells
- Patients plasma/serum
- Normal saline (0.9%)

Procedure

1. Wash the donor's red cells 3 times with normal saline to pack the red cells.
2. Prepare a 5% suspension of donor red cells in normal saline.
3. Label the test tube with the donor unit number. Make separate test tubes and label them accordingly if more than one unit needs to be tested.
4. Add 2 drops of the patient's serum or plasma to each tube.
5. Add 1 drop of the suspension of donor red cells prepared in step 2 to the appropriate test tube.
6. Mix the contents of the tube(s) and centrifuge at 1000 rpm for 1 minute.
7. Examine the tube(s) for haemolysis, gently resuspend the red cell button(s), and examine for agglutination.
8. Read, interpret, and record test results.

Interpretation

- Agglutination or haemolysis constitutes a positive (incompatible) test result.
- A smooth suspension of red cells after resuspension of the red cell button constitutes a negative result and indicates a compatible immediate-spin crossmatch.

4.2 Anti-human globulin technique

This technique will be able to identify incompatibility due to the presence of clinically significant antibodies (IgG type) in the patient's plasma, which are directed against the donor red cells. *For details, see section 7.*

Materials required

- Normal saline
- Antihuman globulin (AHG) reagent
- A 2% to 5% suspension of donor red cells in saline
- IgG-sensitized red cells

Procedure

1. Wash the donor's red cells 3 times with normal saline to pack the red cells.
2. Prepare a 5% suspension of donor red cells in normal saline.
3. Label the test tube with the donor unit number. Make separate test tubes and label them accordingly if more than one unit needs to be tested.
4. Add 2 drops of the patient's serum or plasma to each tube.
5. Add 1 drop of the suspension of donor red cells prepared in step 2 to the appropriate test tube.
6. Incubate at 37°C for 30 to 60 minutes.
7. Centrifuge and observe for haemolysis and agglutination. Grade and record the results.
8. Wash the red cells three times with saline, and completely decant the final wash.
9. Add two drops of AHG to the dry red cell button.
10. Centrifuge and observe for agglutination. Grade and record the results.
11. Confirm the validity of negative results by adding IgG-sensitized red cells.

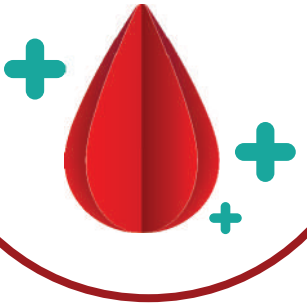
Interpretation

1. The presence of agglutination/haemolysis after incubation at 37°C constitutes a positive test result.
2. The presence of agglutination after the addition of AHG constitutes a positive test result.
3. Antiglobulin test results are negative when no agglutination is observed after initial centrifugation followed by agglutination with the addition of IgG-sensitized red cells and centrifugation. If the IgG-sensitized red cells are not agglutinated, the negative result is considered invalid, and the test must be repeated.

Note: LISS (low ionic strength solution) can also be used to make the 5% donor red cell suspension instead of normal saline in step 2. When LISS is used, the incubation time of 30-60 min as mentioned in step 6 can be reduced to 15 minutes.

Please note that all the centrifugation speeds and incubation times mentioned in the above methods should be standardized by individual laboratories before adopting these methods. The methods described here are indicative of the protocols in most of the laboratories.

APPENDIX 2



Rules and Amendments, Forms, Sample Formats and Tables

A. Drugs and Cosmetics Act, 1940 and Rules, 1945

REGULATORY REQUIREMENTS OF BLOOD AND/OR ITS COMPONENTS INCLUDING BLOOD PRODUCTS

PART X-B

REQUIREMENTS FOR THE COLLECTION, STORAGE, PROCESSING AND DISTRIBUTION OF WHOLE HUMAN BLOOD, HUMAN BLOOD COMPONENTS BY BLOOD BANKS AND MANUFACTURE OF BLOOD PRODUCTS

122-EA. Definitions.- (1) In this Part and in the Forms contained in Schedule A and in Part XII-B and Part XII-C of schedule F, unless there is anything repugnant in the subject or context,-

(a) 'apheresis' means for the process by which blood drawn from a donor, after separating plasma or platelets or leucocytes, is re-transfused – simultaneously into the said donor;

(b) 'autologous blood' means the blood drawn from the patient for re-transfusion unto himself later on;

(c) 'blood' means and includes whole human blood, drawn from a donor and mixed with an anti-coagulant;

(d) 'blood bank' means a place or organization or unit or institution or other arrangements made by such organization, unit or institution for carrying out all or any of the operations for collection, apheresis, storage, processing and distribution of blood drawn from donors and/or for preparation, storage and distribution of blood components;

(e) 'blood component' means a drug prepared, obtained, derived or separated from a unit of blood drawn from a donor;

(f) 'blood product' means a drug manufactured or obtained from pooled plasma or blood by fractionation, drawn from donors;

(g) 'donor' means a person who voluntarily donates blood after he has been declared fit after a medical examination, for donating blood, on fulfilling the criteria given hereinafter, without accepting in return any consideration in cash or kind from any source, but does not include a professional or a paid donor.

EXPLANATION.- For the purposes of this clause, benefits or incentives like pins, plaques, badges, medals, commendation certificates, timeoff from work, membership of blood assurance programme, gifts or little or intrinsic monetary value shall not be construed as consideration;

(h) 'leucapheresis' means the process by which the blood drawn from a donor, after leucocyte concentrates have been separated, is re-transfused simultaneously into the said donor;

(i) 'plasmapheresis' means the process by which the blood drawn from a donor, after the plasma has been

separated, is re-transfused during the same sitting into the said donor;

(j) 'plateletpheresis' means the process by which the blood drawn from a donor, after platelet concentrates have been separated, is re-transfused simultaneously into the said donor.

(k) 'professional donor' means a person who donates blood for a valuable consideration, in cash or kind, from any source, on behalf of the recipient – patient and includes a paid donor or a commercial donor;

(l) 'replacement donor' means a donor who is a family friend or a relative of the patient –recipient.

122-F. Form of application for license for operation of Blood Bank/processing of whole human blood for components/manufacture or Blood Products for sale or distribution – (1) Application for the grant and/or renewal of license for the operation of Blood Bank/processing of Human Blood for components/ manufacture of Blood Products shall be made to the Licensing Authority appointed under Part VII in Form 27-C or Form 27-E as the case may be and shall be accompanied by license fees of rupees six thousand and an inspection fee of rupees one thousand and five hundred for every inspection thereof or for the purpose of renewal of license.

Provided that if the applicant applies for renewal of a license after the expiry, but within six months of such expiry the fee payable for the renewal of the license shall be rupees six thousand and inspection fees of rupees one thousand and five hundred plus additional fees at the rate of rupees one thousand per month or a part thereof in addition to the inspection fee.

Provided further that a licensee holding a license in Form 28-C or Form 28-E as the case may be for the operation of blood bank/processing of whole human blood for components/manufacture of blood products shall apply for a grant of license under sub-rule (1) before the expiry of the said license on Form 27-C or Form 27-E as the case may be and he shall continue to operate the same till the orders on his application are communicated to him.

1. EXPLANATION.- For the purpose of this rule, 'Blood Bank' means a place or organizational unit or an institution, or other arrangement made by such organizational unit or institution for carrying out all or any of the operations of manufacture of human blood components or blood products or whole human blood for its collection, storage, processing, distribution from selected human donors.}
2. A fee of rupees one thousand shall be paid for a duplicate copy of license issued under this rule if the original is defaced, damaged or lost.
3. Application by licensee to manufacture additional drugs listed in the application shall be accompanied by a fee of rupees three hundred for each drug listed in the application.
4. On receipt of the application for the grant or renewal of such license, the Licensing Authority shall -
   (i) verify the statements made in the application form.
   (ii) Cause the manufacturing and testing establishment to be inspected in accordance with the provisions of rules 122-I; and
   (iii) In case the application is for renewal of license, call for information of the past performance of the licensee.
5. If the Licensing Authority is satisfied that the applicant is in a position to fulfil the requirements laid down in the rules, he shall prepare a report to that effect and forward it along with the application and the license (in triplicate) to be granted or renewed, duly completed to the Central license Approving Authority:

   Provided that if the Licensing Authority is of the opinion that the applicant is not in a position to fulfil the requirements laid down in these rules, he may, by order, for a reason to be recorded in writing, refuse to grant or renew the license, as the case may be.
6. If, on receipt of application and the report of the Licensing Authority referred to in Sub-rule 5 and after taking such measures including inspection of the premises, by the inspector, appointed by the Central Govt. under Section 21 of the Act, and/or along with an expert in the field concerned if deemed necessary, the Central license Approving Authority, is satisfied that the applicant is in a position to fulfil the requirement laid down in this rule. He may grant or renew the license, as the case may be:

Provided that if the Central license Approving Authority is of the opinion that the applicant is not in a position to fulfil the requirements laid down in these rules, he may, notwithstanding the report of the Licensing Authority, by order, for a reason to be recorded in writing, reject the application for grant or renewal of license as the case may be and shall supply the applicant with a copy of the inspection report.

122-G. Form of license for the operation of a Blood Bank/Processing of Whole Human Blood for components and manufacture of Blood products and the conditions for the grant or renewal of such license– A license for the operation of a Blood Bank or for processing whole Human Blood for components and manufacture of blood products shall be issued in Form 28-C or Form-28-E or Form 26-G or Form 26-I as the case may be. Before a license in Form 28°C or Form 28-E or Form 26-G or Form 26-I, as the case may be, is granted or renewed, the following conditions shall be complied with by the applicant-

(i) The operation of the Blood Bank and/or processing of whole human blood for components/manufacture of blood product shall be carried out under the active direction and personal supervision of component technical staff consisting of at least one person who is whole-time employee and who is a Medical Officer, and possessing-

a) Post Graduate degree in Medicine-M.D. (Pathology/Transfusion Medicines); or

b) Degree in Medicine (M.B.B.S.) with Diploma in Pathology or Transfusion Medicines having adequate knowledge in blood group serology, blood group methodology and medical principles involved in the procurement of blood and/or preparation of its components; or

c) Degree in Medicine (M.B.B.S.) having experience in Blood Bank for one year during regular service and also has adequate knowledge and experience in blood group serology, blood group methodology and medical principles involved in the procurement of blood and/or preparation of its components, the degree or diploma is from a university recognized by the Central Government.

EXPLANATION- For the purposes of this condition, the experience in Blood Bank for one year shall not apply in the case of persons who are approved by the Licensing Authority and/or Central license Approving Authority prior to the commencement of the Drugs & Cosmetics (Second Amendment) Rules,1999.

(ii) The applicant shall provide adequate space, plant and equipment for any or all the operations of blood collection or blood processing. Space, plant and equipment required for various operations is given in Schedule 'F', Part XII-B and/or XII-C.

(iii) The applicant shall provide and maintain adequate technical staff as specified in Schedule 'F', Part XII-B and/or XII-C.

(iv) The applicant shall provide adequate arrangements for storage of Whole Human Blood, Human Blood Components and blood products.

(v) The applicant shall furnish to the Licensing Authority, if required to do so, data on the stability of Whole Human Blood, its components or blood products which are likely to deteriorate, for fixing the date of expiry, which shall be printed on the labels of such products on the basis of the data so furnished.

122-H. Duration of license. An original license in Form 28-C or Form 28–E or a renewed license in Form 26-G or Form 26-I, unless sooner suspended or cancelled, shall be valid for five years and from the date on which the year in which it is granted or renewed.

122-I. Inspection before grant or renewal of license for operation of Blood Bank, processing of Whole Human Blood for Components and Manufacture of Blood Products– Before a license in Form 28-C or Form 28 –E is granted or a renewal of license in Form 26-G or Form 26-I is made,as the case may be, the Licensing Authority or Central license Approving Authority, as the case may be, shall cause the establishment in which Blood Bank is proposed to be operated/ whole human blood for the component is processed/blood products are manufactured to be inspected by one or more inspectors, appointed under the Act and/or along with the Expert in the field concerned. The Inspector or Inspectors shall examine all portions of the premises and appliances/equipment and inspect the process of manufacture intended to be employed or being employed along with the means to be employed or being employed for the operation of blood bank/processing of whole human blood for components/ manufacture of blood products together with their [testing] facilities and also

enquire into the professional qualification of the expert staff and other technical staff to be employed.

122-J. Report by Inspector- The Inspector or Inspectors shall forward a detailed descriptive report giving his finding on each aspect of inspection along with his recommendation in accordance with the provisions of Rule 122-I to the Licensing Authority or to the Central license Approving Authority.

122-K. Further application after rejection- If within a period of six months from the rejection of application for a license the applicant informs the licensing Authority that the conditions laid down have been satisfied and deposits an inspection fee of rupees two hundred and fifty the Licensing Authority if after causing further inspection to be made is satisfied that the conditions for the grant of a license have been complied with, shall grant or renew a license in Form 28-C or Form 28–E;

Provided that in case of drug notified by the Central Government under rule 68-A, the application, together with the inspection report and the Form of license (in triplicate to be granted or renewed), duly completed shall be sent to the Central license Approving Authority, who may approve the same and return it to the licensing authority for issue of the license.

122-L. Delegation of powers by the Central Licensing Approving Authority- The Central Licensing Approving Authority may, with the approval of the Central Government, by notification delegate his power of signing licenses and any other power under rules to persons under his control having same qualifications as prescribed for Controlling Authority under Rule 50-A, for such areas and for such periods as may be specified.

122-M. Provision for an appeal to the State Government by a Party whose license has not been granted or renewed- Any person who is aggrieved by the order passed by the Licensing Authority or Central license Approving Authority, as the case may be, may with inthirty days from the date of receipt of such order, appeal to the State Government or Central Government, as the case may be, after such enquiry, into the matteras it considers necessary and after giving the said personan opportunity for representing his view in the matter may pass such order in relation there to as it thinks fit.

122-N. Additional information to be furnished by a [applicant] for a license or by a licensee to the Licensing Authority- The applicant for the grant of license or any person granted a license under the part shall, on-demand, furnish to the Licensing Authority, before the grant of the license or during the period the license is in force, as the case may be, documentary evidence in respect of the ownership or occupation, rental or another basis of the premises, specified in the application for a license or in the license granted, the constitution of the firm or any other relevant matter, which may be required for the purpose of verifying the correctness of the statement made by the applicant or the licensee, while applying for or after obtaining the license, as the case may be.

122-O. Cancellation and suspension of licenses- (1) The Licensing Authority or Central license Approving Authority may for such licenses granted or renewed by him after giving the licensee an opportunity to showcause by such an order should not be passed by an order in writing stating the reason thereof, cancel a license issued under this part or suspend it for such period as he thinks fit, either wholly or in respect of some of the substances to which it relates, [or direct the licensee to stop collection, storage, processing, manufacture and distribution of the said substances and [there upon order the destruction of substances and] stocks thereof in the presence of an Inspector] if, in his opinion, the licensee has failed to comply with any of the conditions of the license or with anyprovision of the Act or Rules thereunder.

(2) A licensee whose license has been suspended or cancelled, within three months of the date of the order under sub-rule (1) prefer an appeal against that order to the State Government or Central Government, which shall decide the same.

122-P. Conditions of license- A license in Form 28-C, Form 28-E, Form 26-G or Form 26-I shall be subject to the special conditions set out in Schedule F, Part XII-B and Part XII-C, as the case may be, which relate to the substance in respect of which the license is granted or renewed and to the following general conditions, namely:-

(i) (a) The licensee shall provide and maintain adequate staff, plant and premises for the proper operation of a Blood Bank for processing whole human blood, its components and/or manufacture of blood products.

(b) The licensee shall maintain staff, premises and equipment as specified in Rule 122-G. The licensee shall maintain necessary records and registers as specified in Schedule F, Parts XII-B and XII-C.

(c) The licensee shall test in his own laboratory whole human blood, its components and blood products and [maintain records and] registers in respect of such tests as specified in Schedule F, Part XII-B and Part XII-C. The records and registers shall be maintained for a period of five years from the date of manufacture.

(d) The licensee shall maintain/preserve reference [sample and] supply to the Inspector the reference sample of the whole human blood collected by him in adequate quantity to conduct all the prescribed tests. The licensee shall supply to the Inspector the reference sample for the purpose of testing.

(ii) The licensee shall allow an inspector appointed under the Act to enter, with or [without] prior notice, any premises where the activities of the Blood Bank are being carried out, for the processing of Whole Human Blood and/or Blood Products, to inspect the premises and plant and the process of manufacture and the means employed for standardizing and testing the substance.

(iii) The licensee shall allow an Inspector appointed under the Act to inspect all registers and records maintained under these rules and to take samples of the manufactured product and shall supply to Inspector such information as he may require for the purpose of ascertaining whether the provisions of the Act and Rules thereunder have been observed.

(iv) The licensee shall from time to time report to the Licensing Authority any changes in the expert staff responsible for the operation of a Blood Bank/processing of whole human blood for components and/or manufacture of blood products and any material alterations in the premises or plant used for that purpose which have been made since the date of the last inspection made on behalf of the Licensing Authority before the grant of the license.

(v) The licensee shall on request furnish to the Licensing Authority, or Central license Approving Authority or to such Authority as the Licensing Authority, or the Central license Approving Authority may direct, from any batch unit of drugs as the Licensing Authority or the Central license Approving may from time to time specify, sample of such quantity as may be considered adequate by such Authority for any examination and, if so required, also furnish full protocols of the test which have been applied.

(vi) If the Licensing Authority or the Central license Approving Authority so directs, the licensee shall not sell or offer for sale any batch/unit in respect of which a sample is, or protocols are furnished under the last preceding sub-paragraph until a certificate authorizing the sales of batch/unit has been issued to him by or on behalf of the Licensing Authority or the Central license Approving Authority.

(vii) The licensee shall on being informed by the Licensing Authority or the Controlling Authority that any part of any batch/unit of the substance has been found by the Licensing Authority or the Central license Approving Authority not to conform with the standards of strength, quality or purity specified in these Rules and on being directed so to do so, withdraw, from sales and so far as may in the particular circumstances of the case be practicable recall all issues already made from that batch/unit.

(viii) No drug manufactured under the license shall be sold unless the precautions necessary for preserving its properties have been observed throughout the period after manufacture. Further, no batch/unit manufactured under this license shall be supplied/distributed to any person without prescription of Registered Medical Practitioner.

(ix) The licensee shall comply with the provisions of the Act and of these Rules and with such further requirements, if any, as may be specified in any Rules subsequently made under Chapter IV of the Act, provided that where such further requirements are specified in the Rules, these would come in force four months after publication in the Official Gazette.

(x) The licensee shall maintain an Inspection Book in Form 35 to enable an Inspector to record his impressions and defects noticed.

(xi) The licensee shall destroy the stocks of batch/unit which does not comply with standard tests in such a way that it would not spread any disease/infection by way of proper disinfection method.

(xii) All bio-medical waste shall be treated, disposed of or destroyed as per the provisions of The Bio-Medical Wastes (Management and Handling) Rules 1996.

(xiii) The licensee shall neither collect blood from any professional donor or paid donor nor shall he prepare blood components and/or manufacture blood products from the blood drawn from such a donor.

Note: The Bio-Medical Wastes Management Rules have been amended and notified; refer to Section on Biosafety.

Form 26-G
(See Rule 122-F)

CERTIFICATE OF RENEWAL OF license TO OPERATE A BLOOD BANK FOR PROCESSING OF WHOLE HUMAN BLOOD AND/OR* FOR PREPARATION FOR SALE OR DISTRIBUTION OF ITS COMPONENTS

1 Certified that license number _____granted on _____ to M/s _____ _____ for the operation of a Blood Bank for processing of whole blood and / or for preparation of its components at the premises situated at _____ is hereby renewed with effect from _____ to _____.

2 Name (s) of Items :
1.
2.
3.

3. Name(s) of competent Technical Staff :
1.
2.
3.
4.
5.
6.

Dated _____ Signature _____

Name and Designation _____

Licensing Authority

_____

Central license Approving Authority

* delete, whichever is not applicable.

(b) after Form 26-H, the following Form shall be inserted, namely :-

Form 26-I
(See rule 122-I)

CERTIFICATE OF RENEWAL OF license FOR MANUFACTURE OF BLOOD PRODUCTS

1. Certified that license number _____granted on _____ to M/s _____ for manufacture of blood products at the premises situated at _____ is hereby renewed with effect from _____ to _____.

2. Name(s) of item(s) :
   1.
   2.
   3.

3. Names of competent Technical Staff :

(a) responsible for manufacturing	(b) responsible for testing
1.	1.
2.	2.
3.	3.
4.	4.

Signature _____

Name and Designation _____

Licensing Authority_____

Central license Approving Authority _____

(c) for Form 27-C, the following form shall be substituted, namely:-

Form 27-C

(See rule 122-F)

APPLICATION FOR GRANT / RENEWAL * OF license FOR THE OPERATION OF A BLOOD BANK FOR PROCESSING OF WHOLE BLOOD AND/OR* PREPARATION OF BLOOD COMPONENTS

1. I/We _____of M/s_____hereby apply for the grant of license / renewal of license number _____dated _____ to operate a Blood Bank, for processing of whole blood and/or* for preparation of its components on the premises situated at _____.

2. Name(s) of the item(s):
   1.
   2.
   3.

3. The name(s), qualification and experience of competent Technical Staff are as under :
   (a) Name(s) of Medical Officer.

   (b) Name(s) of Technical Supervisor.

   (c) Name(s) of Registered Nurse.

   (d) Name(s) of Blood Bank Technician.

4. The premises and plant are ready for inspection/ will be ready for inspection on_____.
5. A license fee of rupees _____ and an inspection fee of rupees __ _____ has been credited to the Government under the Head of Account _____ (receipt enclosed).

Signature _____

Dated _____ Name and Designation _____

* delete, whichever is not applicable.

Note 1. The application shall be accompanied by a plan of the premises, list of machinery and equipment for collection, processing, storage and testing of whole blood and its components, memorandum of association/ constitution of the firm, copies of certificate relating to educational qualifications and experience of the competent technical staff and documents relating to ownership or tenancy of the premises.

2. A copy of the application together with the relevant enclosures shall also be sent to the Central license Approving Authority and to the concerned Zonal/Sub- Zonal Officers of the Central Drugs Standard Control Organization.

(d) after Form 27-D, the following Form shall be inserted, namely:-

Form 27-E

(See rule 122-F)

APPLICATION FOR GRANT/RENEWAL*OF license TO MANUFACTURE BLOOD PRODUCTS FOR SALE OR DISTRIBUTION

1. I/We _____of M/s_____hereby apply for the grant of license/renewal of license number _____ dated _____ to manufacture blood products on the premises situated at _____

2. Name(s) of item(s) :
   1.
   2.
   3.
   4.

3. The name(s), qualification and experience of competent Technical Staff as under :

(a) responsible for manufacturing	(b) responsible for testing
1.	1.
2.	2.
3.	3.

4. The premises and plant are ready for inspection / will be ready for inspection on_____

5. A license fee of rupees _____and an inspection fee of rupees _____ has been credited to the Government under the Head of Account _____ (receipt enclosed),

Dated _____ Signature _____

Name & Designation _____

* delete, whichever is not applicable.

NOTE 1. The application shall be accompanied by a plan of the premises, list of machinery and equipment for manufacture of blood products, memorandum of association/constitution of the firm, copies of certificate relating to educational qualifications and experience of the competent technical staff and documents relating to ownership or tenancy of the said premises.

2. A copy of the application together with the relevant enclosures shall also be sent to the Central license Approving Authority and to the concerned Zonal / Sub Zonal Officers of the Central Drugs Standard Control Organization.

(e) for Form 28-C, the following Form shall be substituted, namely :-

Form 28-C

(See rule 122-G)

LICENSE TO OPERATE A BLOOD BANK FOR COLLECTION, STORAGE AND PROCESSING OF WHOLE HUMAN BLOOD AND/OR* ITS COMPONENTS FOR SALE OR DISTRIBUTION

1. Number of license _____ date of issue _____at the premises situated at _____

2. M/s _____ is hereby licensed to collect, store, process and distribute whole blood and / or its components.

3. Name(s) of the item(s) :
   1.
   2.
   3.

4. Name(s) of competent Technical Staff :
   1.
   2.
   3.
   4.
   5.
   6.

5. The license authorizes licensee to manufacture, store, sell or distribute the blood products, subject to the conditions applicable to this license.
6. The license shall be in force from _____ to _____
7. The license shall be subject to the conditions stated below and to such other conditions as may be specified from time to time in the Rules made under the Drugs and Cosmetics Act, 1940.

Dated _____

Signature _____

Name and Designation _____

Licensing Authority

_____

Central license Approving Authority

*delete, whichever is not applicable

CONDITIONS OF LICENSE

1. The licensee shall neither collect blood from any professional donor nor paid donor, nor shall he prepare blood components from the blood collected from such a donor.
2. The license and any certificate of renewal in force shall be displayed on the approved premises, and the original shall be produced at the request of an Inspector appointed under the Drugs and Cosmetics Act, 1940.
3. Any change in the technical staff shall be forthwith reported to the Licensing Authority and/or Central license Approving Authority.
4. The licensee shall inform the Licensing Authority and/or Central license Approving Authority in writing in the event of any change in the constitution of the firm operating under the license. Where any change in the constitution of the firm takes place, the current license shall be deemed to be valid for a maximum period of three months from the date on which the change has taken place unless, in the meantime, a fresh license has been taken from the Licensing Authority and/or Central license Approving Authority in the name of the firm with the changed constitution.

(f) after Form 28-D, the following Form shall be inserted, namely :-

Form 28-E

(See rule 122-G)

LICENSE TO MANUFACTURE AND STORE BLOOD PRODUCTS FOR SALE OR DISTRIBUTION

1. Number of license _____ date of issue _____at the premises situated at _____

2. M/s _____is hereby licensed to manufacture, store, sell or distribute the following blood products :-

3. Name(s) of the item(s) :
   1.
   2.
   3.
   4.
   5.

4. Name(s) of competent Technical Staff :

(a) responsible for manufacturing	(b) responsible for testing
1.	1.
2.	2.
3.	3.

5. The license authorizes licensee to manufacture, store, sell or distribute the blood products, subject to the conditions applicable to this license.

6. The license shall be in force from _____ to _____

7. The license shall be subject to the conditions stated below and to such other conditions as may be specified from time to time in the Rules made under the Drugs and Cosmetics Act, 1940.

Dated _____ Signature _____

Name and Designation _____
Licensing Authority

_____
Central license Approving Authority

*delete, whichever is not applicable

CONDITIONS OF LICENSE

1. The licensee shall not manufacture blood products from any professional donor or paid donor.
2. This license and any certificate of renewal in force shall be displayed on the approved premises and the original shall be produced at the request of an Inspector appointed under the Drugs and Cosmetics Act, 1940.
3. Any change in the technical staff shall be forthwith reported to the Licensing Authority and / or Central license Approving Authority.
4. The licensee shall inform the Licensing Authority and/ or Central license Approving Authority in writing in the event of any change in the constitution of the firm, operating under the license. Where any change in the constitution of the firm takes place, the current license shall be deemed to be valid for maximum period of three months from the date on which the change has taken place unless, in the meantime, a fresh license has been taken from the Licensing Authority and/or Central license Approving Authority in the name of the firm with the changed constitution.

PART XII B

REQUIREMENTS FOR THE FUNCTIONING AND OPERATION OF A BLOOD BANK AND / OR FOR PREPARATION OF BLOOD COMPONENTS.

I. BLOOD BANKS / BLOOD COMPONENTS

A. GENERAL

1. Location and Surroundings: The blood bank shall be located at a place that shall be away from open sewage, drain, public lavatory or similar unhygienic surroundings.
2. Building: The building (s) used for the operation of a blood bank and/or preparation of blood components shall be constructed in such a manner so as to permit the operation of the blood bank and preparation of blood components under hygienic conditions and shall avoid the entry of insects, rodents and flies. It shall be well lighted, ventilated and screened (mesh), wherever necessary. The walls and floors of the rooms, where a collection of blood or preparation of blood components or blood products is carried out shall be smooth, washable and capable of being kept clean. Drains shall be of adequate size and where connected directly to a sewer, shall be equipped with traps to prevent back-siphonage.
3. Health, clothing and sanitation of staff: The employees shall be free from contagious or infectious diseases. They shall be provided with clean overalls, head-gears, foot-wears and gloves, wherever required. There shall be adequate, clean and convenient hand washing and toilet facilities.

B. ACCOMMODATION FOR A BLOOD BANK:

A blood bank shall have an area of 100 square meters for its operations and an additional area of 50 square meters for the preparation of blood components. It shall consist of a room each for –

(1) Registration and medical examination with adequate furniture and facilities for registration and selection of donors;
(2) blood collection (air-conditioned);
(3) blood component preparation. (This shall be air-conditioned to maintain a temperature between 20-degree centigrade to 25-degree centigrade);
(4) laboratory for blood group serology. (air-conditioned)
(5) laboratory for blood transmissible diseases like Hepatitis, Syphilis, Malaria, HIV-antibodies (air-conditioned);
(6) sterilization-cum-washing;
(7) refreshment-cum-rest room (air-conditioned);
(8) store-cum-records.

NOTES :

(1) The above requirements as to accommodation and area may be relaxed, in respect of testing laboratories and sterilization-cum-washing room, for reasons to be recorded in writing by the Licensing Authority and/or the Central license Approving Authority, in respect of blood banks operating in Hospitals, provided the hospital concerned has a pathological laboratory and a sterilization-cum-washing room common with other departments in the said hospital.
(2) Refreshments to the donor after phlebotomy shall be served so that he is kept under observation in the Blood Bank.

C. PERSONNEL

Every blood bank shall have the following categories of whole time competent technical staff:-

(a) Medical Officer, possessing the qualifications specified in the condition of rule 122-G.

(b) Blood Bank Technician(s) ,possessing -

(i) Degree in Medical Laboratory Technology (M.L.T.) with six months experience in the testing of blood and/or its components; or

(ii) Diploma in Medical Laboratory Technology (MLT) with oneyear's experience in the testing of blood and/or its components, the degree or diploma being from a University/Institution recognised by the Central Government or State Government.

(c) Registered Nurse(s).

(d) Technical Supervisor(where blood components are manufactured), possessing -

(i) Degree in Medical Laboratory Technology (M.L.T.) with six months› experience in the preparation of blood components; or

(ii) Diploma in Medical Laboratory Technology (M.L.T) with one year's experience in the preparation of blood components, the degree or diploma being from a University/Institution recognised by the Central Government or State Government.

NOTES:

(1) The requirements of qualification and experience in respect of Technical Supervisor and Blood Bank Technician shall apply in the cases of persons who are approved by the Licensing Authority and/or Central license Approving Authority after the commencement of the Drugs and Cosmetics(Amendment) Rules, 1999.

(2) As regards the number of whole-time competent technical personnel, the blood bank shall comply with the requirements laid down in the Directorate General of Health Services Manual.

(3) It shall be the responsibility of the licensee to ensure thorough maintenance of records and other latest techniques used in the blood banking system that the personnel involved in blood banking activities for collection, storage, testing and distribution are adequately trained in the Current Good Manufacturing Practices/Standard Operating Procedures for the tasks undertaken by each personnel. The personnel shall be made aware of the principles of Good Manufacturing Practices/Standard operating Procedures that affect them and receive initial and continuing training relevant to their needs.

D. MAINTENANCE

The premises shall be maintained in a clean and proper manner to ensure adequate cleaning and maintenance of proper operations. The facilities shall include –

(1) Privacy and thorough examination of individuals to determine their suitability as donors.

(2) Collection of blood from donors with minimal risk of contamination or exposure to activities and equipment are unrelated to blood collection.

(3) Storage of blood or blood components pending completion of tests.

(4) Provision for quarantine, storage of blood and blood components in a designated location, pending repetition of those tests that initially give questionable serological results.

(5) Provision for quarantine, storage, handling and disposal of products and reagents not suitable for use.

(6) Storage of finished products prior to distribution or issue.

(7) Proper collection, processing, compatibility testing, storage and distribution of blood and blood components to prevent contamination.

(8) Adequate and proper performance of all procedures relating to plasmapheresis, plateletpheresis and leucapheresis.

(9) Proper conduct of all packaging, labelling and other finishing operations.

(10) Provision for safe and sanitary disposal of –

(i) Blood and/or blood components not suitable for use, distribution or sale.

(ii) Trash and items used during the collection, processing and compatibility testing of blood and/or blood components.

E. EQUIPMENT:

Equipment used in the collection, processing, testing, storage and sale/distribution of blood and its components shall be maintained in a clean and proper manner and so placed as to facilitate cleaning and maintenance. The equipment shall be observed, standardised and calibrated on a regularly scheduled basis as described in the Standard Operating Procedures Manual and shall operate in the manner for which it was designed so as to ensure compliance with the official requirements (the equipment) as stated below for blood and its components.

Equipment that shall be observed standardised and calibrated with at least the following frequencies:-

	EQUIPMENT	PERFORMANCE	FREQUENCY	FREQUENCY OF CALIBRATION
1.	Temperature recorder	Compare against thermometer	Daily	As often as necessary
2.	Refrigerated centrifuge	Observe speed and temperature	Each day of use	As often as necessary
3.	Haematocrit centrifuge	--	--	Standardise before initial use, after repair or adjustments, and annually.
4.	General lab. centrifuge	--	--	Tachometer. every 6 months,
5.	Automated Blood typing	Observe controls for correct results	Each day of use	---
6.	Haemoglobinometer	Standardise against cyanamethaemo- globulin standard	Each day of use	---
7.	Refractiometer or Urinometer	Standardise against distilled water .	---ditto ---	---
8.	Blood container weighing device	standardise against container of known weight	---ditto --	As often as necessary,
9.	Water Bath	Observe Temperature	---ditto --	----ditto----
10.	Rh view box(wherever necessary)	--ditto --	--ditto--	----ditto----
11.	Autoclave	--ditto --	Each time of use	-- ditto --
12.	Serologic rotators	Observe controls for correct results	Each day of use	speed as often as necessary
13.	Laboratory thermometers	--	--	Before initial use
14.	Electronic thermometers	--	Monthly	--
15.	Blood agitator	Observe the weight of the first container of blood filled for correct results	Each day of use	standardise with a container of known mass or volume before initial use and after repairs or adjustments.

F. SUPPLIES AND REAGENTS:

All supplies and reagents used in the collection, processing, compatibility, testing, storage and distribution of blood and blood components shall be stored at the proper temperature in a safe and hygienic place, in a proper manner and in particular –

(a) All supplies coming and contact with blood and blood components intended for transfusion shall be sterile, pyrogen-free, and shall not interact with the product in such a manner as to have an adverse effect upon the safety, purity, potency or effectiveness of the product.

(b) Supplies and reagents that do not bear an expiry date shall be stored in a manner that the oldest is used first.

(c) Supplies and reagents shall be used in a manner consistent with instructions provided by the manufacturer.

(d) All final containers and closures for blood and blood components not intended for transfusion shall be clean and free of surface solids and other contaminants.

(e) Each blood collecting container and its satellite container(s), if any, shall be examined visually for damage or evidence of contamination prior to its use and immediately after filling. Such examination shall include inspection for breakage of seals, when indicated, and abnormal discolouration. Where any defect is observed, the container shall not be used or, if detected after filling, shall be properly discarded.

(f) Representative samples of each lot of the following reagents and/or solution shall be tested regularly on a scheduled basis by methods described in the Standard Operating Procedures Manual to determine their capacity to perform as required :

Reagents and solutions	Frequency of testing alongwith controls
Anti-human serum	Each day of use
Blood grouping serums	Each day of use
Lectin	Each day of use
Antibody screening and reverse	Each day of use grouping cells
Hepatitis test reagents	Each run
Syphilis serology reagents	Each run
Enzymes	Each day of use
HIV I and II reagents	Each run
Normal saline (LISS and PBS)	Each day of use
Bovine Albumin	Each day of use

G. GOOD MANUFACTURING PRACTICES (GMPs)/STANDARD OPERATING PROCEDURES (SOPs):

Written Standard Operating Procedures shall be maintained and shall include all steps to be followed in the collection, processing, compatibility testing, storage and sale or distribution of blood and/or preparation of blood components for homologous transfusion, autologous transfusion and further manufacturing purposes. Such procedures shall be available to the personnel for use in the concerned areas. The Standard Operating Procedures shall inter alia include :

1. (a) Criteria used to determine donor suitability.
   (b) Methods of performing donor qualifying tests and measurements, Including minimum and maximum values for a test or procedure, when a factor in determining acceptability;
   (c) Solutions and methods used to prepare the site of phlebotomy so as to give maximum assurance of a sterile container of blood;
   (d) Method of accurately relating the product(s) to the donor;
   (e) Blood collection procedure, including in-process precautions taken to measure accurately the quantity of blood drawn from the donor;

(f) methods of component preparation including, any time restrictions for specific steps in processing;

(g) all tests and repeat tests performed on blood and blood components during processing;

(h) pre-transfusion testing, wherever applicable, including precautions to be taken to identify the recipient blood components accurately during processing;

(i) procedures of managing adverse reactions in donor and recipient reactions

(j) storage temperatures and methods of controlling storage temperatures for blood and its components and reagents;

(i) length of expiry dates, if any, assigned for all final products;

(l) criteria for determining whether returned blood is suitable for re-issue;

(m) procedures used for relating a unit of blood or blood component from the donor to its final disposal;

(n) quality control procedures for supplies and reagents employed in blood collection, processing and pre-transfusion testing;

(o) schedules and procedures for equipment maintenance and calibration;

(p) labelling procedures to safeguard its mix-ups, receipt, issue, rejected and in-hand;

(q) procedures of plasmapheresis, plateletphersis and leucapheresis if performed, including precautions to be taken to ensure re-infusion of donor's own cells.

(r) procedures for preparing recovered (salvaged) plasma if performed, including details of the separation, pooling, labelling, storage and distribution.

(s) all records pertinent to the lot or unit maintained pursuant to these regulations shall be reviewed before the release or distribution of a lot or unit of the final product. The review or portions of the review may be performed at appropriate periods during or after blood collection, processing, testing and storage. A thorough investigation, including the conclusions and follow-up, of any unexplained discrepancy or the failure of a lot or unit to meet any of its specifications shall be made and recorded;

2. A licensee may utilise current Standard Operating Procedures, such as the Manuals of the following organizations, so long as such specific procedures are consistent with, and at least as stringent as, the requirements contained in this Part, namely:-

(i) Directorate General of Health Services Manual.

(ii) Other organizations or individual blood bank's manuals, subject to the approval of State Licensing Authority and Central license Approving Authority.

H. CRITERIA FOR BLOOD DONATION:

Conditions for a donation of blood :

(1) General -No person shall donate blood, and no blood bank shall draw blood from a person more than once in three months. The donor shall be in good health, mentally alert and physically fit and shall not be inmates of jail, persons having multiple sex partners and drug addicts. The donors shall fulfil the following requirements, namely:-

(a) The donor shall be in the age group of 18 to 60 years.

(b) The donor shall not be less than 45 kilograms;

(c) Temperature and Pulse of the donor shall be normal;

(d) The systolic and diastolic blood pressures are within normal limits without medication;

(e) Haemoglobin which shall not be less than 12.5 grams;

(f) The donor shall be free from acute respiratory diseases;

(g) The donor shall be free from any skin diseases at the site of phlebotomy ;

(h) The donor shall be free from any disease transmissible by blood transfusion, insofar as can be determined by history and examination indicated above;

(i) The arms and forearms of the donor shall be free from skin punctures or scars indicative of professional blood donors or addiction to self-injected narcotics

(2) Additional qualifications of a donor. -No person shall donate blood, and no blood bank shall draw blood from a donor in the conditions mentioned in column (1) of the Table given below before the expiry of the period of deferment mentioned in column (2) of the said Table.

Table: Deferment of blood donation

CONDITIONS	PERIOD OF DEFERMENT
(1)	(2)
(a) Abortions	6 months
(b) History of Blood transfusion	6 months
(c) Surgery	12 months
(d) Typhoid	12 months after recovery
(e) History of Malaria and duly treated	3 months (endemic) 3 years (non-endemic area)
(f) Tattoo	6 months
(h) Breastfeeding	12 months after delivery
(i) Immunization (Cholera, Typhoid, Diphtheria, Tetanus, Plague, Gammaglobulin)	15 days
(j) Rabies vaccination	1 year after vaccination
(k) History of Hepatitis in family or close contact	12 months
(l) Immunoglobulin	12 months.

(3) No person shall donate blood, and no blood bank shall draw blood from a person suffering from any of the diseases mentioned below, namely .-

a. Cancer
b. Heart disease
c. Abnormal bleeding tendencies
d. Unexplained weight loss
e. Diabetes-controlled on Insulin
f. Hepatitis infection
g. Chronic nephritis
h. Signs and symptoms suggestive of AIDS
i. Liver disease
j. Tuberculosis
k. Polycythemia Vera
l. Asthma
m. Epilepsy
n. Leprosy
o. Schizophrenia
p. Endocrine disorders

I. GENERAL EQUIPMENT AND INSTRUMENTS:

1. For blood collection room:
   (i) Donor beds, chairs and tables: These shall be suitably and comfortably cushioned and shall be of appropriate size.
   (ii) Bedside table.
   (iii) Sphygmomanometer and Stethoscope.
   (iv) Recovery beds for donors.
   (v) Refrigerators, for storing separately tested and untested blood, maintaining the temperature between 2 to 6 degree centigrade with digital dial thermometer, recording thermograph and alarm device, with provision for continuous power supply.
   (vi) Weighing devices for donor and blood containers.

2. For haemoglobin determination :
   (i) Copper sulphate solution (specific gravity 1.053)
   (ii) Sterile lancet and impregnated alcohol swabs.
   (ii) Capillary tube (1.3x1.4x96 mm or pasteur pipettes)
   (iv) Rubber bulbs for capillary tubings.
   (v) Sahli's haemoglobinometer/Colourimetric method.

3. For temperature and pulse determination:
   (i) Clinical thermometers.
   (ii) Watch (fitted with a seconds-hand) and a stop-watch.

4. For blood containers :
   (a) Only disposable PVC blood bags shall be used (closed system) as per the specifications of IP/USP/BP.
   (b) Anti-coagulants: The anti-coagulant solution shall be sterile, pyrogen-free and of the following components that will ensure satisfactory safety and efficacy of the whole blood and/or for all the separated blood components.
   (i) Citrate Phosphate Dextrose Adenine solution (CPDA) or Citrate Phosphate Dextrose Adenine- 1 (CPDA-1) ----14 ml. The solution shall be required for 100 ml of blood.

NOTE 1. (i) In case of single/double/triple/quadruple blood collection bags used for blood component preparations, CPDA blood collection bags may be used.

(ii) Acid Citrate Dextrose solution (A.C.D with Formula-A). I.P. -- 15ml. Solution shall be required for 100ml of blood.

(iii) Additive solutions such as SAGM, ADSOL, NUTRICEL may be used for storing and retaining Red Blood Corpuscles upto 42 days.

NOTE2. The licensee shall ensure that the anti-coagulant solutions are of a licensed manufacturer and the blood bags in which the said solutions are contained have a certificate of analysis of the said manufacturer.

5. Emergency equipment/items.
   (i) Oxygen cylinder with mask, gauge and pressure regulator.
   (ii) 5 percent Glucose or Normal Saline.
   (iii) Disposable sterile syringes and needles of various sizes.

(iv) Disposable sterile I.V. infusion sets.

(v) Ampoules of Adrenaline, Noradrenaline, Mephentin, Betamethasone or Dexamethasone, Metoclopramide injections

(vi) Aspirin.

6. Accessories:

(i) Such as blankets, emesis basins, haemostats, set clamps, sponge forceps, gauze, dressing jars, solution jars, waste cans.

(ii) Medium cotton balls, 1.25 cm. adhesive tapes.

(iii) Denatured spirit, Tincture Iodine, green soap or liquid soap.

(iv) Paper napkins or towels.

(v) Autoclave with temperature and pressure indicator.

(vi) Incinerator

(vii) Stand-by generator.

7. Laboratory equipment:

(i) Refrigerators, for storing diagnostic kits and reagents, maintaining a temperature between 4 to 6-degree centigrade (plus/minus 2-degree centigrade) with digital dial thermometer having provision for continuous power supply.

(ii) Compound Microscope with low and high power objectives.

(iii) Centrifuge Table Model

(iv) Water bath: having a range between 37-degree centigrade to 56-degree centigrade

(v) Rh viewing box in case of slide technique.

(vi) Incubator with thermostatic control.

(vii) Mechanical shakers for serological tests for Syphilis.

(viii) Hand-lens for observing tests conducted in tubes.

(ix) Serological graduated pipettes of various sizes

(x) Pipettes (Pasteur)

(xi) Glass slides

(xii) Test tubes of various sizes/micrometreplates (U or V type)

(xiii) Precipitating tubes 6mmx50mm of different sizes and glass beakers of different sizes

(xiv) Test tube racks of different specifications.

(xv) Interval timer electric or spring-wound.

(xvi) Equipment and materials for cleaning glassware adequately.

(xvii) Insulated containers for transporting blood, between 2-degree centigrade to 10-degree centigrade temperatures, towards and hospitals.

(xviii) Wash bottles

(xix) Filter papers

(xx) Dielectric tube sealer.

(xxi) Plain and EDT A vials

(xxii) Chemical balance (wherever necessary)

(xxiii) ELISA reader with printer, washer and micropipettes.

J. SPECIAL REAGENTS:

(1) Standard blood grouping sera Anti A, Anti B and Anti D with known controls. Rh typing sera shall be in double quantity and each of different brand or if from the same, supplier each supply shall be of different lot numbers.

(2) Reagents for serological tests for syphilis and positive sera for controls.

(3) Anti Human Globulin Serum (Coomb's serum)

(4) Bovine Albumin 22 percent, Enzyme reagents for incomplete antibodies.

(5) ELISA or RPHA test kits for Hepatitis and HIV I & II.

(6) Detergent and other agents for cleaning laboratory glassware.

K. TESTING OF WHOLE BLOOD :

(1) It shall be the responsibility of the licensee to ensure that the whole blood collected, processed and supplied conforms to the standards laid down in the Indian Pharmacopoeia and other tests published, if any, by the Government.

(2) Freedom from HIV antibodies (AIDS) Tests -Every licensee shall get samples of every blood unit tested, before use, for freedom from HIV I and HIV II antibodies either from laboratories specified for the purpose by the Central Government or in his own laboratory. The results of such testing shall be recorded on the label of the container.

(3) Each blood unit shall also be tested for freedom from Hepatitis B surface antigen and Hepatitis C Virus antibody, VDRL and malarial parasite and results of such testing shall be recorded on the label of the container.

NOTE:

(a) Blood samples of donors in a pilot tube and the blood samples of the recipient shall be preserved for 7 days after issue.

(b) The blood intended for transfusion shall not be frozen at any stage.

(c) Blood containers shall not come directly in contact with ice at any stage.

L. RECORDS :

The records which the licensee is required to maintain shall include inter alia the following particulars, namely:-

(1) Blood donor record: It shall indicate serial number, date of bleeding, name, address and signature of donor with other particulars of age, weight, haemoglobin, blood grouping, blood pressure, medical examination, bag number and patient's detail for whom donated in case of replacement donation, category of donation (voluntary/replacement) and deferral records and signature of Medical Officer In-charge.

(2) Master records for blood and its components: It shall indicate bag serial number, date of collection, date of expiry, quantity in ml. ABO/Rh Group results for testing of HIV I and HIV II antibodies, Malaria, V.D.R.L., Hepatitis B surface antigen and Hepatitis C virus antibody and irregular antibodies (if any), name and address of the donor with particulars, utilisation issue number, components prepared or discarded and signature of the Medical Officer Incharge.

(3) Issue register: It shall indicate serial number, date and time of issue, bag serial number, ABO/Rh Group, total quantity in ml, name and address of the recipient, group of recipient, unit/institution, details of cross-matching report, indication for transfusion.

(4) Records of components supplied: quantity supplied; compatibility report, details of recipient and signature of issuing person.

(5) Records of A.C.D./C.P.D/CPD-A/SAGM bags giving details of the manufacturer, batch number, date of supply, and results of testing.

(6) Register for diagnostic kits and reagents used: name of the kits/reagents, details of batch number, date of expiry and date of use.

(7) Blood bank must issue the cross-matching report of the blood to the patient together with the blood unit.

(8) Transfusion adverse reaction records.

(9) Records of purchase, use and stock in hand of disposable needles, syringes, blood bags shall be maintained.

NOTE: The above-said records shall be kept by the licensee for a period of five years.

M. LABELS:

The labels on every bag containing blood and/or component shall contain the following particulars, namely:

(1) The proper name of the product in a prominent place and in bold letters on the bag.

(2) Name and address of the blood bank

(3) license number

(4) Serial number

(5) The date on which the blood is drawn and the date of expiry as prescribed under Schedule P to these rules.

(6) A coloured label shall be put on every bag containing blood. The following colour scheme for the said labels shall be used for different groups of blood:

Blood Group	Colour of the label
O	Blue
A	Yellow
B	Pink
AB	White

(7) The results of the tests for Hepatitis B surface antigen, and Hepatitis C virus antibody, syphilis, freedom from HIV I and HIV II antibodies and malarial parasite.

(8) The Rh group.

(9) Total volume of blood, the preparation of blood, nature and percentage of anti-coagulant.

(10) Keep temperature continuously at 2-degree centigrade to 6 degrees centigrade for whole human blood and/or components as contained under III of Part XII B.

(11) Disposable transfusion sets with filter shall be used in administration equipment.

(12) Appropriate compatible cross-matched blood without a typical antibody in the recipient shall be used.

(13) The contents of the bag shall not be used if there is any visible evidence of deterioration, like, haemolysis, clotting or discolouration.

(14) The label shall indicate the appropriate donor classification like “voluntary donor” or “replacement donor” in no less prominence than the proper name.

NOTES:

1. In the case of blood components, particulars of the blood from which such components have been prepared shall be given against item numbers (5), (7),(8), (9) and (14).
2. The blood and/or its components shall be distributed on the prescription of a Registered Medical Practitioner.

II. BLOOD DONATION CAMPS.

A blood donation camp may be organized by -

(a) a licensed designated Regional Blood Transfusion Centre; or

(b) a licensed Government blood bank; or

(c) the Indian Red Cross Society; or

(d) a licensed blood bank run by registered voluntary or charitable organizations recognized by State or Union Territory Blood Transfusion Council.

NOTE:

(i) Designated Regional Blood Transfusion Centre» shall be a centre approved and designated by a Blood Transfusion Council constituted by a State Government to collect, process and distribute blood and its components to cater to the needs of the region and that centre has also been licensed and approved by the Licensing Authority and Central license Approving Authority for the purpose.

(ii) The designated Regional Blood Transfusion Centre, The Government blood bank and Indian Red Cross Society shall intimate within a period of seven days the venue where blood camp was held and details of group-wise blood units collected in the said camp to the licensing Authority and Central license Approving Authority.

For holding a blood donation camp, the following requirements shall be fulfilled/complied with, namely:-

(A) PREMISES, PERSONNEL ETC.

(a) Premises under the blood donation camp shall have sufficient area, and the location shall be hygienic so as to allow proper operation, maintenance and cleaning.

(b) All information regarding the personnel working, equipment used and facilities available at such a Camp shall be well documented and made available for inspection, if required, and ensuring—

(i) continuous and uninterrupted electrical supply for equipment used in the Camp;

(ii) adequate lighting for all the required activities;

(iii) hand-washing facilities for staff;

(iv) reliable communication system to the central office of the Controller/Organiser of the Camp;

(v) furniture and equipment arranged within the available place;

(vj) refreshment facilities for donors and staff;

(vii) facilities for medical examination of the donors;

(viii) proper disposal of waste.

(B) PERSONNEL FOR OUT-DOOR BLOOD DONATION CAMP:

To collect blood from 50 to 70 donors in about 3 hours or from 100 to 120 donors in 5 hours, the following requirements shall be fulfilled/complied with:-

(i) One Medical Officer and two nurses or phlebotomists for managing 6-8 donor tables;

(ii) two medico-social workers;

(iii) three blood bank technicians;

(iv) two attendants;

(v) vehicle having a capacity to seat 8-10 persons, with provision for the carriage of donation goods including facilities to conduct a blood donation camp.

(C) EQUIPMENT:

1. BP apparatus.
2. Stethoscope.
3. Blood bags (single, double, triple, quadruple)
4. Donor questionnaire.
5. Weighing device for donors.
6. Weighing device for blood bags,
7. Artery forceps, scissors.
8. Stripper for blood tubing.

9. Bedsheets, blankets/mattress.
10. Lancets, swab stick/toothpicks.
11. Glass slides.
12. Portable Hb meter/copper sulphate.
13. Test tube (big) and 12x100 mm (small)
14. Test tube stand.
15. Anti-A, Anti-B and Anti.AB, Antisera and Anti-D
16. Test tube sealer film.
17. Medicated adhesive tape.
18. Plastic wastebasket
19. Donor cards and refreshment for donors.
20. Emergency medical kit
21. Insulated blood bag containers with provisions for storing between 2 degree centigrade to 10-degree centigrade.
22. Dielectric sealer or portable sealer
23. Needle destroyer (wherever necessary)

III. PROCESSING OF BLOOD COMPONENTS FROM WHOLE BLOOD BY A BLOOD BANK

The Blood components shall be prepared by blood banks as a part of the Blood Bank services. The conditions for grant or renewal of a license to prepare blood components shall be as follows: -

(A) ACCOMMODATION :

(1) Rooms with adequate area and other specifications for preparing blood components depending on the quantum of workload shall be as specified in item B under the heading "I. BLOOD BANKS/BLOOD COMPONENTS" of this Part.

(2) Preparation of Blood components shall be carried out only under a closed system using single, double, triple or quadruple plastic bags except for preparation of Red Blood Cells Concentrates, where single bags may be used with transfer bags.

(B) EQUIPMENT :

(i) Air conditioner;
(ii) Laminar air flow bench;
(iii) Suitable refrigerated centrifuge;
(iv) Plasma expresser;
(v) Clipper and clips and or dielectric sealer;
(vi) Weighing device;
(vii) Dry rubber balancing material;
(viii) Artery forceps, scissors;
(ix) Refrigerator maintaining a temperature between 2-degree centigrade to 6-degree centigrade, a digital dial thermometer with recording thermograph and alarm device, with provision for continuous power supply;
(x) Platelet agitator with incubator (wherever necessary)
(xi) Deep freezers maintaining a temperature between minus 30-degree centigrade to minus 40-degree centigrade and minus 75-degree centigrade to minus 80-degree centigrade;
(xii) Refrigerated Water bath for Plasma Thawing;
(xiii) Insulated blood bag containers with provisions for storing at the appropriate temperature for transport purposes:

(C) PERSONNEL:

The whole time competent technical staff meant for processing of Blood Components (that is Medical Officer, Technical Supervisor, Blood Bank Technician and Registered Nurse) shall be as specified in item C, under the heading "I. BLOOD BANKS/BLOOD COMPONENTS" of this Part.

(D) TESTING FACILITIES:

General: Facilities for A,B, AB and O groups and Rh(D) grouping.

Hepatitis: B Surface antigen and Hepatitis C virus antibody, VDRL, HIV I and HIV II antibodies and malarial parasites shall be mandatory for every blood unit before it is used for the preparation of blood components. The results of such testing shall be indicated on the label.

(E) CATEGORIES OF BLOOD COMPONENTS:

(1) **CONCENTRATED HUMAN RED BLOOD CORPUSCLES:** The product shall be known as "Packed Red Blood Cells" that is Packed Red Blood Cells remaining after separating plasma from human blood.

General Requirements :

(a) Storage: Immediately after processing, the Packed Red Blood Cells shall be kept at a temperature maintained between 2-degree centigrade to 6-degree centigrade.

(b) Inspection: The component shall be inspected immediately after separation of the plasma, during storage and again at the time of issue. The product shall not be issued if there is any abnormality in colour or physical appearance or any indication of microbial contamination.

(c) Suitability of Donor: The source of blood for Packed Red Blood Cells shall be obtained from a donor who meets the criteria for Blood Donation as specified in item H under the heading "I. BLOOD BANKS/ BLOOD COMPONENTS" of this Part.

(d) Testing of Whole Blood: Blood from which Packed Red Blood Cells are prepared shall be tested as specified in item K relating to Testing of Whole Blood under the heading "I. BLOOD BANKS/BLOOD COMPONENTS" of this Part.

(e) Pilot samples: Pilot samples collected in integral tubing or in separate pilot tubes shall meet the following specifications:

(i) One or more pilot samples of either the original blood or of the Packed Red Blood Cells being processed shall be preserved with each unit of Packed Red Blood Cells which is issued.

(ii) Before they are filled, all pilot sample tubes shall be marked or identified so as to relate them to the donor of that unit or Packed Red Blood Cells.

(iii) Before the final container is filled or at the time the final product is prepared, the pilot sample tubes accompanying a unit of Packed Red Blood Cells shall be attached in a tamper-proof manner that shall conspicuously identify removal and re-attachment.

(iv) All pilot sample tubes, accompanying a unit of packed red blood cells, shall be filled immediately after the blood is collected or at the time the final product is prepared, in each case, by the person who performs the collection of preparation.

(F) PROCESSING :

(i) Separation: Packed Red Blood Cells shall be separated from the whole blood-

(a) if the whole blood is stored in ACD solution within 21days, and

(b) if the whole blood is stored in CPDA-1 solution, within 35 days, from the date of collection. Packed Red Blood Cells may be prepared either by centrifugation done in a manner that shall not tend to increase the temperature of the blood or by normal undisturbed sedimentation method. A portion of the plasma, sufficient to ensure optimal cell preservation, shall be left with the Packed Red Blood Cells.

(ii) Packed Red Blood Cells Frozen: Cryophylactic substance may be added to the Packed Red Blood Cells for extended manufacturer's storage not warmer than minus 65-degree centigrade provided the manufacturer submits data to the satisfaction of the Licensing Authority and Central license Approving

Authority, as adequately demonstrating through in-vivo cells survival and other appropriate tests that the addition of the substance, the material used and the processing methods results in a final product meets the required standards of safety, purity and potency for Packed Red Blood Cells and that the frozen product shall maintain those properties for the specified expiry period.

(iii) Testing: Packed Red Blood Cells shall conform to the standards as laid down in the Indian Pharmacopoeia.

(2) PLATELETS CONCENTRATES:

The product shall be known as "Platelets Concentrates" that is, platelets collected from one unit of blood and re-suspended in an appropriate volume of original plasma.

General Requirements :

(i) Source:

The source material for platelets shall be platelet-rich plasma or buffy coat which may be obtained from the whole blood or by plateletpheresis.

(ii) Processing:

(a) Separation of buffy-coat or platelet-rich plasma and platelets and re-suspension of the platelets shall be in a closed system by-centrifugal method with appropriate speed, force and time.

(b) Immediately after collection, the whole blood or plasma shall be held in storage between 20-degree centigrade to 24-degree centigrade. When it is to be transported from the venue of blood collection to the processing laboratory, during such transport action, the temperature as close as possible to a range between 20-degree centigrade to 24-degree centigrade shall be ensured. The platelet shall be separated within 6 hours after the time of collection of the unit of whole blood or plasma.

(c) The time and speed of centrifugation shall be demonstrated to produce an unclamped product, without visible haemolysis, that yields a count of not less than $3.5x10^{10}$ (3.5x10 raised to the power of 10) and $4.5x10^{10}$(4.5x10 raised to the power ten), i.e. platelets per unit from a unit of 350 ml and 450 ml blood respectively. One percent of total platelets prepared shall be tested, of which 75 percent of the units shall conform to the above said platelet count.

(d) The volume of original plasma used for re-suspension of the platelets shall be determined by the maintenance of the pH of not less than 6 during the storage period. The pH shall be measured on a sample of platelets that have been stored for the permissible maximum expiry period at 20-degree centigrade to 24-degree centigrade.

(d) Final containers used for platelets shall be colourless and transparent to permit visual inspection of the contents. The caps selected shall maintain a hermetic seal to prevent contamination of the contents. The container material shall not interact with the contents, under the normal conditions of the storage and use, in such a manner as to have an adverse effect upon the safety, purity, potency, or efficacy of the product. At the time of filling, the final container shall be marked or identified by number so as to relate it to the donor.

(iii) Storage:

Immediately after re-suspension, platelets shall be placed in storage not exceeding for a period 5 days, between 20 degrees centigrade to 24-degree centigrade, with continuous gentle agitation of the platelet concentrates maintained throughout such storage.

(iv) Testing:

The units prepared from different donors shall be tested at the end of the storage period for -

(a) Platelet count;

(b) the pH of not less than 6 measured at the storage temperature of the unit;

(c) measurement of actual plasma volume;

(d) one percent of the total platelets prepared shall be tested for sterility;

(e) the tests for functional viability of the platelets shall be done by swirling movement before issue;

(f) If the testing results indicate that the product does not meet the specified requirements, immediate corrective action shall be taken and records maintained.

(v) Compatibility Test:

Compatible transfusion for a variable number of Red Blood Cells, A, B, AB and O grouping shall be done if the platelets concentrate is contaminated with red blood cells.

(3) GRANULOCYTE CONCENTRATES:

(i) Storage: It shall be kept between 20-degree centigrade to 24-degree centigrade for a maximum period of 24 hours.

(ii) Unit of granulocytes shall not be less than 1 $x10^{10}$ (i.e. 1x10 raised to the power of 10) when prepared on cell separator.

(iii) Group-specific tests/HLA test wherever required shall be carried out.

(4) FRESH FROZEN PLASMA:

Plasma is frozen within 6 hours after blood collection and stored at a temperature no warmer than minus 30-degree centigrade shall be preserved for a period of not more than one year.

(5) CRYOPRECIPITATE:

Concentrate of anti-haemophiliac factor shall be prepared by the thawing of the fresh plasma frozen stored at minus 30-degree centigrade.

(a) Storage:

Cryoprecipitate shall be preserved at a temperature not higher than minus 30-degree centigrade and may be preserved for not more than one year from the date of collection.

(b) Activity:

Anti-haemophiliac factor activity in the final product shall be not less than 80 units per bag. One per-cent of the total cryoprecipitate prepared shall be tested, of which seventy-five percent of the unit shall conform to the said specification.

(6) PLASMAPHERESIS, PLATELETPHERESIS, LEUCAPHERESIS USING A CELL SEPARATOR.

An area of 10 square meters shall be provided for apheresis in the Blood Bank. The blood banks specifically permitted to undertake the said apheresis on the donor shall observe the criteria as specified in item H relating to Criteria for blood donation under the heading “I. Blood Banks/Blood Components” of this Part. The written consent of the donor shall be taken, and the donor must be explained the hazards of apheresis. The Medical Officer shall certify that donor is fit for apheresis, and it shall be carried out by a trained person under the supervision of the Medical Officer.

(A) PLASMAPHERESIS, PLATELET PHERESIS AND LEUCAPHERESIS:

The donors subjected to plasmapheresis, plateletpheresis and leucopheresis shall, in addition to the criteria specified in item H relating to the CRITERIA FOR BLOOD DONATION, under the heading “I. BLOOD BANKS/ BLOOD COMPONENTS” of this Part being observed, be also subjected to protein estimation on post-pheresis/ first sitting whose results shall be taken as a reference for subsequent Pheresis/Sitting. It shall also be necessary that the total plasma obtained from such donor and periodicity of Plasmapheresis shall be according to the standards described under validated Standard Operating Procedures.

NOTE:

(i) At least 48 hours must elapse between successive apheresis and not more than twice a week.

(ii) Extracoporeal blood volume shall not exceed 15% of the donor’s estimated blood Volume.

(iii) Platelet pheresis shall not be carried out on donors who have taken medication containing Asprin within 3 days prior to donation.

(iv) If during plateletpheresis or leucapheresis, RBCs cannot be re-transfused, then at least 12 weeks shall elapse before a second cytapheresis procedure is conducted.

(B) MONITORING FOR APHERESIS:

Before starting apheresis procedure, haemoglobin or haematocrit shall be done. Platelet count, WBC counts, the differential count may be carried out. In repeated plasmapheresis, the serum protein shall be 6 gm /100 ml.

(C) COLLECTION OF PLASMA:

The quantity of plasma separated from the blood of a donor shall not exceed 500 ml per sitting and once in a fortnight or shall not exceed 1000 ml per month.

PART XII C

I. REQUIREMENTS FOR MANUFACTURE OF BLOOD PRODUCTS

The blood products shall be manufactured in separate premises other than that meant for the blood bank. The requirements that are essential for grant or renewal of a license to manufacture blood products such as Albumin, Plasma Protein Fraction, Immunoglobins and Coagulation Factor Concentrates, shall be as follows, namely:-

A. GENERAL REQUIREMENTS

1. Location and surroundings, buildings and water supply:

   The requirements as regards location and surrounding buildings and water supply as contained in paragraphs 1.1.1, 1.1.2, 1.1.3 of Part I of Schedule M shall apply mutatis mutandis to the manufacture of blood products.

2. Disposal of waste and infectious materials:

   (i) The requirement as regards disposal of waste and infectious materials as contained in paragraph 1.1.4 of Part I of Schedule M shall apply mutatis mutandis to the manufacture of blood products.

   (ii) Proper facility shall also be provided for potentially infectious materials, particularly HIV I & HIV II, Hepatitis B (surface antigen and Hepatitis C virus antibody) through autoclaving, incineration or any other suitable validated methods.

3. Health, clothing and sanitation of personnel:

   (i) The requirement is contained in paragraph 3 of Part I of Schedule M shall be complied with.

   (ii) The personnel working in the manufacturing areas shall be vaccinated against the Hepatitis B virus and other infectious transmitting diseases.

4. Requirements for manufacturing area for Blood Products:

   (i) For the manufacture of blood products, separate enclosed areas specifically designed for the purpose shall be provided. These areas are provided with airlocks for entry and shall be essentially dust free and ventilated with an air supply. Air supply for the manufacturing area shall be filtered through bacteria retaining filters (HEPA Filters) and shall be at a pressure higher than in the adjacent areas.

   The filters shall be checked for performance on installation and periodically thereafter, and records thereof shall be maintained.

   (ii) Interior surfaces (walls, floors and ceilings) shall be smooth and free from cracks;they shall not shed matter and shall permit easy cleaning and disinfection. Drains shall be excluded from aseptic areas.

   Routine microbial counts of the manufacturing area shall be carried out during manufacturing operations. The results of such counts shall be checked against well documented in-house standards and records maintained.

   Access to the manufacturing areas shall be restricted to a minimum number of authorised personnel. In addition, special procedures for entering and leaving the manufacturing areas shall be prominently

displayed.

(iii) Sinks shall be excluded from aseptic areas. Any sink installed in other clean areas shall be of a suitable material such as stainless steel, without an overflow, and be supplied with water of potable quality. Adequate precautions shall be taken to avoid contamination of the drainage system with dangerous effluents and airborne dissemination of pathogenic microorganisms.

(iv) Lighting, air-conditioning, ventilation shall be designed to maintain a satisfactory temperature and relative humidity to minimise contamination and to take account of the comfort of personnel working with protective clothing.

(v) Premises used to manufacture blood products shall be suitably designed and constructed to facilitate good sanitation.

(vi) Premises shall be carefully maintained, and it shall be ensured that repair and maintenance operations do not present any hazard to the quality of products. Premises shall be cleaned and, where applicable, disinfected according to detailed written validated procedures.

(vii) Adequate facilities and equipment shall be used for the manufacture of blood products derived from blood plasma.

(viii) All containers of blood products, regardless of the stage of manufacture, shall be identified by securely attached labels. Cross-contamination shall be prevented by the adoption of the following measures, namely:-

(a) processing and filling shall be in segregated L areas ;

(b) manufacture of different products at the same time shall be avoided;

(c) simultaneous filling of the different products shall be avoided;

(d) ensure transfer, containers/materials using airlocks, air extraction, clothing change and careful washing and decontamination of equipment;

(e) protecting containers/materials against the risk of contamination caused by re-circulation of untreated air or by accidental re-entry of extracted air;

(f) using containers that are sterilised or are of documented low “bioburden”.

(ix) Positive pressure area shall be dedicated to the processing area concerned;

(x) Air-handling units shall be dedicated to the processing area concerned;

(xi) Pipework, valves and vent filters shall be properly designed to facilitate cleaning and sterilisation. Valves on fractionation / reacting vessels shall be completely steam-sterilisable. Air vent filters shall be hydrophobic and shall be validated for their designated use.

5. Ancillary Areas:

(i) Rest and refreshment rooms shall be separated from other areas.

(ii) Facilities for changing and storing clothes and for washing and toilet purposes shall be easily accessible and appropriate for the number of users. Toilets shall not be connected directly with production or storage areas.

(iii) Maintenance workshops shall be separated from production areas. Wherever parts and tools are stored in the production area, they shall be kept in rooms or lockers reserved for that use.

(iv) Animal houses shall be well isolated from other areas, with separate entrance.

B. COLLECTION AND STORAGE OF PLASMA FOR FRACTIONATION:

(a) Collection :

(1) Plasma shall be collected from the licensed Blood Banks through a cold chain process and stored in a frozen condition not warmer than minus twenty-degree centigrade;

(2) Individual plasma shall remain in quarantine till it is tested for Hepatitis B surface antigen and Hepatitis C virus antibody HIV I and HIV II.

(3) A sample from pooled -lot plasma of about 10-12 units of different donors shall be tested for Hepatitis B surface antigen and Hepatitis C virus antibody, HIV I and HIV II and if the sample found negative, only then it shall be taken up for fractionation.

(b) Storage Area :

(1) Storage areas shall be of sufficient space and capacity to allow orderly storage of the various categories of materials, intermediates, bulk and finished products, products in quarantine, released, rejected, returned, or recalled products.

(2) Storage areas shall be designed or adapted to ensure good storage conditions. In particular, they shall be clean, dry and maintained within the temperature required for such storage, and where special storage conditions are required (e.g. temperature, humidity), these shall be provided, checked and monitored.

(3) Receiving and dispatch bays shall protect materials and products from the weather and shall be designed and equipped to allow containers of incoming materials to be cleaned, if necessary, before storage.

(4) Where quarantine status is ensured by storage in separate areas, these areas shall be clearly marked, and their access restricted only to authorised personnel.

(5) There shall be a separate sampling area for raw materials. If sampling is performed in the storage area, it shall be conducted in such a way so as to prevent contamination or cross-contamination.

(6) Segregation shall be provided for the storage of rejected, recalled, or returned materials or products.

(7) Adequate facility shall be provided for the supply of ancillary material, such as ethanol, water, salts and polyethene glycol. Separate facilities shall be provided for the recovery of organic solvents used in fractionation.

C. PERSONNEL:

(1) Manufacture:

The manufacture of blood products shall be conducted under the active direction and personal supervision of competent technical staff, consisting of at least one person who shall be a whole-time employee, with one-year practical experience in the manufacture of blood products/plasma fractionation and possesses –

(a) Post-graduate degree in Medicine -M.D. (Microbiology/ Pathology/ Bacteriology / Immunology / Biochemistry); or

(b) Post-graduate degree in Science (Microbiology); or

(c) Post-graduate degree in Pharmacy (Microbiology), from a recognised University or Institution.

(2) Testing:

The head of the testing unit shall be independent of the manufacturing unit, and testing shall be conducted under the active direction and personal supervision of competent technical staff consisting of at least one person who shall be a whole-time employee. The Head of the testing unit shall have eighteen months of practical experience in the testing of drugs, especially the blood products, and possesses –

(a) Post-graduate degree in Pharmacy or Science - (Chemistry/Microbiology/Biochemistry); or

(b) Post-graduate degree in Medicine-M.D. (Microbiology /Pathology / Biochemistry),from a recognised University or Institution.

D PRODUCTION CONTROL :

(1) The production area and the viral inactivation room shall be centrally air-conditioned and fitted with HEPA Filters having Grade C (Class 10,000) environment as given in the table below.

(2) The filling and sealing shall be carried out under aseptic conditions in centrally air-conditioned areas fitted with HEPA Filters having Grade A or, as the case may be, grade B (Class 100) environment given in the said Table

TABLE AIR CLASSIFICATION SYSTEM FOR MANUFACTURE OF STERILE PRODUCTS.

Maximum number of particles permitted per m^3

	MAXIMUM NUMBER OF PARTICLES PERMITTED PER m^3		MAXIMUM NUMBER OF VIABLE MICROORGANISM PERMITTED PER m^3
GRADE	0.5 -5 micron	Less than 5 micron	
A (Class 100) (Laminar-Airflow workstation)	3500	None	Less than 1
B (Class 100)	3500	None	Less than 5
C (Class 10000)	350000	2000	Less Than 100

(3) The physical and chemical operations used for the manufacture of plasma fractionation shall maintain a high yield of safe and effective protein.

(4) The fractionation procedure used shall give a good yield of products meeting the in-house quality requirements as approved by the Licensing Authority and Central license Approving Authority, reducing the risk of microbiological contamination and protein denaturation to the minimum.

(5) The procedure adopted shall not affect the antibody activity and biological half-life or biological characteristics of the products.

E. VIRAL INACTIVATION PROCESS:

The procedure used by the licensee to inactivate the pathogenic organisms such as enveloped and non-enveloped virus, especially infectivity from HIV I & HIV II, Hepatitis B surface antigens and Hepatitis C virus antibody the viral inactivation and validation methods adopted by the licensee, shall be submitted for approval to the Licensing Authority and Central license Approving Authority

NOTES:

(1) No preservative (except stabiliser to prevent –protein denaturation such as glycine, sodium chloride or sodium caprylate) shall be added to Albumin, Plasma Protein Fraction, Intravenous Immunoglobulins or Coagulation Factor Concentrates without the prior approval of Licensing Authority and Central license Approving Authority.

(2) The licensee shall ensure that the said stabilisers do not have a deleterious effect on the final product in quantity present to not cause any untoward or adverse reaction in human beings.

F. QUALITY CONTROL :

Separate facilities shall be provided for Quality Control such as Hematological, Bio-chemical, Physico-chemical, Microbiological, Pyrogens, Instrumental and Safety testing. The Quality Control Department shall have inter alia the following principal duties, namely.-

(1) To prepare detailed instructions in writing for carrying out test and analysis.

(2) To approve or reject raw material, components—containers, closures, in-process materials, packaging material, labelling and finished products.

(3) To release or reject a batch of finished products which are ready for distribution.

(4) To evaluate the adequacy of the conditions under which raw materials, semi-finished products and finished products are stored.

(5) To evaluate the quality and stability of finished products and, when necessary, of raw materials and semi-finished products.

(6) To review production records to ensure that no errors have occurred or if errors have occurred that they have been fully investigated.

(7) To approve or reject all procedures or specifications impacting the produc's identity, strength, quality, and purity.

(8) To establish shelf-life and storage requirements based on stability tests related to storage conditions.

(9) To establish and, when necessary revise, control procedures and specifications.

(10) To review complaints, recalls, returned or salvaged products and investigations conducted there under for each product.

(11) To review Master Formula Records/Cards periodically.

G. TESTING OF BLOOD PRODUCTS:

The products manufactured shall conform to the standards specified in the Indian Pharmacopoeia, and where the standard of any product is not specified in the Pharmacopoeia, the standard for such product shall conform to the standard specified in the United States Pharmacopoeia or the British Pharmacopoeia. The final products shall be tested for freedom from HIV I and HIV II antibodies, Hepatitis B surface antigen and Hepatitis C virus antibody.

H. STORAGE OF FINISHED PRODUCT:

(i) The final products shall be stored between two-degree centigrade to eight-degree centigrade unless otherwise specified by the Central license Approving Authority.

(ii) The shelf-life assigned to the products by the licensee shall be submitted for approval to the Licensing Authority and Central license Approving Authority.

I. LABELLING:

The products manufactured shall be labelled as specified in the Indian Pharmacopoeia, the British Pharmacopoeia ofthe United States Pharmacopoeia, which shall be in addition to any other requirement stated under Part IX or Part X of these rules. The labels shall indicate the results of tests for Hepatitis B surface antigen and Hepatitis C Virus antibody, freedom from HIV I and HIV II antibodies.

J. RECORDS:

The licensee shall maintain records as per Schedule U and also comply with Batch manufacturing records as specified in Paragraph 9 of Part I of Schedule M and any other requirement as may be directed by Licensing Authority and Central license Approving Authority.

K. MASTER FORMULA RECORDS:

The licensee shall maintain Master Formula Records relating to all manufacturing and quality control procedures for each product, which shall be prepared and endorsed by the competent Technical Staff, i.e., Head of the manufacturing unit. The Master Formula Records shall contain --

(i) the patent or proprietary name of the product alongwith the generic name, if any, strength and the dosage form;

(ii) a description or identification of the final containers, packaging materials, labels and closures to be used;

(iii) the identity, quantity and quality of each raw material to be used irrespective of whether or not it appears in the finished product. The permissible overage that may be included in a formulated batch shall be indicated;

(iv) a description of all vessels and equipment and the sizes used in the process;

(v) manufacturing and control instructions along with parameters for critical steps such as mixing, drying, blending, sieving and sterilising the product;

(vi) the theoretical yield to be expected from the formulation at different stages of manufacture and permissible yield limits;

(vii) detailed instructions on precautions to be taken in the manufacture and storage of drugs and of semi-finished products; and

(viii) the requirements in-process quality-control tests and analysis to be carried out during each stage of manufacture, including the designation of persons or departments responsible for the execution of such tests and analysis.

II. REQUIREMENTS FOR MANUFACTURE OF BLOOD PRODUCTS FROM BULK FINISHED PRODUCTS

Where the blood products, such as Albumin, Plasma Protein Fraction, Immunoglobulins and Coagulation Factor Concentrates are manufactured through the manufacturing activities of filling and sealing the blood products from bulk powder or solution or both, the requirements as they apply to the manufacture of blood products from whole blood shall apply mutatis mutandis to such manufacture of blood products, unless other requirements have been approved by the Central license Approving Authority.

GUIDELINES FOR APPROVAL OF BLOOD AND/OR ITS COMPONENTS TO STORAGE CENTRES AND FIRST REFERRAL UNIT, COMMUNITY HEALTH CENTRE, PRIMARY HEALTH CENTRE OR ANY HOSPITAL

Ministry of Health & Family Welfare (Deptt. of Health) vide Notification No. GSR 909(E) dated 20th December 2001 exempted blood storage centres run by FRU, Community Health Centre, PHC or any hospital from the purview of obtaining a license for operation. This notification has been inserted under Schedule K of Drugs & Cosmetics rules, 1945 under serial no. 5B. The main aim of this notification is to make abundant availability of whole human blood or its components to the said hospitals without taking license. However, this exemption applies to those centres which are transfusing blood and/or its components less than 2000 units per annum.

To ensure the safety and quality of blood and/or its components to be stored in such blood storage centres, the following conditions are applicable before getting exemption from the purview of taking of a license from the respective State Drugs Controllers:-

"5B. Whole Human Blood I.P. and/or its components stored for transfusion by a First Referral Unit, Community Health Centre, Primary Health Centre and a Hospital

The provisions of Chapter IV of the Act and the Rules made there under which require obtaining of a license for operation of a blood bank or processing Whole Human Blood and/or its components, subject to the following conditions, namely:-

(1) The First Referral Unit, Community Health Centre, Primary Health Centre and/or any Hospital shall be approved by the State / Union Territory Licensing Authority after satisfying the conditions and facilities through inspection.

(2) The captive consumption or Whole Human Blood I.P. or its components in the First Referral Unit, Community Health Centre, Primary Health Centre and/or any Hospital shall not be more than 2000 units annually.

(3) The Whole Human Blood and/or its components shall be procured only from Government Blood Bank and/or Indian Red Cross Society Blood Bank and/or Regional Blood Transfusion Centre duly licensed.

(4) The approval shall be valid for a period of two years from the date of issue unless sooner suspended or cancelled, and First Referral Unit, Community Health Centre, Primary Health Centre or the Hospital shall apply for renewal to the State Licensing Authority three months prior to the date of expiry of the approval.

(5) The First Referral Unit, Community Health Centre, Primary Health Centre and/or any Hospital shall have the following technical staff for storage of blood or its components:-

  (a) A trained Medical Officer for proper procurement, storage and cross-matching of blood and/or its components. He/she shall also be responsible for identifying haemolysed blood and ensure non-supply of date expired blood or its components.

  (b) A blood bank Technician with the qualification and experience as specified in Part XII B of Schedule F or an experienced laboratory technician trained in blood grouping and cross-matching.

(5) The First Referral Unit, Community Health Centre, Primary Health Centre and Hospital shall have an area of 10 sq. metres. It shall be well lighted, clean and preferably air-conditioned. Blood bank refrigerator of appropriate capacity fitted with alarm device and temperature indicator with regular temperature monitoring shall be provided to store blood units between 2°C to 8°C, and if the components are proposed to be stored, specified equipment as specified in Part XII B of Schedule F shall also be provided.

(6) The First Referral Unit, Community Health Centre, Primary Health Centre and Hospital shall maintain records and registers, including details of procurements of Whole Human Blood I.P. and/or blood components, as required under Part XII B of Schedule F.

(7) The First Referral Unit, Community Health Centre, Primary Health Centre and Hospital shall store samples of donors blood as well as patients sera for seven days after transfusion."

[No. X – 11014/3/2001-DMS & PFA] (DEEPAK GUPTA)
JOINT SECRETARY OF SECRETARY OF INDIA

GUIDELINES BEFORE GRANT OF APPROVAL FOR OPERATION OF WHOLE HUMAN BLOOD AND/OR ITS COMPONENTS STORAGE CENTRES RUN BY FIRST REFERRAL UNIT, COMMUNITY HEALTH CENTRE, PRIMARY HEALTH CENTRE OR ANY HOSPITAL.

*****

The following guidelines may be followed before exempting the said institutions for obtaining a license for operation of a Blood Bank or processing Whole Human Blood / or its components:

1. The applicant shall be First Referral Unit, Community Health Centre, Primary Health Centre or any Hospital.

2. The applicant shall furnish an undertaking to the licensing authority that the captive consumption of Whole Human Blood or Components shall not be more than 2000 units annually.

3. The applicant shall enclose a list of equipment needed for storage viz blood bank refrigerator with alarm system & temperature indicator. A separate list of equipment for blood components would be enclosed if proposed to be stored.

4. The applicant shall furnish the following :
   a Name of the medical officer responsible for conducting the operation of the blood storage centre.
   b Attested certified copies of MBBS or MD qualification
   c Name, certified copies of qualification and experience of the blood bank technician.
   d Name attested certified copies of qualification and experience of the blood bank technician having non-DMLT qualification

5. The applicant shall furnish the source of procurement of Whole Human Blood / Blood Components, namely the name and address of the Blood Banks.
   a. The source of procurement of blood/components shall be from licensed Blood Banks run by Govt. Hospitals / Indian Red Cross Society / Regional Blood Transfusion Centres only.
   b. A letter of consent from the above Blood Banks who intend to supply Whole Human Blood / Blood Components to the Blood Storage Centres shall be furnished along with the application.

6. The applicant shall submit the plan of the premises. A minimum area of 10 sq. meter is essential for the Blood Storage Centre.

7. In order to satisfy the conditions and facilities, an inspection of the proposed Blood Storage Centre may be carried out by the respective State Drug Control Department.

8. The Inspection team shall also inspect the Blood Banks that have given consent letters for the supply of Whole Human Blood / Components. The inspection team may verify whether the Blood Banks have sufficient quantity of blood units to be supplied to the Blood Storage Centres and also verify the mode of shipper or containers used for supply of blood units/components to ensure that the proper storage condition is maintained as per the pharmacopoeia. The Blood Bank shall label the blood units/components as per the Drugs & Cosmetics Rules, 1945.

9. The Blood Banks who intend to supply the blood units/components shall test the following mandatory tests before supplying to Blood Storage Centres.
   a. Blood Grouping
   b. Anti Body Testing

c. Haemoglobin Content
d. HIV I & II Anti Bodies
e. Hepatitis B Surface antigen
f. Hepatitis C Anti Body
g. Malarial Parasite
h. Syphillis or VDRL

The label of the tested blood unit shall contain the above particulars with the date of testing before supplying to Blood Storage Centres.

The Blood Bank shall maintain a separate register for the supply of blood units/components to Blood Storage Centres with all necessary details.

10. The validity of approval shall be for 2 years from the date of issue of the approval.

11. The State Licensing Authority shall forward the approved Blood Storage Centres to the concerned Zonal Officer immediately.

12. A format of the approval proforma is enclosed.

CERTIFICATE OF APPROVAL TO BLOOD STORAGE CENTRE FOR STORAGE OF WHOLE HUMAN BLOOD AND* / OR ITS COMPONENTS

No. _____ Date of Issue _____

M/s _____ is hereby approved to store the following items on the premises situated at _____ under the supervision of the following technical staff :

1. Names of the approved medical officer:
1. Names of the items:
2. Name of the qualified Blood Bank Technician:
3. Name & address of the licensed Blood :
   Bank from whom the blood units would be procured.
5. The approval shall be inforce from to

Signature

Designation

Licensing Authority

Dated

* Delete whichever is not applicable.

CONDITIONS

The Blood storage center shall comply with the conditions as stipulated under item 5B of Schedule K of the Drugs and Cosmetics Rules which also includes as under :-

1. The captive consumption of Whole Human Blood or its components in the above said center shall not be more than 2000 units annually.
2. In the event of any change in the technical staff shall be forthwith reported to the licensing authority.
3. In the event of any change in the name of the licensed blood bank from whom the blood units are procured, the same shall be intimated to the licensing authority for approval.
4. The centre shall apply for renewal of the approval to the licensing authority three months prior to the date of expiry of the approval.
5. The centre shall maintain records and registers, including the details of blood procurement* / its components.
6. The centre shall store samples of donors' blood as well as patients' sera for 7 days after transfusion.

B. Drugs and Cosmetics (Amendment) Rules, 2020

MINISTRY OF HEALTH AND FAMILY WELFARE

(Department of Health and Family Welfare)

NOTIFICATION

New Delhi, the 11th March 2020

G.S.R. 166(E).—Whereas a draft of certain rules further to amend the Drugs and Cosmetics Rules, 1945, was published as required under subsection (1) of section 12 and sub-section (1) of section 33 of the Drugs and Cosmetics Act, 1940 (23 of 1940) *vide* notification of the Government of India in the Ministry of Health and Family Welfare (Department of Health and Family Welfare) number G.S.R. 1152(E), dated the 29th November 2018, in the Gazette of India, Extraordinary, Part II, Section 3, Sub-section (i), inviting objections and suggestions from persons likely to be affected thereby before the expiry of a period of forty-five days from the date on which the copies of the Official Gazette containing the said notification were made available to the public; And whereas copies of said Official Gazette were made available to the public on the 30th November 2018; And whereas objections and suggestions received from the public on the said rules have been considered by the Central Government.

Now, therefore, in the exercise of the powers conferred under sections 12 and 33 of the Drugs and Cosmetics Act,1940 (23of1940), the Central Government, after consultation with the Drugs Technical Advisory Board, hereby makes the following rules further to amend the Drugs and Cosmetics Rules, 1945, namely:

1. (1) These rules maybe called the Drugs and Cosmetics (Second Amendment) Rules, 2020.

   (2) They shall come into force on the date of their publication in the Official Gazette.

2. In the Drugs and Cosmetics Rules, 1945 (here in after referred to as the said rules), in Part X B, in the heading, for the words –"Blood Banks", the words –"Blood Centers" shall be substituted.

3. In the said rules, in rule 122 EA, in sub-rule (1),—

   (i) For clause (d) following shall be substituted, namely,—

—(d) Blood Centre' is an authorized premise in an organization or institution as the case maybe, for carrying out all or any of the operations including collection, apheresis, processing, storage and distribution of blood drawn from donors or received from another licensed Blood Centre and for preparation, storage and distribution of blood components;

   (ii) In clause (g), after the word accepting ', the words 'against donated unit' shall be inserted.

   Amendment- "Donor" means a person who voluntarily donates blood after he has been declared fit after a medical examination, for donating blood, on fulfilling the criteria given hereinafter, without accepting against the donated unit in return any consideration in cash or in-kind from any source but does not include a professional or a paid donor.

   (iii) after clause(m), the following clause shall be inserted, namely,—

   (n) 'Erythrocytapheresis' means a selective collection of one or two units of red cells from a donor or patient using a cell separator and re-transfusing the remaining blood in to the donor or patient."

4. In the said rules, in rule 122 EA, rule 122 F, rule 122 G, rule 122 I and rule 122 P, for the words –"BloodBank", the words-"Blood Centre" wherever they occur shall be substituted.

5. In the said rules, in rule 122 G, in sub-rule (1), for condition (i) the following shall be substituted, namely,—

—(i)The operation of Blood Centre or processing or both of whole human blood for components shall be conducted under the active direction and personal supervision of competent technical staff consisting of at least one person who is whole-time employee and who is Medical Officer, and possessing—

   (a) Degree in Medicine M.B.B.S. having experience of working in Blood Centre, not less than one year during regular service and also has adequate knowledge and experience in blood group serology,

blood group methodology and medical principles involved in the procurement of blood or preparation of its components or both; or

(b) Degree in Medicine M.B.B.S. with Diploma in Clinical Pathology or Diploma in Pathology and Bacteriology with six months experience in a licensed Blood Centre; or

(c) Degree in Medicine M.B.B.S. with Diploma in Transfusion Medicine or Diploma in Immunohaematology or Blood Transfusion with three months experience in a licensed Blood Centre; or

(d) Doctor of Medicine Pathology or Diplomate of National Board Pathology with three months experience in a licensed Blood Centre; or

(e) Postgraduate degree in Transfusion Medicine - Doctor of Medicine Transfusion Medicine or Diplomate of National Board Transfusion Medicine, Doctor of Medicine Immuno hematology and Blood Transfusion, the Central Government or State Government from a University recognizes the degree or diploma.

Explanation— For this condition, the experience in Blood Centre shall not apply in the case of persons who are approved by the Licensing Authority or Central license Approving Authority or both before the commencement of the Drugs and Cosmetics (Second Amendment) Rules, 1999.

6. In the said rules, in rule 122 G, for sub-rule (2) and Explanation to that, the following shall be substituted, namely,—

—[(2)Applications for grant or renewal of license for operation of Blood Centre or processing of Human blood components shall be made by the Blood Centre run by the Government, Indian Red Cross Society, Hospital, Charitable Trust or Voluntary organization and Blood Centre run by Charitable Trust or Voluntary organization need to be approved by a State or Union territory Blood Transfusion Council as per procedure laid down in this regard by the National Blood Transfusion Council.

Explanation:— For the purpose of this sub-rule, renewal shall include renewal of any license issued after the commencement of the Drugs and Cosmetics (Sixth Amendment) Rules, 2005).

7. In the said rules, in Schedule A, in Form 26 G, Form 27 C and Form 28 C, for the words –Blood Bank, the words-Blood Centre wherever they occur shall be substituted.

8. In the said rules, in Schedule F, in Part XII B,—

(1) (a) for the words —Blood Bank, the words —Blood Centre wherever occur shall respectively be substituted.

(b) For the words —Blood Banks, the words —Blood Centres wherever they occur shall respectively be substituted.

(c) For the words— Blood Banking, the words —Blood Centres wherever they occur shall respectively be substituted.

(d) For the words— Blood Bank 's, the words –Blood Centre's shall be substituted.

(2) Under the heading—I. BLOOD CENTRES/BLOOD COMPONENTS, under sub-heading

—B. ACCOMMODATION FOR A BLOOD CENTRES,—

(i) for serial number (8),the following shall be inserted,namely,-

9. Store-cum-records.

10. Counselling area with adequate privacy.

11. Identified Quality Control area with component preparation area may be provided.

(ii) Under sub-heading—C. PERSONNEL

(a) for clause (b), the following shall be substituted, namely,—

—(b)Blood Centre Technician(s) possessing—

(i) Diploma in Medical Laboratory Technology (DMLT) or Transfusion Medicine or Blood Bank Technology after 10+2 with one-year experience in the testing of blood and/or its components in licensed Blood Centre; or

(ii) Degree in Medical Laboratory Technology (M.L.T.) or Blood Bank Technology with six month's experience in the testing of blood and/or its components in licensed Blood Centre; or

(iii) B.Sc. in Hematology and Transfusion Medicine with six month's experience in the testing of blood and/or its components in licensed Blood Centre; or

(iv) M.Sc. in Transfusion Medicine with six months experience in the testing of blood and/or its components in licensed Blood Centre; or

(v) Post Graduate Diploma in Medical Laboratory Technology (PGDMLT)/Post Graduate Diploma in Medical Laboratory Science (PGDMLS) with six months experience in the testing of blood and/or its components in licensed Blood Centre.

(b) For clause (d), the following clause shall be substituted, namely:—

—(d) Technical supervisor (where blood components are manufactured), possessing-–

(i) Diploma in Medical Laboratory Technology or Transfusion Medicine or Blood Bank Technology after 10+2 with one year experience in the testing of blood or its components or both in licensed Blood Centre; or

(ii) Degree in Medical Laboratory Technology or Blood Bank Technology with six month's experience in the testing of blood or its components or both in licensed Blood Centre; or B.Sc.in Hematology and Transfusion Medicine with six month's experience in the testing of blood or its components or both in licensed Blood Centre; or

(iii) M.Sc. in Transfusion Medicine with six month's experience in the testing of blood or its components or both in licensed Blood Centre; or

(iv) Post Graduate Diploma in Medical Laboratory Technology or Post Graduate Diploma in Medical Laboratory Science with six month's experience in the testing of blood or its components or both in licensed Blood Centre; or

(v) Post Graduate Diploma in Transfusion Technology (PGDTT) approved by the Central Government or State Government with experience of 6 months in the testing of blood or its components or both in licensed blood centre.

(c) After clause (d) so amended, the following paragraph shall be inserted, namely,—

—Blood Centre organizing blood donation camps shall have whole following time or part-time counselling staff (Counselor or Medical Social Worker) possessing,—

(a) Master's degree in social work, sociology, psychology with six months of experience; or

(b) Degree in Science or Health Science with one year of experience; or

(c) A person with 10+2 having three years of experience in counselling in the Blood centres collecting blood less than 3000 units per annum can share a counsellor or medical social worker within the institution.

(iii) under sub-heading —E, EQUIPMENT—, after entry no. 15, the following entries shall be inserted, namely,—

16. Standard Certified Weight(s). --- Once in a year

17. Equipment for Transfusion ---- Each Run Once in a year

Transmitted Infection (TTI) laboratory like ELISA Plate Reader if ELISA is used

or

Chemiluminescence Immuno Assay (CLIA)---- Each day of use

or Enzyme-Linked Fluorescence Assay (ELFA)

18. Micropipettes if ELISA is used. ---- Once in a year

(iv) For sub-heading H., the following shall be substituted, namely,—

—H. CRITERIA FOR BLOOD DONATION

S.No.	Condition	Criteria
1.	Well-being	The donor shall be in good health, mentally alert and physically fit and shall not be inmates of jail or any other confinement. –Differently-abled or donor with communication and sight difficulties can donate blood provided that clear and confidential communication can be established and he/she fully understands the donation process and gives valid consent.
2.	Age	Minimum age 18 years Maximum age 65 years First time donor shall not be over 60 years of age; for repeat donor upper limit is 65 years. For a pheresis donors18-60 years
3.	Whole Blood Volume Collected and weight of the donor	350ml-45kg 450ml– more than 55 kg Apheresis– 50kg
4.	Donation Interval	For whole blood donation, once in three months (90 days) for males and four months (120 days) for females. For apheresis, at least 48 hours interval after platelet/plasma – apheresis shall be kept (not more than 2 times a week, limited to 24 in one year) After whole blood donation, a platelet pheresis donor shall not be accepted before 28 days. Apheresis platelet donor shall not be accepted for whole blood donation before 28 days from the last platelet donation provided reinfusion of red cell was complete in the last platelet pheresis donation. If the reinfusion of red cells was not complete, then the donor shall not be accepted within 90 days. A donor shall not donate any type of donation within 12 months after a bone marrow harvest, within 6 months after a peripheral stem cell harvest.
5.	Blood Pressure	100-140mm Hg Systolic 60-90mm Hg diastolic with or without medications. There shall be no findings suggestive of end-organ damage or secondary complication (cardiac, renal, eye or vascular) or history of feeling giddiness, fainting made out during history and examination. Neither the drug nor its dosage should have been altered in the last 28 days.
6.	Pulse	60-100 Regular
7.	Temperature	Afebrile;37°C/98.4°F
8.	Respiration	The donor shall be free from acute respiratory disease.
9.	Haemoglobin	>or=12.5g/dL Thalassemia trait may be accepted, provided Haemoglobin is acceptable.
10.	Meal	The donor shall not be fasting before the blood donation or observing fast during the period of blood donation and last meal should have been taken at least 4 hours prior to donation. Donor shall not have consumed alcohol and show signs of intoxication before the blood donation. The donor shall not be a person having regular heavy alcohol intake.
11.	Occupation	The donor who works as air crew member, long distance vehicle driver, either above sea level or below sea level or in emergency services or where strenuous work is required, shall not donate blood at least 24 hours prior to their next duty shift. The donor shall not be a night shift workers without adequate sleep.

S.No.	Condition	Criteria
12	Risk behaviour	The donor shall be free from any disease transmissible by blood transfusion, as far as can be determined by history and examination. The donor shall not be a person considered "at risk" for HIV, Hepatitis B or C infections (Transgender, Men who have sex with men, Female sex workers, Injecting drug users, persons with multiple sexual partners or any other high risk as determined by the medical officer deciding fitness to donate blood)
13.	Travel and residence	The donor shall not be a person with history of residence or travel in a geographical area which is endemic for diseases that can be transmitted by blood transfusion and for which screening is not mandated or there is no guidance in India.
14.	Donor Skin	The donor shall be free from any skin diseases at the site of phlebotomy. The arms and forearms of the donor shall be free of skin punctures or scars indicative of professional blood donors or addiction of self-injected narcotics.
Physiological Status for Women		
15.	Pregnancy or recently delivered	Defer for 12 Months after delivery
16.	Abortion	Defer for 6 months after abortion
17.	Breastfeeding	Defer for a total period of lactation
18.	Menstruation	Defer for the period of menstruation
Non-specific illness		
19.	Minor non-specific symptoms including but not limited to general malaise, pain, headache	Defer until all symptoms subside, and the donor is a febrile
Respiratory (Lung) Diseases		
20.	Cold, flu, cough, sore throat or acute sinusitis	Defer until all symptoms subside, and the donor is a febrile
21.	Chronic sinusitis	Accept unless on antibiotics
22.	Asthmatic attack	Permanently Defer
23.	Asthmatics on steroids	Permanently Defer
Surgical Procedures		
24.	Major surgery	Defer for12 months after recovery. (Major surgery being defined as that requiring hospitalisation, anaesthesia (general/spinal) had Blood Transfusion and/or had significant Blood loss)
25.	Minor surgery	Defer for 6 months after recovery
26.	Received Blood Transfusion	Defer for 12 months
27.	Open heart surgery Including By pass surgery	Permanently defer
28.	Cancer surgery	Permanently defer
29.	Tooth extraction	Defer for 6 months after tooth extraction
30.	Dental surgery under anaesthesia	Defer for 6 months after recovery
Cardio-Vascular Diseases (Heart Disease)		
31.	Has any active symptom (Chest Pain, Shortness of breath, swelling of feet)	Permanently defer

S.No.	Condition	Criteria
32.	Myocardial infection (Heart Attack)	Permanently defer
33.	Cardiac medication (digitalis, nitro-glycerine)	Permanently defer
34.	Hypertensive heart disease	Permanently defer
35.	Coronary artery disease	Permanently defer
36.	Angina pectoris	Permanently defer
37.	Rheumatic heart disease with residual damage	Permanently defer
	Central Nervous System/Psychiatric Diseases	
38.	Migraine	Accept if not severe and occurs at a frequency of less than once a week
39.	Convulsions and Epilepsy	Permanently defer
40.	Schizophrenia	Permanently defer
41.	Anxiety and mood disorders	Accept person having anxiety and mood(affective) disorders like depression or bipolar disorder, but is stable and feeling well on the day regardless of medication.
	Endocrine Disorders	
42.	Diabetes	Accept person with Diabetes Mellitus well controlled by diet or oral hypoglycaemic medication, with no history of orthostatic hypotension and no evidence of infection, neuropathy or vascular disease (in particular peripheral ulceration)– Permanently defer person requiring insulin and/or complications of Diabetes with multi-organ involvement– Defer if oral hypoglycaemic medication has been altered/dosage adjusted in the last 4 weeks
43.	Thyroid disorders	Accept donations from individuals with Benign Thyroid Disorders if euthyroid (Asymptomatic Goitre, History of Viral Thyroid it is, Auto Immune Hypo Thyroidism) Defer if under investigation for Thyroid Disease or thyroid status is not known Permanently defer if: 1) Thyrotoxicosis due to Graves 'Disease 2) Hyper/HypoThyroid History of malignant thyroid tumours
44.	Other endocrine disorders	Permanently defer
	Liver Diseases and Hepatitis infection	
45.	Hepatitis	Known Hepatitis B, C – Permanently defer Unknown Hepatitis -Permanently defer Known hepatitis A or E -Defer for 12 months
46.	Spouse/ partner/ close contact of individual suffering from hepatitis,	Defer for 12 months
47.	At risk for hepatitis by tattoos, acupuncture or body piercing, scarification and any other invasive cosmetic procedure by self or spouse/ partner	Defer for12 months

S.No.	Condition	Criteria
48.	Spouse/ partner of individual receiving transfusion of blood/ components	Defer for 12 months
49.	Jaundice	Accept donor with a history of jaundice that was attributed to gall stones, Rh disease, mono nucleosis or in the neonatal period.
50.	Chronic Liver disease/ Liver Failure	Permanently defer
	HIV Infection/AIDS	
51.	At risk for HIV infection (Transgender, Men who have Sex with Men, Female Sex Workers, Injecting drug users, persons with multiple sex partners)	Permanently defer
52.	Known HIV positive person or spouse/ partner of PLHA (the person living with HIV AIDS)	Permanently defer
53.	Persons having symptoms suggestive of AIDS	Permanently defer person having lymphadenopathy, prolonged and repeated fever, prolonged & repeated diarrhoea irrespective of HIV risk or status
	Sexually Transmitted Infections	
54.	Syphilis (Genital sore, orgeneralized skin rashes)	Permanently defer
55.	Gonorrhoea	Permanently defer
	Other Infectious diseases	
56.	History of Measles, Mumps, Chickenpox	Defer for 2 weeks following full recovery
57.	Malaria	Defer for 3 months following full recovery.
58.	Typhoid	Defer for 12 Months following full recovery
59.	Dengue/Chikungunya	In case of history of Dengue/Chikungunya: Defer for 6 Months following full recovery. Following a visit to Dengue/Chikungunya endemic area: 4 weeks following return from a visit to dengue-endemic area if no febrile illness is noted.
60.	Zika Virus/ West Nile Virus	In case of Zika infection: Defer for 4 months following recovery. In case of history of travel to West Nile Virus endemic area or Zika virus outbreak zone: Defer for 4 months.
61.	Tuberculosis	Defer for 2 years following confirmation of cure
62.	Leishmaniasis	Permanently defer
63.	Leprosy	Permanently defer
	Other infections	
64.	Conjunctivitis	Defer for the period of illness and continuation of local medication.
65.	Osteomyelitis	Defer for 2 years following completion of treatment and cure.
	Kidney Disease	
66.	Acute of kidney infection (pyelonephritis)	Defer for 6 months after complete recovery and last dose of medication
67.	Acute infection (cystitis)/ UTI of	Defer for 2 weeks after complete recovery and last dose of medication

S.No.	Condition	Criteria
68.	Chronic infection of kidney/ kidney disease/renal failure	Permanently defer
	Digestive System	
69.	Diarrhoea	A person having a history of diarrhoea in the preceding week, particularly if associated with fever: Defer for 2 weeks after complete recovery and last dose of medication
70.	Glendoscopy	Defer for 12 months.
71.	Acid Peptic disease	Accept person with acid reflux, mild gastro-oesophageal reflux, mild hiatus hernia, gastro-oesophageal reflux disorder (GERD), hiatus hernia: Permanently defer person with stomach ulcer with symptoms or with recurrent bleeding:
	Other diseases/disorders	
72.	Auto immune disorders like Systemic lupus erythematosus, scleroderma, dermatomyositis, ankylosing spondylitis or severe rheumatoid arthritis	Permanently defer
73.	Polycythaemia Vera	Permanently defer
74.	Bleeding disorders and unexplained bleeding tendency	Permanently defer
75.	Malignancy	Permanently defer
76.	Severe allergic disorders	Permanently defer
77.	Haemoglobinopathies and red cell enzyme deficiencies with a known history of haemolysis	Permanently defer
	Vaccination and inoculation	
78.	**Non live vaccines and Toxoid:** Typhoid, Cholera, Papilloma virus, Influenza, Meningococcal, Pertussis, Pneumococcal, Polio injectable, Diphtheria, Tetanus, Plague	Defer for 14 days
79.	**Live attenuated vaccines:** Polio oral, Measles (rubella) Mumps, Yellow fever, Japanese encephalitis, influenza, Typhoid, Cholera, Hepatitis A	Defer for 28 days
80.	Anti-tetanus serum, anti-venom serum, anti-diphtheria serum, and anti-gas gangrene serum	Defer for 28 days
81.	Anti-rabies vaccination following animal bite, Hepatitis B Immunoglobulin, Immunoglobulins	Defer for 1 year
82.	Swine Flu	Defer for 15 days

S.No.	Condition	Criteria
	Medications are taken by prospective blood donor	
83.	Oral contraceptive	Accept
84.	Analgesics	Accept
85.	Vitamins	Accept
86.	A mild sedative and tranquillizers	Accept
87.	Allopurinol	Accept
88.	Cholesterol-lower in medication	Accept
89.	Salicylates (aspirin) other NSAIDs	Defer for 3 days if blood is to be used for Platelet preparation
90.	Ketoconazole, Antihelminthic drugs including mebendazole,	Defer for 7 days after the last dose if the donor is well
91.	Antibiotics	Defer for 2 Weeks after the last dose if the donor is well
92.	Ticlopidine, clopidogrel	Defer for 2 Weeks after the last dose
93.	Piroxicam, dipyridamole	Defer for 2 Weeks after the last dose
94.	Etretinate, Isotretinoin. (Used for acne)	Defer for 1 month after the last dose
95.	Finasteride used to treat benign prostatatic hyperplasia	Defer for1 month after the last dose
96.	Radio active contrast material	8 weeks deferral
97.	Dutasteride used to treat benign prostatatic hyperplasia	Defer for 6 months after the last dose
98.	Any medication of unknown nature	Defer till details are available
99.	Oral anti-diabetic drugs	Accept if there is no alteration in dose within last 4 weeks.
100.	Insulin	Permanently defer
101.	Anti-arrhythmic, Anti-convulsions, Anticoagulant, Anti-thyroid drugs, Cytotoxic drugs, Cardiac Failure Drugs (Digitalis)	Permanently defer
	Other conditions requiring Permanent deferral	
102.	Recipients of organ, stem cell and tissue transplants Donors who have had an unexplained delayed faint or delayed faint with injury or two consecutive faints following a blood donation.	Permanently defer
	Residents of other countries	
103.	Residents of other countries	Accept only after staying in India for three continuous years

Under the heading—II. BLOOD DONATION CAMPS—

(i) in —Notes, at serial number (i), after the words —constituted by a State Government — the following words shall be inserted, namely,—

—in accordance with the procedure laid down by the National Blood Transfusion Council in this regard,

Designated Regional Blood Transfusion Centre— shall be a centre approved and designated by a Blood Transfusion Council constituted by a State Government per the procedure laid down by the National Blood Transfusion Council in this regard to collect, process and distribute blood and its components to cater to the needs of the region and that centre has also been licensed and approved by the Licensing Authority and Central license Approving Authority for the purpose

(ii) under the sub-heading —(B) Personnel for Out-door Blood Donation Camp, for serial number (ii), the following shall be substituted, namely, —(ii) two counsellors or medical social workers;

(iii) under sub-heading —(C) Equipment, for item 12, the following shall be substituted, namely, —12.A Portable Hb Hb meter or copper sulphate method or any quantitative method can be used for the determination of Hemoglobin estimation.

(1) Under the heading—III. Processing of Blood Components from Whole Blood by a Blood Centre,

(i) under sub-heading—(B) Equipment,—

(a) for the item (iv), the following shall be substituted, namely,—

—(iv) Plasma Expresser or Automated Extractor or Multi-Head Tube Sealer.

(b) for the item (xi), the following shall be substituted, namely,—

—(xi) Deep Freezer or Snap Freezer maintaining a temperature between minus 30-degree centigrade to minus 40-degree centigrade and minus 75-degree centigrade to minus 80-degree centigrade.

(c) After the item (xiii), the following shall be inserted, namely, (xiv) Cryobath and any better equipment or technology.

(ii) under sub-heading—(E) Categories of Blood Components,—

(a) in clause (1), for the portion beginning with the words —The product shall be'' and ending with the words —from human blood, the following shall be substituted, namely,—

—The product shall be known as —Packed Red Blood Cells — that is packed red blood cells remaining after separating plasma from human blood which also includes modified packed red blood cells including semi-packed red blood cells, washed red blood cells, leukoreduced red blood cells, irradiated red blood cells and frozen red blood cells. Types of Red Cell components: —

(i) Saline washed Red Cells: Red cells washed with sterile Normal Saline by centrifugation at 2 to 8 degrees centigrade

(ii) Leucodepleted red cells: Shall be prepared by a method known to reduce leucocytes in the final component to less than $5x10^8$ when intended to prevent febrile reactions and to less than $5x10^6$ when required to prevent alloimmunisation or cytomegalovirus infection. For achieving a level of less than $5x10^6$ leucocyte filters are necessary.

(iii) Irradiated red cells: prepared by gamma cell or x-irradiation at 25 Gy to prevent graft versus host disease due to proliferation of lymphocytes.

(iv) Frozen Packed Red Blood Cells: Cryoprotective substance may be added to the Packed Red Blood Cells for extended storage between minus 80 to minus 196 degrees centigrade.

(v) Packed red cell aliquot prepared for transfusion to paediatric patients by technique to preserve sterility.

(vi) The quality control criteria for validation of the processes should be as follows:

1% of Packed Red cells may be tested, of which atleast 75% of the packed red cells shall conform to the following quality control criteria-

(a) Volume:

250 ml +/- 10% from 450 ml bag

150 ml+/- 10% from 350 ml bag

(b) Hematocrit: 65-70% when stored in CPDA1 solution 50-60% when stored in SAGM solution

(c) Culture: Sterile

(b) in clause (2) relating to the Platelets Concentrates, after the first paragraph, the following shall be inserted, namely, —

—Types of Platelets: —

i. Platelet Rich Plasma: plasma which is rich in platelets and separated from whole blood

ii. Random Donor Platelet Concentrate

(a) prepared from platelet-rich plasma

(b) prepared from Buffy Coat

iii. Pooled Platelets

(a) prepared by pooling of 6 units of random donor platelet, preferably ABO or Rh type matched are pooled into one bag of "Pooled Platelets".

(c) in clause (2), after sub-clause (v) relating to compatibility tests, the following shall be inserted, namely, —

—Preparation of pooled platelet concentrate: —

One single unit of random donor platelets is not enough to provide an adequate haemostatic dose in an adult patient. Therefore, up to 6 units of random donor platelets, preferably ABO or Rh type matched, are pooled into one bag of "Pooled Platelet Concentrate". The pooled platelets may be prepared by pooling buffy coats and then processed into one unit of pooled buffy coats— pooled platelet concentrate. Alternatively, pooling can be done after the preparation of random donor platelets by platelet-rich plasma method or buffy coat method. If the pooling is done in an open system (using spikes for pooling), the shelf life of the pooled platelets will be 6 hours, while for a closed system (using the sterile connecting device) the expiry date will be that of the platelet unit having the shortest expiry date. The labelling requirements for the final pooled product shall remain the same as any other platelet product except that the final pack should have a unique pool number or donation numbers of all contributing units.

The platelet content in the pooled product should be $\geq 2 \times 10^{11}$/unit. Modified platelet component includes: leucodepleted, irradiated, washed platelets or platelets suspended in additive solution.

(d) in clause (3) relating to Granulocyte Concentrates, for sub-clause (i) and (ii), the following sub-clauses shall be substituted, namely, —

—(i) Granulocyte concentrates prepared either by pooling multiple units of buffy coat or by apheresis as described under the apheresis section. The same shall be stored at 20-24^{o}C and used within a maximum period of 24 hours.

(ii) Pooled granulocytes shall meet the same Quality Control requirements as that for apheresis granulocytes. (at least 1 x 10 raised to the power 10).

(e) in clause (4) relating to Fresh Frozen Plasma, after the first paragraph, the following shall be inserted, namely, —

—The quality control criteria for validation of the processes should be as follows:

Volume:

180-220 ml from 350 ml bag

220-300 ml from 450 ml bag Factor VIII: at least 70 iu / bag

Excess and expired plasma may be issued for fractionation to the licensed fractionation centre in the Country with justification to be recorded in writing.

(f) in clause (5), for the words —Concentrate of anti-haemophiliac factor shall be prepared by the thawing of the fresh plasma frozen stored at minus 30-degree centigrade, the following shall be substituted, namely:

—Concentrate of anti-haemophiliac factor shall be prepared by thawing FFP at 4^{o}C in a cold room or blood bank refrigerator or 4-10^{o}C in a cryobath. Minus 80^{o}C deep freezer should be used for faster freezing of plasma for preparation of cryoprecipitate.

The quality control criteria for validation of the processes should be as follows:

Volume: 15 – 20 ml Fibrinogen: at least 150 mg/bag Factor VIII: at least 80 iu/bag

Preparation of pooled cryoprecipitate:

One single unit of cryoprecipitate is not enough to provide an adequate haemostatic dose in an adult

patient. Therefore, multiple units of cryoprecipitate may be pooled in one bag. If the pooling is done in an open system (using spikes for pooling), the shelf life of the pooled cryoprecipitate will be 6 hours.

The labelling requirements for the final pooled product shall remain the same as any other cryoprecipitate product except that the final pack should have a unique pool number or donation numbers of all contributing units.

(2) For sub-heading —"F. Plasmapheresis, Plateletpheresis, Leucapheresis, Using a Cell Separator", the following shall be substituted, namely, —

—(**F) APHERESIS USING A CELL SEPARATOR**

General requirements:

(a) Accommodation: An air-conditioned area of 10 square meters shall be provided for apheresis/therapeutic procedures in the blood Centre.

(b) Equipment:

i. Cell separator

ii. Dielectric tube sealer

iii. Other emergency equipment/ items

– Oxygen cylinder with mask, gauge and pressure regulator. (ii) 5 per cent Glucose or Normal Saline.

– Disposable sterile syringes and needles of various sizes.

– Disposable sterile I.V. infusion sets.

– Ampoules of Adrenaline, Noradrenaline, Mephentin, Betamethasone or Dexamethasone, Metoclorpropamide injections.

– Aspirin.

(c) Criteria for selection of donors:

At least 48 hours must elapse between successive apheresis and not more than twice a week. For haematopoietic stem cells, the procedures can be done daily.

Types of Apheresis:

1. Plasmapheresis
2. Plateletpheresis for harvesting Platelet concentrate (Single Donor Platelets)
3. Leucapheresis for harvesting
   – Granulocyte concentrate
   – Lymphocytes
   – Mononuclear cells
4. Erythrocytapheresis- Red cell apheresis including double unit red cell collection
5. Haematopoietic stem cells (Peripheral Blood Stem Cells)

1. Plasmapheresis:

The total serum protein shall be 6 gm/dl before the first plasmapheresis procedure. In repeated plasmapheresis:

a. It should be tested before the third procedure if done within four weeks, and it shall be 6 gm/dl.

b. The quantity of plasma separated from the donor's blood shall not exceed 500 ml per sitting and once in a fortnight or shall not exceed 1000 ml per month.

2. Plateletpheresis (Single Donor Platelets):

(i) Plateletpheresis shall not be carried out on donors who have taken medication containing aspirin within 3 days prior to donation

(ii) Platelet count, WBC counts, the differential count may be carried out.

The term plateletpheresis includes platelets collected by apheresis, using a cell separator, and the product is called single donor platelets and includes washed single donor platelets, Modified single donor platelets (with replacement of compatible plasma), leukoreduced single donor platelets and double single donor platelets collected from a single donor. Single donor platelets should have a platelet count of $\geq$ 3X 10^{11} / unit.

i. Storage: Shall be kept up to 5 days between 20ºC to 24ºC with continuous agitation.

ii. Apheresis platelet should contain a minimum of 3 x 10^{11} platelets in 75% of the units tested amongst 1% of monthly production, or 4 platelet concentrates per month, whichever is higher.

iii. The pH must be 6 or higher at the end of the permissible storage period.

3. Leucapheresis

This procedure includes a collection of Granulocytes (Granulocytopheresis), Lymphocytes or Peripheral blood stem cells or Haematopoietic stem cells to treat traditional conditions followed by their preservation.

4. Erythrocytapheresis

This is the collection of 2 units of Red cells from a single donor meeting the specified requirement.

5. Therapeutic Plasmapheresis and Cytapheresis:

Therapeutic Apheresis activity is allowed in the Blood Centre attached to the Hospital having Apheresis facilities under the responsibility of Registered Medical Practitioner (RMP) who has obtained the consent of patient and record of which shall be maintained and signed by the RMP & blood bank medical officer.

This shall be done only at the written request of the patient‘s physician. The patient‘s informed consent shall be taken. Records of the procedure shall be maintained. Provisions for emergency care shall be available by the patient‘s physician.

6. In the said rules, in Schedule K, in Serial Number 5B and in Serial Number 30, for the words

—Blood Bank—, the words —Blood Centre— wherever they occur shall be substituted.

[F. No.X.11014/34/2018-DR] Dr. MANDEEP K. BHANDARI, Jt. Secy.

Note : The principal rules were published in the Official Gazette vide notification number F.28-10/45- H (1), dated 21st December 1945 and last amended vide notification number

G.S.R. 101(E), dated the 11th February, 2020.

C. Sample format of blood donor questionnaire

Blood Centre, रक्त कोष,

Hospital name with address (bilingual)

(Blood Donor Questionnaire & consent form / *रक्ताधान प्रश्नावली एवं सहमती प्रपत्र*)

License No.: 1204/87 Date: _____

For Office Use Only

Donation No.:_____ **Blood Bag Segment No.**_____ **Type of Bag:** **S/ D / T / Q /FB**

Volume Collected: _____(ml) **Phlebotomy Site:** **Right / Left**

Blood Collection Time: Start: _____ **End:** _____ **Signature of Phlebotomist:** _____

Confidential / *गोपनीय*

Pl. answers the following questions correctly. This will help to protect you and the patient who receives your blood.

कृपया निम्न लिखित प्रश्नों का सही उत्तर दें, इससे आपको तथा आपका रक्त प्राप्त करने वाले रोगी को सुरक्षा प्रदान करने में सहायता प्राप्त होगी।

Donor's Details/ रक्तदाता का विवरण

Name(नाम) : _____ **Age**(आयु):_____

Father's/Husband's Name (पिता/ पति का नाम):_____ **Male**(पुरुष)☐ / **Female**(महिला)☐

Occupation(व्यवसाय):_____

Address for communication (स्थायी पता):_____

_____

State(राज्य):_____**Pin Code** (पिन कोड):_____ **Mobile No**(मोबाईल नं.):_____

Patient's Name (रोगी का नाम) : _____ **UHID No.**_____ **(CTVS / NS)**

Relationship with Patient (रोगी से रिश्ता) _____

[√] Tick wherever applicable / *उपर्युक्त स्थान पर सही का निशान लगाए*

1a. Have you donated blood previously / **क्या आपने पहले कभी रक्तदान किया है?**: Yes हाँ / No नही

1b. If yes, on how many occasions / **(अगर हाँ तो कितनी बार)**:_____

When last / **(अन्तिम बार कब दिया था?)** :_____

1c. Did you have any discomfort during/after donation in your previous donations? Yes हाँ / No नही

क्या आपको पहले कभी रक्तदान करते समय या बाद में कोई असुविधा हुई है?

2. Do you feel well today?/ **क्या आज आप ठीक महसूस कर रहे हैं?** Yes हाँ / No नही

3. Did you have something to eat in the last 4 hours?/ **क्या आपने पिछले 4 घंटे में कुछ खाया हैं?** Yes हाँ / No नही

4. Did you sleep well last night? /**क्या आप पिछली रात ठीक से सोये थे?** Yes हाँ / No नही

5. Have you any reason to believe that you may be infected by either Hepatitis, Malaria, HIV/AIDS, and/or venereal disease? / **क्या आपको किसी कारण से ऐसा लगता है कि आप हेपेटाईटीस, मलेरिया, एच. आई. वी. एड्स, अथवा रतिज रोग आदि में से किसी रोग से संक्रमित है ?** Yes हाँ / No नही

6. In the last 6 months have you had any history of the following:

क्या आपको पिछले छः महिनो में निम्न लिखित में से किसी रोग से पीड़ित हुए है?

☐ Unexplained weight loss **(बिना कारण वजन कम होना)** ☐ Repeated Diarrhea **(बार बार उल्टी दस्त होना)**

☐ Swollen glands **(ग्रंथियों में सुजन)** ☐ Continuous low-grade fever**(लगातार निम्न ग्रेड बुखार)**

7. In the last 6 months have you had any / **पिछले छः महिनो में निम्न लिखित में से कुछ करवाया है?**: -

☐ Tattooing **(शरीर गोदना)** ☐ Ear Piercing **(कान छिदवाना)**

☐ Dental Extraction **(दांत निकलवाना)**

8. Is there any history of surgery or blood transfusion in the past 6 months? / **क्या आपका पिछले छः महिनो में कोई ओपरेशन या आपको रक्ताधान हुआ है?**

☐ Major Surgery **(बडा ओपरेशन)** ☐ Minor Surgery **(छोटा ओपरेशन)** ☐ Blood Transfusion **(रक्त चढाना)**

9. Do you suffer from or have suffered from any of the following diseases? / **क्या आप निम्न लिखित बिमारियों से पीडित हैं या पीड़ित रह चुके है?**

☐ Heart Disease **(हृदय रोग)** ☐ Lungs disease **(फेफडों का रोग)**

☐ Kidney Disease **(गुरदे का रोग)**
☐ Epilepsy **(मिरगी)**
☐ Tuberculosis **(छय, टी. बी.)**
☐ Hepatitis B/C **(हिपेटाईटीस बी. सी)**
☐ Jaundice **(पीलिया)**
☐ Malaria **(मलेरिया)**
☐ Fainting spells **(बार बार बेहोश होना)**
☐ Cancer/Malignant Disease **(कैंसर रोग)**
☐ Diabetes **(मधुमेह)**
☐ Abnormal bleeding tendency **(असामान्य रक्तस्त्राव)**
☐ Allergic Disease **(एलर्जी)**
☐ Sexually Trans. Diseases **(योन संचरित रोग)**
☐ Typhoid (last 1 yr.)**(टाईफाइड, पिछले एक साल में)**
☐ Mental illness **(मानसिक रोग)**

10. Are you taking or have taken any of these in the past 72 hours? **/ क्या आपने पिछले 72 घंटो में निम्न में से किसी का सेवन किया था?**

☐ Antibiotics **(एंटीबायोटीक्स)** ☐ Aspirin **(एस्परीन)** ☐ Alcohol **(शराब)** ☐ Hormones (हॉर्मोन)
☐ Steroids **(स्टीरोईडस)** ☐ Vaccinations **(टीकाकरण)** ☐ Anticoagulent (स्कन्दंरोधी)
☐ Dog Bite/Rabies vaccine (1 yr.) **(कुत्ते के काटने का टीकाकरण, पिछले एक साल में)**

11. For women donors, **/ केवल महिला रक्तदाताओ के लिए**

(a) Are you pregnant **/ क्या आप गर्भवती हैं?** Yes **हाँ** / No **नही**
(b) Have you had an abortion in the last 3 months **/ क्या आपने पिछले 3 महिनो मे गर्भपात करवाया हैं?** Yes **हाँ** / No **नही**
(c) Do you have a child less than one year old? **/ क्या आपका शिशु 1 साल से कम का हैं?** Yes **हाँ** / No **नही**
(d) Is the child still breast-feeding? **/ क्या आप अपने शिशु को स्तनपान करवा रही हैं?** Yes **हाँ** / No **नही**
(e) Are you having your periods today? **/ क्या आपको आज मासिक धर्म हो रहा है?** Yes **हाँ** / No **नही**

12. Have you read and understood all the information presented and answered all the questions truthfully, as any incorrect statement or concealment may affect your health or may harm the recipient. Yes **हाँ** / No **नही**
क्या आपने प्रस्तुत कि गई समस्त सुचनाओ को पढ़ लिया है और उनका उतर ईमानदारी से दिया है, क्योंकि कोई भी गलत विवरण अथवा सुचना छुपाने से आपके स्वास्थ्य पर प्रभाव पड़ सकता है अथवा रक्त प्राप्त करने वाले को नुकसान पहुच सकता है ?

13. I understand that **/ मैं समझता हूँ कि।**

(a) Blood donation is a totally voluntary act and no inducement or remuneration has been offered.
रक्तदान बिल्कल स्वेच्छिक कार्य है और इसके लिए कोई प्रलोभन या धन का प्रस्ताव नही दिया गया है ।
(b) Donation of blood/components is a medical procedure and that by donating voluntarily, I accept the risk associated with the procedure. **/ रक्तदान करना एक चिकित्सकीय प्रक्रिया है, और स्वेछिकता से किये गए रक्तदान की प्रकिया से जुड़े हुए जोखिमो को मैं स्वीकार करता / करती हूँ ।**
(c) My blood will be tested for Hepatitis B, Hepatitis C, Malarial parasite, HIV/AIDs and venereal diseases in addition to any other screening tests required to ensure blood safety and inform me if needed.
रक्त को सुरक्षित सुनिश्चित करने के लिए आवश्यक हेपेटाईटीस बी. सी, मलेरिया, एच. आई. वी. और रतिज तथा अन्य रोगों कि जॉच के लिए मेरे रक्त का परिक्षण कर सकते है और आवशकता अनुसार मुझे सूचित करे ।
(d) I give my consent to the blood centre for blood donation, to use my collected blood for component preparation, testing, transfusion to patient and to outsource my unused excess plasma for fractionation.
मैं रक्त कोष को मेरे रक्तदान के लिए और एकत्रित किये रक्त से अवयव तैयार करने, उसे जांच करने, जरूरतमंद रोगी को देने और अप्रयुक्त अतिरिक्त प्लाज्मा को फ्रेक्सेनेस्न हेतु देने की अनुमति प्रदान करता/करती हूँ ।
I prohibit any information provided by me or about my donation to be disclosed to any individual or government agency without my prior permission. **/ मैं, मेरे रक्तदान के बारे में प्रदान कि गई सुचना को किसी व्यक्ति या सरकारी एजेंसी को मेरी पूर्वानुमति के बिना प्रकट करने कि अनुमति नहीं देता हूँ।**

Date **/ *तिथि*:** _____ Time **/ *समय*:** _____ Donor's signature **/ *रक्तदाता के हस्ताक्षर*:** _____

For Office Use Only

Pre – Donation Counselling: Done/ Not- Done **Signature of Counsellor:** _____

General Physical Examination

Systemic Examination: _____
Weight (Kg) _____ **Height(cm)** _____ **Hb(gm%)** _____
BP (mm of Hg) _____ **Pulse (/Min.)** _____ **Temperature(^{0}C)** _____
Multiple Pricks at phlebotomy Site: **YES/ NO**

Accept ☐ **Defer** ☐ **Reason for deferral** _____
Signature of Medical Officer: _____

Blood safety begins with a Healthy Donor ***रक्त की सुरक्षा स्वस्थ रक्तदाता से ही शुरू होती है।***

D. Sample format for informed consent before autologous blood donation

CONSENT FOR PREDEPOSIT AUTOLOGOUS BLOOD DONATION

I, Mr./Mrs./Miss……..…son/daughter/wife of……isadmitted under (Physician/surgeon's name) ……...…… in the department of ……..…… for…… disease. I have been explained in my own language that

- My surgery/ therapeutic procedure requires the possibility of blood/ blood component transfusion and transfusion of my own blood is a possibility.
- The procedure of autologous blood collection and transfusion along with its possible benefits and complications has been explained to me.
- I give my consent for withdrawal of my blood by authorized medical personnel for autologous transfusion. If I do not require transfusion of the blood withdrawn for autologous transfusion, I understand that it may be disposed of as per the hospital policy.

(Signature/left thumb impression) Patient / Pt. Representative name: _____ Relation to patient: _____ Date: Place:	(Signature/left thumb impression) Witness name: _____ _____ Date: Place:	(Signature/left thumb impression) Doctor name: _____ _____ Date: Place:

E. Sample format of referral slip for TTI reactive blood donors.

Name & address of referring blood centre: _____

_____

Date of referral: _____ Blood centre License no_____

__

Name of donor: _____Age: _____years

Gender: Male/ Female, Blood donor ID no: _____

Contact details: _____

Name & designation of referring person: _____

Referred for (Write TTI for whom donor screened reactive)	Serology result	S/C/O	Assay used	NAT result	Assay used

Address of the referral centre: _____

(ICTC / Clinician)

Blood Centre seal with contact details

Consent for referral

(To be retained by the referring blood centre)

- I understand that during the blood donation process, I have been counselled regarding the importance of safe blood donation and have consented to test my blood and be informed of any abnormal test results.
- I understand that these screening tests conducted at blood centre are not diagnostic and may yield false-positive results.
- I understand that any willful misrepresentation of facts could endanger my health or that of patient's receiving my blood and may lead to litigation.
- I understand that I have been contacted, counselled, and referred by the blood centre for confirmation and management to the appropriate facility.

Signature of Referring Blood Centre Staff

Signature of Donor

Date: _____

F. Sample format of informed consent form before transfusion

INFORMED CONSENT FOR BLOOD TRANSFUSION

I, Mr./Mrs./Miss……..…son/daughter/wife of……admitted under (Physician/ surgeon's name) ……...…… in the department of ……..…… for…… disease. I have been explained in a language that I understand that

1. I/ My patient's condition/ surgery/ therapeutic procedure requires the possibility of blood/ blood component transfusion.
2. I / My patient understand that the blood/blood component hasbeen prepared and tested in accordance with rules established under the national regulations.
3. I / My patient have been explained the benefits as well as risks (including the transmission of HIV, Hepatitis B, Hepatitis C, syphilis/malaria and other transfusion related adverse events) of such transfusion. I understand that these risks remain despite the testing mentioned in point no 2.
4. I/ my patient have also been explained the alternatives to transfusion and their benefits and limitations.
5. I / My patient had the opportunity to ask any questions/clarifications related to the need/benefits/risks/ alternatives to transfusion.
6. I / My patient believe that i/we have been sufficiently informed to decide to give consent for transfusion of blood or blood component.

I / My patient consent to the transfusion of blood and blood component as deemed necessary by the treating physician/surgeon.

(Signature/left thumb impression) Patient / Pt. Representative name: _____ Relation to patient: _____ Date: Place:	(Signature/left thumb impression) Witness name: _____ _____ Date: Place:	(Signature/left thumb impression) Doctor name: _____ _____ Date: Place:

G. Sample format of blood/blood component requisition form

Blood / blood component requisition form (front side)

(Hospital name and address)

To be filled by requesting hospital / doctor

Patient details:

Name: _____Age: _____Gender: _____

Hospital ID: _____ I.P. No: _____ Ward/Bed no: _____

Consultant / doctor Incharge: _____ Requesting hospital: _____

Diagnosis: _____

Blood group (if Known): _____ Transfusion History: _____

Indication for Transfusion: _____

Lab values (if available): Hb_____ Plt count _____PT/APTT_____

Remarks: _____

Blood / Blood component details:

Component	W.B./Packed red cells	Platelet – WB derived	F.F.P.	Cryo Precipitate	Cryo Poor Plasma	Platelets - Apheresis
Qty. (Units)						

Required on:

Date: _____Time:_____ Type of Request: Routine / Emergency

Certified that the blood samples & details in the requisition formare correct. I have explained the necessity of Blood Transfusion or any procedure and the risk associated with it to the patient/relatives.

The informed consent for this has been taken from the patient/relative.

Name of Doctor Date Time Signature of Doctor

INSTRUCTIONS FOR SENDING REQUISITION FOR TRANSFUSION

- All requests for cross-matching (compatibility testing) of routine cases should be sent 24 hours in advance.
- Send a minimum of 5ml. of a whole blood sample for cross-matching in a clean plain sterile capped vial having a self-sticking non-removable label.
- The blood sample should be labelled properly & correctly with the patient's particulars which should match with the requisition form.
- In case of urgent transfusion, please indicate the nature of the emergency.
- Always make sure that blood/blood components are arranged before undertaking any major surgery.
- Blood / Blood components once issued will not be received back.
- Please ensure appropriate & rational use of blood.The Dept. does not advocate the use of whole blood.
- In case of neonates kindly send a 3ml EDTA sample of the mother if available. Please mention the date and time of dispatch of samples sent earlier.

IMPORTANT: This Blood Request form will not be accepted if it is not signed or any section is left blank.

Blood / blood component requisition form (Reverse side)

For use by Transfusion Medicine department only

Request No._____

Requisition received on Date: _____ Time: _____ By: _____

(Name & Signature of Blood Centre staff)

Investigations on patient sample:

Blood group: ABO & Rh (D)_____

Antibody screen: _____

Tested By: _____ Date: _____ Time: _____

Blood bag ID	Pilot Tube Number	Component	ABO&Rh of bag	IS Xmatch	LISS/AHG Xmatch	Auto Control	Date & Time of X Match	Initials of X-matching Staff	Date & Time of Issue	Initials of Issuing Staff

COMPATIBILITY TESTING

Blood component issued to: _____Signature of person receiving: _____

H. Sample format of monitoring of transfusion

XYZ Blood Centre										
Patient details: Name: Age / Gender: Hospital ID: Patient Blood group: Department / Ward / Bed no:										
SN	Component type & Blood group	Blood bag number	Start date & time	End date & time	Pre-transfusion vitals	Post transfusion Vitals at			Signature of person monitoring	Remarks / reaction
						15 min	1 hr	Tx completion		
1.										
2.										
3.										
4.										
5.										
6.										
7.										
8.										
9.										
10.										
11.										
12.										

I. Haemovigilance forms for recipient and donor adverse event reporting

National Institute of Biologicals
Ministry of Health & Family Welfare, Govt. of India

NATIONAL BLOOD DONOR VIGILANCE PROGRAMME

(Haemovigilance Programme of India)
Adverse Blood Donor Reaction Reporting Form

Version-2

A) Donor Information

Donor Id *: _____

Type of Donation* (a) Whole Blood (b) Apheresis__ (Platelets/Plasma/ Plasma + Platelets/RBC/Granulocyte/ Peripheral Blood Stem Cells/ COVID-19 Convalescent Plasma)

Sex * _____ (Male/Female/Other)

Weight of Donor (kg) * _____ Height of Donor (cm)* _____

Donor Type* (a) Voluntary (b) Replacement (c) Family Donor (d) Autologous (First Time/Repeat)

Age/ Date of Birth * Yrs: ____ Month: ____ Days: ____ OR _____

Site of Donation* _____ (Blood Centre/Camp)

Pre-Donation Vitals* Pulse: ____ per min BP (Systolic): ____ mmHg BP (Diastolic): ____ mmHg

Date of Donation * _____

Time of Donation Hr ____ Min ____

B) Whole blood Details of Blood Collected/Apheresis Details of Blood Collected

(a) Whole Blood

Lot No. of Blood Bag* _____

Volume Collected (ml)* _____

Manufacturer of Blood Bag* _____ (Terumo Penpol Limited/Mitra Industries Pvt. Ltd/ HLL Lifecare Ltd/Fresenius Kabi AG/Fenwal Inc/Polymed/Other)

Expiry Date of Blood Bag* _____

(b) Apheresis

Lot No. Kit* _____

Volume Collected (ml)* _____

Expiry Date of Kit* _____

C) Adverse Reaction Details

Date and Time of reaction* _____ Hr____Min____

Type of Reaction* _____ (Localised/Generalized/Both/ Other Reactions)

Vitals at the time of Reaction Pulse: _____ per min BP (Systolic): ____ mmHg BP (Diastolic): ____ mmHg

Data Captured* _____ (Onsite/Call back by donor/ Call back by Blood Centre)

Reaction Time* _____ (Pre-Donation/During Donation/After Donation)

Venipuncture Site* _____ (Left/Right/Both)

Injury* _____ (Yes/No)

Venipuncture* _____ (1/2/>2)

Site of Reaction* _____ (At Donation Site/ Outside Donation Site)

Donation Completed* _____ (Yes/No)

D) Type of Complications:*

Localised Complications

☐ **A1-Complications mainly characterized by the occurrence of blood outside the vessels**
- (a) ☐ Haematoma (bruise)
- (b) ☐ Arterial puncture
- (c) ☐ Delayed(bleeding/Re-bleeding) ☐ (Within 30 minutes of Donation/After 30 minutes of Donation)

☐ **A2-Complications mainly characterized by pain**
- (a) ☐ Nerve injury/irritation
- (b) ☐ Other Painful arm

☐ **A3-Localised infection/inflammation along the course of a vein**
- (a) ☐ Thrombophlebitis
- (b) ☐ Cellulitis

☐ **A4- Allergy (local): Itching and redness at the** ☐ (Venipuncture Site/Medical Adhesive Medicated Tape/Skin Disinfection Area)

☐ **A5-Other major blood vessel injury -Serious conditions needing specialist medical diagnosis and attention**
- (a) ☐ Deep venous thrombosis (DVT)
- (b) ☐ Arteriovenous fistula
- (c) ☐ Compartment syndrome
- (d) ☐ Brachial artery pseudoaneurysm

National Institute of Biologicals
Ministry of Health & Family Welfare, Govt. of India

NATIONAL BLOOD DONOR VIGILANCE PROGRAMME

(Haemovigilance Programme of India)

Adverse Blood Donor Reaction Reporting Form

Version-2

Generalized Complications

☐ **B1-Vasovagal reactions**

(a) ☐ Generalized Weakness (b) ☐ Anxiety (c) ☐ Dizziness (d) ☐ Nausea
(e) ☐ Vomiting (f) ☐ Pallor(skin and lips) (g) ☐ Rapid Pulse (h) ☐ Convulsions
(i) ☐ Cold extremities (j) ☐ Hyperventilation (k) ☐ Hypotension (l) ☐ Low Vol Pulse
(m) ☐ Feeling of warmth (n) ☐ Tetany (o) ☐ Loss of bowel or bladder control (p) ☐ Cyanosis
(q) ☐ Sweating (r) ☐ Loss of Consciousness(LOC) ☐ (<60 Sec/>60 Sec)

☐ **B2-Allergic reactions (Generalized)**

(a) ☐ Cyanosis (b) ☐ Wheezing (c) ☐ Flushing,swelling of eyes,lips or tongue
(d) ☐ Chest tightness (e) ☐ Cardiac a rrest

☐ **B3-Other serious complications related to blood donation**

(a) ☐ Acute cardiac symptoms(other than myocardial infarction or cardiac arrest) (b) ☐ Myocardial infarction(MI)
(c) ☐ Cardiac arrest (d) ☐ Transient Ischemic attack (TIA) (e) ☐ Death

Apheresis Complication Yes/No

☐ **C-Complications related to apheresis**

(a) ☐ Citrate reaction
☐ tingling/vibrations-lips,fingers ☐ light-headedness ☐ Metallic taste ☐ Muscle twitching
☐ Carpopedal spasm ☐ Shock ☐ Cardiac arrest ☐ Tetany
☐ Prophylactic Calcium given before reaction ☐ (Yes/No)
(b) ☐ Haemolysis during procedure
(c) ☐ Air embolism
(d) ☐ Unable to return red cell(>200ml)

Other Complication

☐ **D-Other Reactions** Please Specify _____

Outcome* ☐ Resolved on donation site ☐ Resolved on follow up ☐ Recovered with Sequelae
☐ Permanently disabled ☐ Death following the adverse reactions ☐ Unknown

Imputability* ☐ Definite (Certain) ☐ Probable (Likely) ☐ Possible
☐ Unlikely (Doubtful) ☐ Excluded

Any Other Information: ☐

Reporter…….. **Date of Report**……..

Denominator Data about All Donor

Total Donation in the month (of reporting)

☐ **Whole blood** ☐

Volume of donation (Total)* No. of 350 ml bags ☐ No. of 450 ml bags ☐

☐ **Apheresis if apheresis** ☐ RBC ☐ Platelets ☐ Plasma ☐
Plasma+Platelets ☐ Granulocyte ☐ Peripheral Blood Stem Cells ☐
COVID-19 Convalescent Plasma ☐

Gender of Donor(Total)* Male ☐ Female ☐ Other ☐
Type of Donation(Total)* Voluntary ☐ Replacement ☐ Family Donor ☐ Autologous ☐
Donor Types(Total)* First-Time Donors ☐ Repeat Donors ☐
Site of Donation(Total)* Blood Centre ☐ Camp ☐

National Institute of Biologicals
Ministry of Health & Family Welfare, Govt. of India
(National Coordinating Center)
HAEMOVIGILANCE PROGRAMME OF INDIA

Transfusion Reaction Reporting Form (TRRF) For Blood & Blood Components & Plasma Products (Version-2)

* Mandatory Field

(A) Patient Information

Hospital Code No.:

Patient Initials*: | Gender*: | Blood Group*:

Hospital Admission No.*: | Age/Date of Birth*: | .....Yrs | .....Month | .....Days | .....Hrs | .....Mins

Primary Diagnosis*:

Medical History:

(B) Transfusion Reaction Details*

Was the patient under anaesthesia during transfusion: Yes/No if Yes type : GA/Spinal/LA

	Temp:	Pulse:	BP:	RR:	SPO2:
Pre-transfusion Vitals:					
Vitals at the time of reaction:					

Please tick mark the relevant signs and symptoms listed below

Generalised		Pain	Respiratory	Renal	Circulatory
☐ Fever	☐ Anxiety	☐ Chest Pain	☐ Dyspnoea	☐ Haematuria	☐ Tachycardia
☐ Chills	☐ Itching (Pruritus)	☐ Abdominal	☐ Wheeze	☐ Haemoglobinuria	☐ Hypertension
☐ Rigors	☐ Edema (Site)_____	☐ Back/Flank Pain	☐ Cough	☐ Oliguria	☐ Hypotension
☐ Nausea	☐ Juandice	☐ Infusion Site Pain	☐ Hypoxemia	☐ Other_____	☐ Raised JVP
☐ Urticaria	☐ Other_____	☐ Other_____	☐		☐ Arrhythmias
☐ Flushing			Bilateral Infiltrates on		☐ Other_____
☐ Restlessness			Chest X-ray		
☐ Vomiting			☐ Other_____		

Any Other(Specify) : .....

(C) Transfusion Product(s) Details*

Select*	Select Component	Select Indication	Date & Time of Issue of Blood Component	Date & Time of onset Transfusion	Unit Id (Transfused)	Blood Group	Volume Transfused (ml)	Expiry date of Blood Component	Manufacturer of Blood Bag	Batch / Lot No. of the Blood Bag	1st time/ repeat Transfusion
☐	COVID-19 Convalescent Plasma										☐ 1st Time ☐ Repeat 1 to 10 ☐ Repeat > 10
☐	Whole blood										
☐	Packed Red blood cells (PRBC)										
☐	Buffy coat depleted PRBC										
☐	Leucofiltered PRBC										
☐	Random Donor platelets/ pooled										
☐	Apheresis Platelets										
☐	Fresh Frozen Plasma										
☐	Cryoprecipitate										
☐	Any Other										

Add New Plasma Product

Select	Plasma Product	Indication	Date of Administration	Manufacturer	Expiry Date of the Plasma Product	Batch No. / Lot No.	1st Time / Repeat
							☐ 1st Time ☐ Repeat 1 to 10 ☐ Repeat > 10

(D) Investigations			
☐	Clerical Checks	Specify Error Found if any: _____	
	Investigation	**Pre-transfusion sample**	**Post-transfusion sample**
☐	Visual Check		
* ☐	Repeat Blood Grouping	O+ /A+ /B+ /AB+ /O- /A- /B- /AB-	O+ /A+ /B+ /AB+ /O- /A- /B- /AB-
* ☐	Repeat Crossmatch	☐ Compatible ☐ InCompatible ☐ Not Done	☐ Compatible ☐ InCompatible ☐ Not Done
* ☐	Repeat Antibody screen	☐ Negative ☐ Positive ☐ Not Done	☐ Negative ☐ Positive ☐ Not Done
☐	Antibody Identification		
* ☐	Direct antiglobulin test	☐ Negative ☐ Positive ☐ Not Done	☐ Negative ☐ Positive ☐ Not Done
☐	Hemoglobin		
☐	Plasma Hemoglobin		
☐	Urine hemoglobin		
☐	Bilirubin (Total/conjugated)		
☐	Platelet count		
☐	PT/INR		
* ☐	Blood culture of Blood Bag	☐ Negative ☐ Positive ☐ Not Done	Specify Organism if positive _____
* ☐	Blood culture of Patient	☐ Negative ☐ Positive ☐ Not Done Specify Organism if positive _____	☐ Negative ☐ Positive ☐ Not Done Specify Organism if positive _____
☐	Chest X-ray of the patient in case of suspected TRALI		
In case of Non-immune hemolysis (which of the following was the case?)			
☐	Hemolysis due to freezing of PRBC Units		
☐	Hemolysis due to inappropriate warming of PRBC Units		
☐	Hemolysis due to infusion of any other fluid through same BT set.	Specify Fluid: _____	
☐	Mechanical damage		
In Case of ABO Mismatch (which of the following was the case?)			
☐	Wrong Blood in tube		
☐	Grouping error		
☐	Labelling error		
☐	Wrong unit transfused		

(E) Nature of Adverse Reaction(s)*

Select	Reaction	Date & Time of Onset of Reaction	Date & Time of Recovery	Outcome
☐	Febrile Non Haemolytic Reactions (FNHTR) 1° C rise in temperature ☐ 2° C rise in temperature ☐ Only Chills & Rigors ☐			☐ 1. Death following the Adverse Reaction(s)
☐	Allergic reaction			
☐	Anaphylaxis			
☐	Immunological Haemolysis due to ABO Incompatibility			
☐	Immunological Haemolysis due to other Allo-Antibodies			
☐	Non Immunological Haemolysis			☐ 2. Recovered
☐	Hypotensive Transfusion Reaction			
☐	Transfusion Related Acute Lung Injury (TRALI) Definite ☐ Possible ☐			
☐	Transfusion Associated Dyspnoea (TAD)			
☐	Transfusion Associated Circulatory Overload (TACO)			☐ 3. Recovered with Sequelae
☐	Transfusion Transmitted Bacterial Infection			
☐	Transfusion Transmitted Parasitic Infection (Malaria)			
☐	Post Transfusion Purpura			
☐	Transfusion Associated Graft versus Host Disease (TAGvHD)			☐ 4. Unknown
☐	Other Reaction (s) Add New			

IMPUTABITLITY ASSESSMENT

(F) Imputability Assessment*

S. No.	Reaction Term	Transfusion Product/ Component	*Imputability Assessment (Please mention from the below list)

***Imputability: 1. Definite (Certain), 2. Probable (Likely), 3. Possible, 4. Unlikely (Doubtful), 5. Excluded, 6. Not Assessed**

Monthly Denominator Reporting Form *

Hospital Code :	Month/Year:
Blood Component	**No.of Units Issued**
1) COVID-19 Convalescent Plasma	
2) Fresh Frozen Plasma	
3) Whole Blood	
4) Packed Red Blood Cells (PRBC)	
5) Buffy Coat Depleted PRBC	
6) Leucofiltered PRBC	
7) Random Donor Platelets/ Pooled	
8) Apheresis Platelets	
9) Cryoprecipitate	
10) Any Other_____	

J. Imputability and severity of adverse transfusion and donor reactions

Imputability levels

Imputability means the likelihood that an adverse reaction in a recipient can be attributed to the blood/blood components transfused. The imputability levels are given below

Term	Assessment scale
Definite (certain)	When there is conclusive evidence beyond reasonable doubt that the adverse event can be attributed to transfusion
Probable (likely)	When the evidence is clearly in favour of attributing the adverse event to the transfusion
Possible	When the evidence is indeterminate for attributing the adverse event to transfusion or an alternate cause
Unlikely (doubtful)	When the evidence is clearly in favour of attributing the adverse event to causes other than transfusion
Excluded	When there is conclusive evidence beyond reasonable doubt that the adverse event can be attributed to causes other than the transfusion

Grades of severity

Grade 1 (Non-severe): The recipient may have required medical intervention (e.g., symptomatic treatment), but lack of such would not result in permanent damage or impairment of a body function.

Grade 2 (Severe): The recipient required in-patient hospitalization or prolongation of hospitalization directly attributable to the event OR persistent or significant disability or incapacity OR medical or surgical intervention required to preclude permanent damage or impairment of bodily function.

Grade 3 (Life-threatening): The recipient required major intervention following the transfusion (vasopressors, intubation, transfer to intensive care) to prevent death.

Grade 4 (Death): The recipient died following an adverse reaction, and the death is possible, probably or definitely related to transfusion.

If the recipient died of another cause, the severity of the reaction should be graded as 1, 2, 3.

K. Tables showing phenotype prevalence of minor blood group antigens in Indian blood donors

Antigen		Prevalence in the population (%)			
Traditional	ISBT symbol	Indian	Caucasian	Blacks	Asians
C	RH2	83.8	68	27	93
c	RH3	58.1	80	96	47
E	RH4	19.4	29	22	39
e	RH5	98.4	98	98	96
K	KEL1	4.4	9	2	-
k	KEL2	99.9	>99.99	>99.99	-
Jk^a	JK1	80.8	77	92	73
Jk^b	JK2	67.8	74	49	76
Fy^a	FY1	85.5	66	10	99
Fy^b	FY2	57.1	83	23	18
M	MNS1	87.2	78	74	-
N	MNS2	62.9	72	75	-
S	MNS3	54.2	55	31	-
s	MNS4	88.2	89	93	-
P1	P1	65.3	79	94	-
Le^a	LE1	17.4	22	23	-
Le^b	LE2	45.6	72	55	-

L. Formula to calculate antigen-negative blood units using phenotype prevalence

The phenotype prevalence of common blood group antigens (available in Annexure V) is useful in calculating the average number of blood units which need to be screened to find the requisite number of phenotype negative units for a patient with identified alloantibodies. The following formula may be used for this purpose:

$N=X/\{(1-PA1)(1-PA2).....(1-PAy)\}$ Where, N = Average number of units to be screened

X = No of antigen negative units required

PA1 = Phenotype prevalence of antigen of 1st alloantibody

PA2 = Phenotype prevalence of antigen of 2nd alloantibody

PAy = Phenotype prevalence of antigen of yth alloantibody

For example, consider a patient with anti-Jk^a, Anti-c, and Anti-K for whom a blood request has been sent to arrange three units of red cells. By applying the formula above we can easily get the average number of units we should screen to search three units negative for Jk^a, c and K antigens as below.

$n=3 / \{(1-P\ Jk^a)\ (1-P\ c)\ (1-P\ K)\}$

$n = 3 \div \{(1-0.808)\ (1-0.581)\ (1-0.044)\}$

$n = 3 \div \{0.192 \times 0.419 \times 0.956\}$

$n = 3 \div 0.077 = 38.96$, rounded to 39 units

Thus, one needs to screen an average of 39 units to search three units negative for all three antigens (Jk^a, c and K antigens) in Indian settings.

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