

Training Module For
Blood Bank
Medical Officers
And Lab
Technicians

2015

Ministry of Health and Family Welfare
Government of India



भानु प्रताप शर्मा
सचिव
B.P. SHARMA
Secretary



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

Foreword

Blood Transfusion Services in India have advanced significantly through the Blood Safety Program which has been an integral part of all phases of the National AIDS Control Programme since 1992. This has contributed immensely in improving access to safe and quality blood, and in promotion of Voluntary Blood Donation, and has also led to several advancements in terms of better policies, improved infrastructure and adoption of modern technologies.

Capacity building and training are a vital part of service delivery. Regular and standardized training leads to improvements in the knowledge, skills and standards of personnel providing blood transfusion services. The scope of a standardized training curriculum on Blood Transfusion Service is to train the Medical Officers, Staff Nurses, Counselors and Lab Technicians of the Blood Bank to become totally familiar with the basic techniques of Blood Banking, and to help them adopt techniques which comply with the regulatory framework in the field of Blood Transfusion.

The set of training modules is intended to emphasize Good Laboratory Practices (GLP) and Quality Management Systems (QMS) in Blood Transfusion Services. Training would be imparted through identified centres identified by National AIDS Control Organization, Ministry of Health and Family Welfare. These training centres will work towards capacity building for all cadres of the Blood bank staff across all facilities situated in different regions of the country.

I am confident that this training module would be very useful for all in the field of blood transfusion services.


(B.P. Sharma)



सत्यमेव जयते

एन. एस. कंग, भा.प्र.से
अपर सचिव

NAVREET SINGH KANG, IAS
Additional Secretary



भारत सरकार

स्वास्थ्य एवं परिवार कल्याण मंत्रालय

राष्ट्रीय एड्स नियंत्रण संगठन

Government of India

Ministry of Health & Family Welfare

National AIDS Control Organisation

Preface

The goals and objectives of the Blood Safety Programme are to ensure the provision of safe and quality blood, even to remote areas of the country. National AIDS Control Organisation (NACO) supports a network of about 1200 Blood Banks in the Government and Charitable sectors through provision of equipment, consumables, manpower and capacity building. Nearly 70% of the country's blood requirement is met through this network. The scenario of blood banking in India owes much of its modernization due to the efforts made during various phases of the National AIDS Control Programme (NACP).

During NACP IV, NACO has identified specific areas for strengthening technical and service quality standards, management structures, partnership mechanisms, and monitoring and evaluation systems to achieve the objective of catering to the country's blood requirements through a nationally coordinated and well networked Blood Transfusion Service.

Blood Transfusion Services Division, NACO and the National Blood Transfusion Council are committed to improve all aspects of Blood Transfusion Services in coordination with State AIDS Control Societies and State Blood Transfusion Councils. With rapid technological advancement in transfusion medicine, there is a felt need for an elaborative and standardized training curriculum so as to comply with the Indian health Policy Framework. The current training curriculum is based on changing needs of transfusion professionals.

This training module has been prepared with an objective of introducing uniform standards in all aspects of blood banking for medical officers, staff nurses, counselors and laboratory technicians working in the blood banks. The module is designed for the better understanding and comprehension of blood banking processes and procedures, in order to improve technical and managerial skills of the personnel.

Appropriate in-service training programmes will facilitate provision of trained manpower to enhance quality of transfusion services and to keep abreast with the latest developments in this fast changing field.

(N.S. Kang)

6th Floor, Chandralok Building, 36 Janpath, New Delhi -110001, Telefax : 011-23325331

E-mail : nacoasdg@gmail.com

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IAS
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एड्स नियंत्रण विभाग
राष्ट्रीय एड्स नियंत्रण संगठन
9वां तल, चन्द्रलोक बिल्डिंग, 36 जनपथ, नई दिल्ली -110001
Government of India
Ministry of Health & Family Welfare
Department of AIDS Control
National AIDS Control Organisation
9th Floor, Chandralok Building, 36 Janpath, New Delhi - 110 001

Message


Access to safe blood and maintaining standards in Blood Transfusion Services (BTS) is the predominant responsibility of NBTC. Currently, a network of 1161 blood banks is under the umbrella of NACO support and it is essential to provide regular Training to the Blood Bank staff, throughout the country.

This series of training modules is designed to train the blood bank staff on the basic techniques of Blood Banking which comply with worldwide standards in the field of Blood Transfusion.

The module is intended to incorporate Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP) and quality systems for Blood Bank personnel.

18 Training Institutes have been identified under NACP IV. These Training Institutes will work towards capacity building of the Blood bank staff through standardized training curriculum for all cadres of staff in Blood Banks.

I would like to place on record my appreciation to Dr. R.S. Gupta, DDG BTS Division, Dr. S. D. Khaparde, ex- DDG, the BTS team at NACO and other organizations who contributed to the development of these guidelines.


(K B Agarwal) 29.5.15

Dr. Jagdish Prasad
M.S., M.Ch., FIACS
Director General of Health Services



भारत सरकार
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
स्वास्थ्य सेवा महानिदेशालय
निर्माण भवन, नई दिल्ली-110 108
GOVERNMENT OF INDIA
MINISTRY OF HEALTH & FAMILY WELFARE
DIRECTORATE GENERAL OF HEALTH SERVICES
NIRMAN BHAWAN, NEW DELHI-110 108
Tel.: 23061063, 23061438 (O), 23061924 (F)
E-mail : dghs@nic.in

दिनांक/Dated.....

Message

Ensuring the safety and availability of Blood and Blood products is an essential Public Health responsibility. Measures to ensure blood safety also play a major role in preventing the transmission of HIV, Hepatitis virus and other Blood borne pathogens in health care settings.

Access to sufficient and safe Blood and Blood products provided within a National Blood System is a vital component in achieving Universal health coverage. So far, Blood Transfusion Services were available only through established Blood Banks extending up to district level. In 2003, under National Health Mission these services were made available at sub-district level through Blood Storage Units at First Referral Units. These Centres were aimed at meeting the requirements of blood for pregnant women requiring blood transfusions during pregnancy or labour.

For quality, safety and efficacy of Blood and Blood products, well equipped Blood Centres with adequate infrastructure and trained manpower is an essential requirement. To ensure effective clinical use of Blood and to maintain quality standards in Blood Banking procedures, training of clinical staff is important.

18 Training Institutes are identified to enhance quality services in Blood Transfusion Services. These Training Institutes will work towards capacity building of the Blood Banks through standardized Training Curriculum for all cadres of staff in the Blood Banks.

These revised editions of training modules for Blood Bank staff will be a useful resource for standardizing Blood Transfusion services across blood banks situated in different regions of the country. My congratulations to, Dr. R.S. Gupta, DDG, the BTS team at NACO and other organizations who contributed in the development of these modules.

(Dr Jagdish Prasad)



सत्यमेव जयते

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9वां तल, चन्द्रलोक बिल्डिंग, 36 जनपथ, नई दिल्ली -110001
Government of India
Ministry of Health & Family Welfare
National AIDS Control Organisation

Dr. R S Gupta

M.D.

Deputy Director General
(NACO)

Tel. : 91-11-23736851
Fax : 91-11-23731746
E-mail : rsgupta.naco@gmail.com

9th Floor, Chandralok Building,
36 Janpath, New Delhi - 110 001

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(Dr. R.S Gupta)

LIST OF CONTRIBUTORS

EXPERT MEMBERS (IN ALPHABETICAL ORDER):

Dr. Ashish Jain

Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh.

Dr. Atul Sonkar

Sanjay Gandhi Post Graduate Institute of Medical Science (SGPGIMS), Lucknow.

Dr. Charusmita Modi

King Edward Medical Hospital (KEM), Mumbai.

Dr. Debasish Gupta

Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum.

Dr. Dolly Daniel

Christian Medical College (CMC), Vellore.

Dr. Gajendra Gupta

Santokba Duriabhji Memorial Hospital, Jaipur.

Dr. Hari Krishan Dhawan

Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh.

Dr. Harprit Singh

National Programme Officer , Blood Transfusion Services, NACO, MoHFW and National Consultant Blood Safety, WHO - India

Dr. Jayashree Sharma

King Edward Memorial Hospital, Mumbai.

Dr. Joy Mammen

Christian Medical College(CMC), Vellore.

Dr. Kabita Chatterjee

All India Institute of Medical Sciences (AIIMS), New Delhi.

Dr. K. Selvarajan

Rajiv Gandhi Government General Hospital, Chennai.

Dr. Manisha Srivastava

Bhopal Memorial Hospital and Research Centre (BMHRC), Bhopal.

Dr. M. D. Gajjar

BJ Medical College, Ahmedabad.

Dr. Neelam Marwah

Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh.

Dr. Narinder Naidu

Indian Red Cross Society, Mumbai.

Dr. Pauline Harvey

U.S. Centers for Disease Control and Prevention - Division of Global HIV / AIDS.

Dr. P. Arumugam
Tamil Nadu Dr. MGR Medical University, Chennai.

Dr. P. Mahalingam
Christian Medical Association of India(CMAI), New Delhi.

Dr. Rekha Hans
Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh.

Dr. R. K. Chaudhary
Sanjay Gandhi Post Graduate Institute Medical Science (SGPGIMS), Lucknow.

Dr. R. N. Makroo
Apollo Hospital, Mathura Road, New Delhi.

Dr. R. R. Sharma
Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh.

Dr. Sabita Basu
Tata Medical Centre, Kolkata.

Dr. Sarika
Christian Medical Association of India (CMAI), New Delhi.

Dr. Sanjay Kumar Jadhav
National Adviser (BTS), NACO, MoHFW, & (Senior Technical Advisor, CMAI / CDC /QMS / BB Project)

Dr. Shanoo Mishra
PO-QC, (BTS), NACO, MoHFW.

Dr. Shobini Rajan
ADG, (BTS), NACO, MoHFW.

Dr. Suchet Sachdev
Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh.

Dr. Sunder Periyavan
National Institute of Mental Health and Neuro-Sciences (NIMHANS), Bangalore.

Dr. Sunil D. Khaparde
DDG, (BTS), NACO, MoHFW.

Dr. Sunil Rajadhyaksha
Tata Memorial Hospital, Mumbai

Dr. Sunita Upadhyaya
U.S. Centers for Diseases Control and Prevention – Division of Global HIV/ AIDS.

Dr. Veena Doda
Ram Manohar Lohia Hospital(RMLH), New Delhi.

Dr. Zarin S. Bharucha
Federation of Mumbai Blood Bank, Mumbai.



CONTENTS

	Acronyms	v
	List of Tables	vii
	List of Figures	ix
	Introduction to blood transfusion services	x
01	Blood donor selection	1
	1.1. Components of donor selection	2
	1.2. Donor room procedures	4
	1.3. Organization of blood donation camps	23
02	Immunohaematology	30
	2.1. Basics of red cell serology	31
	2.2. ABO and Rh blood group typing	34
	2.3. ABO grouping discrepancies	39
	2.4. Anti globulin test	42
	2.5. Compatibility testing (Cross matching)	44
	2.6. Quality control of immunohematology reagents	50
03	Transfusion Transmissible Infections (TTIs)	59
	3.1. Characteristics of transfusion transmissible pathogens	60
	3.2. Human immunodeficiency virus	61
	3.3. Hepatitis 'B' Virus	62
	3.4. Hepatitis 'C' Virus	63
	3.5. Syphilis	64
	3.6. Malaria	65
	3.7. Generalised course of TTI	66
	3.8. Types of assay	69
	3.9. Selection of assays	72
	3.10. Blood screening process	72
	3.11. Preventive strategies for transfusion transmissible infections	80
04	Biosafety	84
	4.1. Risk to healthcare workers (HCW)	85
	4.2. Good laboratory practices	86
	4.3. Biomedical waste management	89
	4.4. Disinfection	91
	4.5. Autoclaving	92
	4.6. Disposal of blood and laboratory material	92

05

Blood components	95
5.1. Constituents of blood	96
5.2. Blood component separation	98
5.3. Steps of component preparation	100
5.4. Plateletpheresis	103
5.5. Storage and shelf life of components	103
5.6. Preparation of leucodepleted blood components	103
5.7. Quality control of blood / blood components	104
5.8. Storage of blood and blood components prior to transfusion	112
5.9. Criteria for reissue of received back blood bags	113
5.10. Procedure for tracing a unit of blood	113

06

Pre-transfusion issues and bedside practices	116
6.1. Procedure for collection of blood sample of the patient for pre-transfusion testing	117
6.2. Procedure for arranging / requisitioning of blood/ component from the BTS	117
6.3. Administration of blood and blood component	118
6.4. Disposable equipment for administration of blood	121
6.5. Paediatric patients	121
6.6. Blood warmers	121
6.7. Concurrent fluids and medications	122
6.8. Role of Hospital Transfusion Committee (HTC)	122

07

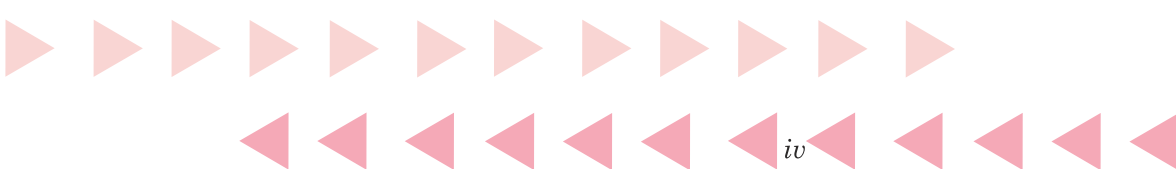
Transfusion reactions	126
7.1. Immune transfusion reactions	127
7.2. Non immune transfusion reactions	134
7.3. Laboratory investigations	135
7.4. Haemovigilance	136

08

Quality management of blood transfusion service	139
8.1. Terms and definitions in Quality Management System	140

References	155
-------------------	------------

Annexures	156
------------------	------------



ACRONYMS

ACD	Acid Citrate Dextrose	▲	CRYO	Cryoprecipitate
AIDS	Acquired Immune Deficiency Syndrome	▲	CuSO ₄	Copper Sulphate
AMC	Annual Maintenance Contract	▲	DAT	Direct Anti-Globulin Test
APh	Apheresis	▲	D&CA	Drugs and Cosmetics Act, 1940
AZT/ZDV	Zidovudine	▲	DGHS	Directorate General of Health Services
BTS	Blood Transfusion Services	▲	DHTR	Delayed Haemolytic Transfusion Reaction
CD4	Cluster Of Differentiation	▲	DIC	Disseminated Intravascular Coagulation
CDC	Centers For Disease Control and Prevention (United States)	▲	EBV	Epstein Barr Virus
CDP/CP	Cryo Deficient Plasma / Cryo Poor Plasma	▲	EDTA	Ethylene Diamine Tetraacetic Acid
CMC	Comprehensive Maintenance Contract	▲	EIA	Enzyme Immuno Assay
CMV	Cytomegalovirus	▲	ELISA	Enzyme-Linked Immuno Sorbent Assay
CMAI	Christian Medical Association of India	▲	FFP	Fresh Frozen Plasma

GA	General Anaesthesia	▲	PEP	Post Exposure Prophylaxis
Hb	Hemoglobin	▲	PRBC	Packed Red Blood Cells
HBIG	Hepatitis Immunoglobulin	▲	PRO	Public Relation Officer
HbsAg	Hepatitis B Surface Antigen	▲	PRP	Platelet Rich Plasma
Hct	Hematocrit	▲	PTP	Post Transfusion Purpura
HDN	Haemolytic Disease of the Newborn	▲	QMS	Quality Management System
Hg	Mercury	▲	SAGM	Sodium-chloride, Adenine, Glucose, Mannitol
HTC	Hospital Transfusion Committee	▲	SOP	Standard Operating Procedure
HTR	Haemolytic Transfusion Reaction	▲	SBTC	State Blood Transfusion Council
IAT	Indirect Antiglobulin Test	▲	TA-GVHD	Transfusion Associated Graft Versus Host Disease
IH	Immunoematology	▲	TPHA	Treponema Pallidum Haemagglutination Assay
ICTC	Integrated Counselling and Testing Centre	▲	TRALI	Transfusion Related Acute Lung Injury
MSW	Medical Social Worker	▲	TTIs	Transfusion transmissible infections
NACO	National AIDS Control Organization	▲	VDRL	Venereal Disease Research Laboratory
NBTC	National Blood Transfusion Council	▲	WB	Whole Blood
PC	Platelet Concentrate	▲		

LIST OF TABLES

Table 1.1	Hemoglobin concentration and Interpretation	6
Table 1.2	Quality Control of CuSO ₄	6
Table 1.3	Deferment of blood donation	9
Table 1.4	Recommended blood bags for various components	16
Table 1.5	Classification of Donor Adverse Reactions	19
Table 1.6	Materials required during an emergency in the post donation period	22
Table 1.7	Required number of beds according to the expected number of donors	24
Table 2.1	Characteristics of Antibodies or Immunoglobulins	31
Table 2.2	Tube Agglutination grading chart	32
Table 2.3	Reagents and action of potentiators	34
Table 2.4	Interpretation of forward and reverse grouping results	37
Table 2.5	Direct Antiglobulin Test (DAT) (Used to detect in-vivo sensitization of RBCs)	42
Table 2.6	Indirect Antiglobulin Test (IAT) (Used to detect in-vivo sensitization of RBCs)	42
Table 2.7	Choice of blood for Double Volume Exchange Transfusion	49
Table 2.8	Choice of Blood / Blood Components	50
Table 2.9	Quality control of ABO reagents	52
Table 2.10	Quality Control of Anti-A, Anti-B and Anti-AB (Monoclonal antisera) - Frequency of control	52
Table 2.11	Quality Control of Anti-A, Anti-B and Anti-AB (Monoclonal antisera) - Titre & Intensity	52
Table 2.12	Quality control of anti D	53
Table 2.13	Avidity, intensity and titre of anti D	53
Table 2.14	Quality control of anti-human globulin (AHG)	54
Table 2.15	Quality control of reagents red cells	55
Table 2.16	Quality control of enzymes	55
Table 2.17	Quality control of bovine serum albumin	55
Table 3.1	Transfusion Transmitted Agents	60
Table 3.2	TTIS with various methods of detection	67
Table 3.3	Comparison of ELISA with Rapid Assay	71
Table 3.4	Specimen problems	78

LIST OF TABLES

Table 3.5	Clerical errors	78
Table 3.6	Kit dependent errors	79
Table 3.7	Technologist dependent errors	79
Table 3.8	Equipment based errors	79
Table 3.9	Environment dependent errors	80
Table 3.10	Non repeatable / Non-results	80
Table 4.1	Modes of exposure to blood borne pathogens in the laboratory	85
Table 4.2	PEP for HBV	88
Table 4.3	HIV Post-Exposure Prophylaxis Evaluation	88
Table 4.4	Post-Exposure Chemoprophylaxis	89
Table 4.5	Categories of Bio-Medical Waste	89
Table 4.6	Segregation and Disposal	90
Table 5.1	Components - Storage Temperature and Shelf Life	103
Table 5.2	Quality control of whole blood	105
Table 5.3	Quality control of Red cell concentrate	105
Table 5.4	Quality control of Red cell concentrate in preservative solution	105
Table 5.5	Quality Control of Fresh Frozen Plasma (FFP)	106
Table 5.6	Quality control of Cryoprecipitate	106
Table 5.7	Quality control of Platelet concentrates, prepared from whole blood	109
Table 5.8	Quality control of Platelet concentrate prepared from buffy coat	109
Table 5.9	Quality control of Platelet Concentrate prepared by apheresis	110
Table 6.1	Time limit for transfusion	121
Table 8.1	Schedules and procedures for equipment maintenance and calibration	146

LIST OF FIGURES

Figure 1	Copper Sulphate Solution	7
Figure 2	Copper Sulphate solution - Specific gravity measurement (1.053)	7
Figure 3	Single Blood Bag	15
Figure 4	Double Blood Bag	15
Figure 5	Triple Blood Bag System	15
Figure 6	Quadruple Blood Bag System	15
Figure 7	Penta Blood Bag System	15
Figure 8	Cleaning phlebotomy site with Povidone Iodine	17
Figure 9	Cleaning phlebotomy site with Isopropyl Alcohol	17
Figure 10	Sample layout of blood donation camp layout	25
Figure 11	Internal Panoramic View of Blood Mobile	26
Figure 12	Quality control of antisera by time estimation	51
Figure 13	Appearance of serological markers in HBV infection	63
Figure 14	Generalized course of Transfusion Transmissible Infections (TTIs)	66
Figure 15	Universal Bio Hazard Symbol	91
Figure 16	Colour Coded Bins	91
Figure 17	Red Bin with Infected Waste Discard	91
Figure 18	Black Bin with General Waste Discard	91
Figure 19	Constituents of Blood	96
Figure 20	Distribution of blood components by size and weight	99
Figure 21	Whole Blood showing color coded label and segments	100
Figure 22	Red Cell Concentrate color coded label and segments	100
Figure 23	Fresh Frozen Plasma Unit	100
Figure 24	Preparation of Packed Red Cells and Plasma	101
Figure 25	Preparation of Packed Red Cells in Additive Solution	101
Figure 26	Preparation of Blood Components by Buffy Coat method	102
Figure 27	Transfusion reaction	128
Figure 28	Concept of Quality Management	141
Figure 29	Deviation and Adverse Events	149
Figure 30	Levels of documentation	150

INTRODUCTION TO BLOOD TRANSFUSION SERVICES

Blood transfusion is an essential and life-saving support within the health care system but the safety and availability of blood and transfusion is not assured. The threats include:

- An insufficient number of voluntary non-remunerated repeat regular blood donors to ensure an adequate supply of safe blood and blood components.
- Risk of the transmission of infections such as HIV, hepatitis B and C, and syphilis through unsafe transfusion.
- Unnecessary transfusions, which needlessly expose patients to the risk of acute or delayed reactions and transfusion-transmitted infections.
- Errors in the transfusion of blood and blood components.

In order to ensure sustainability and overall safety of the entire blood transfusion process, an effective quality management system needs to be implemented.

There are many definitions of quality, one of the simplest and most appropriate being “fit for purpose”. In the context of blood transfusion, this means the setting and meeting of basic national quality standards and then continual quality improvement to ensure the safety of the transfusion process.

This training program is designed to improve the safety and quality of blood transfusion by capacity building in all aspects of blood transfusion.

The fundamental aim of this training is therefore to instill an understanding in the participants that for blood safety, a culture of quality has to be created in BTSs and that all staff needs to be fully aware of this culture and play their part in putting it into practice. An important part of the training course, and a measure of its effectiveness, is to ensure that course participants are aware of their central role in this process and their interest and enthusiasm for quality is aroused. One of the fundamental objectives of the courses is to help participants develop a realistic plan of action for establishing an effective quality system in their blood banks.



Blood Safety

Blood safety depends on the following factors

- The incidence and prevalence of transfusion - transmissible infections in the blood donor population.
- The effectiveness of the blood donor education and recruitment programme and procedures for donor selection and screening, including the deferral or exclusion of unsuitable donors.
- The quality of screening of all donated blood for transfusion-transmissible infections.
- The quality of blood grouping, compatibility testing, component preparation and the storage and transportation of blood products.
- The extent to which blood and blood products are prescribed only when there is no alternative to transfusion for the particular patient.
- The reliability of the system for ensuring that patients receive blood that is compatible with their blood group, red cell antibodies and other special requirements.

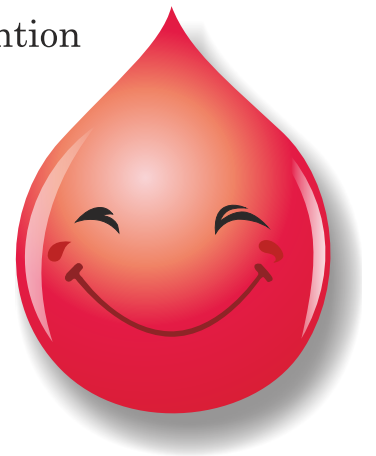
It is therefore important that the quality and safety of all blood and blood products be assured throughout the process from the selection of blood donors to the administration of the product to the patient.

BLOOD DONOR SELECTION

Learning Objectives

When you have completed this chapter you should be able to:

- Identify the donors for blood collection
- Describe the types of donors and the reasons for the deferral of donors
- Perform estimation of hemoglobin
- Recognize the donor's reaction and its prevention
- Organize blood donation camps



1.1 COMPONENTS OF DONOR SELECTION

Blood collection is the most essential function of a blood transfusion service and proper attention should be given to its arrangement. Blood donation must be made a pleasant and rewarding experience for the blood donor.

1 Donor Recruitment and Retention

Recruitment and Retention of voluntary blood donors is the key to safe and sufficient blood supply. The goal to be achieved is a panel / registry of repeat donors who are well informed, committed and regularly screened for markers of transfusion transmissible diseases. The universal principle is careful planning, organization, knowledge about the communities and their motivating factors, effective communication and campaign. Good service and support of donors as well as good public reputation goes a long way in increasing the voluntary safe blood donors or donation.

The other vital principle is that everyone involved in Blood Transfusion Services (BTS) should advocate for voluntary blood donation. Doctors, nurses, technicians, donor recruiters, PRO/MSW, driver, attendants as well as paramedics are all part of a team with a common purpose and an important message to get across. The task is to cause 100% voluntary donation, which will take time, but we have the greatest resource - PEOPLE. Success lies in harnessing “PEOPLE POWER”.

2 Donor Education

- Awareness of the kind of information people need before deciding to donate blood is an important basis for Donor education, motivation and recruitment activities
- Donor education about the need for safe blood is essential for donor recruitment strategy
- Community organizations and individual volunteers play an important role in donor recruitment
- Educational materials in the form of leaflets, posters, films do support donor education. Educational talks delivered at important forums on safe blood will help in motivating the public to donate blood.
- Effectiveness of donor education, motivation and recruitment should be monitored and evaluated on a regular basis.

The donors are the most important people in any blood transfusion set up as without them the services cannot continue to operate.

- Everyone involved in interviewing and counselling should develop a friendly and tactful approach that encourages donors to be honest and accurate in their answers to questions about their medical history
- The health check should always be handled professionally so that the donors feel they are in good hands
- The environment around the blood donation area should be safe, pleasant and convenient
- It is always essential for the staff to show a high degree of professionalism, to be smart, and to maintain personal hygiene
- They should be good mannered and be capable of conversing freely with the donors at the time of donation.
- An act of carelessness or lack of professionalism by staff during or after donation can be detrimental to the donors coming back to donate blood.

It is important to make the experience of blood donation pleasant for donors by providing

- Hygienic safe environment
- Short waiting time
- Privacy during pre - donation counselling
- Adequate explanation of the procedures
- Reasons for temporary or permanent deferral
- Individual care for each donor
- Sensitivity to their feelings of fear and embarrassment
- A word of appreciation and thankfulness to motivate them to come again.
- Indiscreet comments about donor's previous health check results and deferral should not be made.
- Chatting with other staff and ignoring the donor should be avoided.

1.2 DONOR ROOM PROCEDURES

1 Location

The blood donation complex should be located at a place which is easily accessible to the general public and the patient's relatives.

2 Types Of Donors

- (i). Voluntary blood donors
- (ii). Replacement / Relative donors
- (iii). Professional donors
- (iv). Autologous blood donors
- (v). Donors for special procedures (Plateletpheresis / Plasmapheresis / Erythrocytapheresis / Haematopoietic stem cell collection / Leucopheresis etc)
- (vi). Directed or designated donors

(i). Voluntary Non-remunerated Donors

The voluntary donors give blood voluntarily without receiving any remuneration in the form of money or a substitute for money. Their primary motivation is to help unknown recipients and not to obtain any personal benefit.

They are the safest donors because they are more likely to be free from TTI as they have been educated about the importance of safe blood. Once they become repeat donors, they are screened each time they donate blood thereby reducing the chances of window period donation.

(ii). Replacement Donors

The replacement donors come to the BTS with a request from the physician treating the patient, giving particulars of the patient like name, ward, hospital ID, diagnosis, and an estimate of the blood and blood components likely to be required. Members of the patient's family are under pressure to donate blood and may conceal potentially important information about their health status, particularly the risk of transmitting an infectious disease.

Relatives who cannot find suitable or willing donors within the family may seek replacement donors who are prepared to give their blood for payment. Donors who are paid by the patient's family are less likely to reveal any reasons why they may be unsuitable as donors.

(iii). Professional donors

They give blood in return for money. Though strictly banned by regulations, they still continue to donate blood under the garb of replacement donors. They do not reveal their unsuitability to donate blood. Paid donors present a major risk to the safety of the blood supply for the following reasons.

- Paid donors undermine the voluntary non remunerated system of blood donation which is the foundation of a safe blood supply.
- The highest incidence and prevalence of transfusion-transmissible infections are generally found among commercial or paid donors.
- They are often under nourished, in poor health and may donate their blood more frequently than it is recommended. This may have harmful effects on their own health as well as present a risk to the recipients.

Professional donors should be rejected and may be identified by the following criteria:

- Nervousness and aggressive behaviour
- Vague answers
- Needle marks / scars on antecubital areas of both arms
- Mismatched in socioeconomic status of donor and patient
- Unwillingness to bring other donors

(iv). Autologous Donors

They are donors who donate blood for themselves, to be used at a later date. Recipients who serve as their own donors receive the safest possible blood since the risks of TTI and alloimmunization are completely eliminated.

(v). Aphaeresis donors

They are a special category of donors who willingly donate only the specific blood component required. They need to be screened thoroughly before putting them on the aphaeresis machine.

3 Measurement of Hemoglobin

(i). Hemoglobin Estimation by Copper Sulphate (CuSO_4) Test

Principle: This is a semi quantitative test based on specific gravity a drop of blood with hemoglobin concentration of not less than 12.5 g/dL sinks in a CuSO_4 solution with a specific gravity of 1.053.

30 ml of copper sulphate working solution (sp.gr.1.053) in a clean, dry beaker is used for determining hemoglobin. The working solution is changed after testing 25 samples.

Procedure:

1. The fingertip is cleaned thoroughly with a spirit swab and allowed to dry.
2. Medial side of the left ring finger of the donor is pricked using a sterile lancet.
3. The first drop of blood is wiped and next drop of blood is allowed to fall into a beaker containing CuSO_4 solution of specific gravity 1.053 from a height of at least 1 cm.
4. If blood drop sinks to the bottom of the solution then the hemoglobin is more than 12.5 g/dL.
5. If blood drop floats for more than 15 seconds then the hemoglobin is less than 12.5 g/dL.

Preparation of CuSO_4 for Hemoglobin Estimation

To prepare 100 ml of copper sulphate working solution (Sp.gr.1.053)

- i. Carefully weigh 159.6gms of pure air dried crystals of CuSO_4 and put it in a conical flask and add distilled water to make it to 1000ml of stock solution.
- ii. Cap the flask and mix well to ensure that the copper sulphate has dissolved.
- iii. Specific gravity of the stock solution made is 1.100.
- iv. Add 52 ml of prepared stock solution to 48 ml of distilled water to make 100 ml of working solution.
- v. Check the specific gravity (of 1.053) by hydrometer.
- vi. Solution should be stored at room temperature in tightly capped containers to prevent evaporation.

Quality control of CuSO_4

1. Check the copper sulphate working solution against a light source for presence of precipitate and cloudiness.
2. Check the specific gravity of the solution using a hydrometer.
3. Copper sulphate being a coloured solution, the marking on the hydrometer. Corresponding to the upper meniscus of the solution should be 1.053 for 12.5g/dL of hemoglobin.
4. Arrange the blood samples according to hemoglobin concentration in a rack
5. Obtain samples of known hemoglobin values.
6. Transfer 30 ml of copper sulphate working solution in a tube.
7. Mix the sample of known hemoglobin concentration by inversion technique.
8. Allow one drop of blood to fall gently into the copper sulphate solution.
9. Repeat the procedure for all blood samples.
10. Record the results in the record book.

Table 1.1: Hemoglobin concentration and Interpretation

Results	Hemoglobin concentration	Interpretation
Blood drop floats	Hemoglobin < 12.5gm/dL	Defer
Blood drop sinks	Hemoglobin \geq 12.5gm/dL	Accept

Note: Drop to be observed in 15 seconds

Table 1.2: Quality Control of CuSO_4

Date of Preparation of working solution _____ Technician _____

Date	Specific gravity CuSO_4	Hemoglobin estimation			Signature of Doctor
		CuSO_4 method (Hb < or \geq 12.5 gm/dL)	By Cell Counter	Any other – Hemoglobin meter / Colour strip	

CuSO₄ (stock solution)

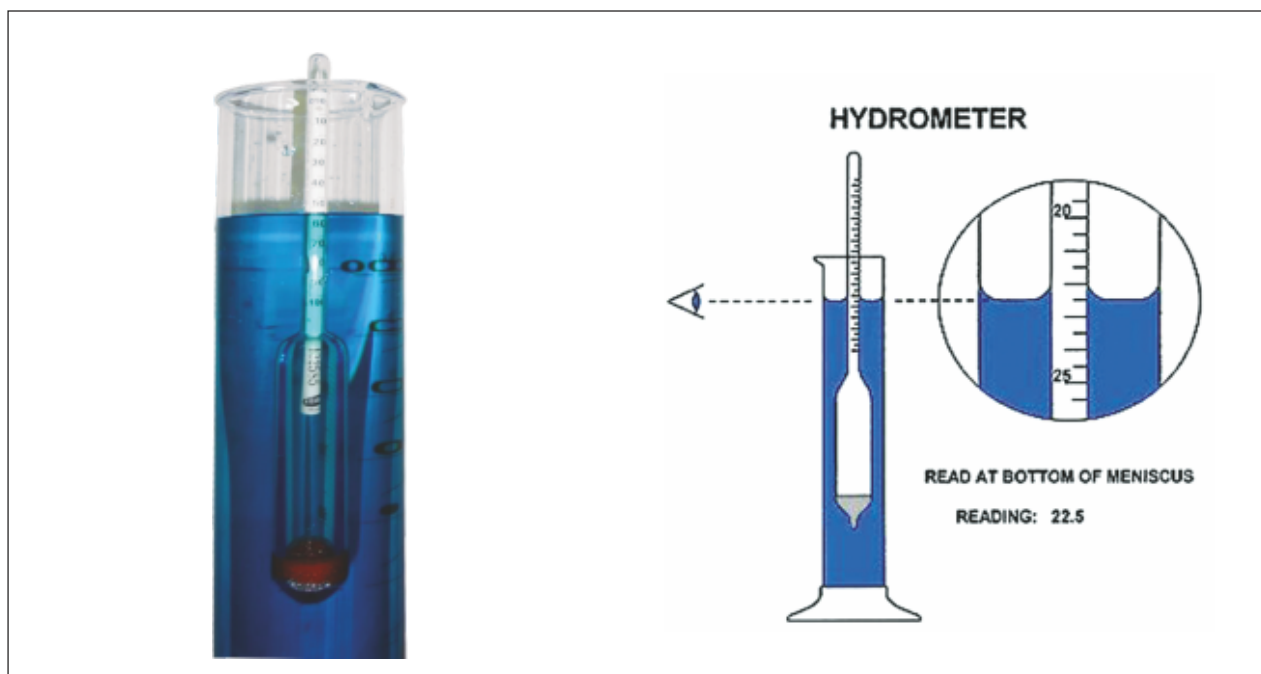
Date of preparation: _____

Volume of stock solution prepared: _____

Weight of dry crystal of CuSO₄ used: _____

Specific gravity: _____

Technician name: _____



*Figure 1:
Copper Sulphate Solution*

*Figure 2: Copper Sulphate solution -
Specific gravity measurement*

(ii). Hemoglobin estimation by portable digital Hemoglobinometer

This is a quantitative test.

Principle: Azide methemoglobin method to measure Hb in undiluted blood.

Microcuvettes used for Hb estimation has;

Sodium desoxycholate that dissolves and disperses the cell membrane of the red blood corpuscles thereby releasing the hemoglobin formerly contained in the erythrocytes.

Sodium nitrite it oxidises the bivalent iron of the oxyhemoglobin and the deoxyhemoglobin to trivalent iron, in methemoglobin.

Sodium azide existing and formed methemoglobin and azide ions from sodium azide NaN_3 form a coloured complex which exhibits maximal absorption at 540 and 575 nm and hence it can be quantitatively determined photo metrically.

Microcuvettes can be stored for a period of two years from the date of manufacture but once opened they are stable only for three months.

Method

- i. The ring finger tip is cleaned thoroughly with spirit swab and allowed to dry.
- ii. The finger is then punctured firmly near the tip with sterile disposable lancet. A good free flow of blood is ensured; the finger is not to be squeezed repeatedly since it may dilute the blood drop with excess tissue fluid and give false low results.
- iii. The first drop of blood is wiped and the next drop of blood is allowed to fill the cuvette completely in one continuous process.
- iv. Wipe off the excess blood on the outside of cuvette. Make sure that no blood is withdrawn out of the cuvette during this procedure.
- v. Place the filled cuvette into the cuvette holder immediately and push it in a measuring position.
- vi. Results are displayed in 15-45 seconds in g/dL.
- vii. Dispose the cuvettes and lancets in 1% sodium hypochlorite solution in a puncture proof container.

Interpretation

1. Donors with hemoglobin $\geq 12.5\text{gm/dL}$ are accepted for blood donation.
2. However, if the hemoglobin level is less than 12.5gm/dL the donor is deferred. Refer the donor to a physician for advice.

4

Donor selection for whole blood donation (as per Drugs and Cosmetics Act, 1940)

Donor selection is critical to the supply of safe blood and its products.

The purpose of donor selection is to identify any factors that might make an individual unsuitable as a donor, either temporarily or permanently. The following guidelines should be observed in selection of donors.

Donor selection is done by a qualified medical officer and is based on medical history, limited physical examination and simple laboratory tests. It should be done very carefully to avoid any untoward effect on the donor or the recipient. The prospective donor should appear to be in good health.

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 inmate 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 65 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) Hemoglobin 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 of 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 the column (2) of the said Table

Table 1.3: Deferment of blood donation

CONDITIONS (1)	PERIOD OF DEFERMENT (2)
(a) Abortions	6 months
(b) History of Blood transfusion	6 months
(c) Surgery	12 months
(d) Typhoid	12 months
(e) History of malaria and duly treated	3 months (endemic) 3 years (non-endemic area)
(f) Tattoo	6 months
(g) Breast feeding	12 months after delivery
(h) Immunization (Cholera Typhoid, Diphtheria, Tetanus, Plague, Gammaglobulin)	15 days
(i) Rabies vaccination	1 year after vaccination
(j) History of Hepatitis in family or close contact	12 months
(k) 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) Polycythaemia Vera
 - (l) Asthma
 - (m) Epilepsy
 - (n) Leprosy
 - (o) Schizophrenia
 - (p) Endocrine disorders

(For details refer WHO guidelines:

www.who.int/bloodsafety/publications/bts_guideline_donor_suitability/en)

Guidelines on Assessing Donor suitability for blood donation

Physical Examination

- A prospective donor should be in good health.
- Donors weighing more than 45 kg (for 350ml) and more than 50 kg (for 450ml) are acceptable (8-9 ml/kg body weight).
- Blood pressure should be within acceptable range for a particular age group with or without medication. Diastolic BP: 60 to 90mm of Hg; Systolic BP: 100 to 160 mm of Hg.
- Hemoglobin should not be less than 12.5g/dL
- Pulse of the donor should be observed for one minute and should be between 60 to 100. Any irregularity in the pulse or pulse deficit more than 10 should be considered for deferral
- Phlebotomy site is examined for any infective lesions and scar of needle pricks indicative of intravenous drug abuse or frequent blood donations.
- Systemic examination of heart, lungs and abdomen should be normal.
- There should be no generalized lymphadenopathy or epitrochlear nodes.

5

Donor registration

It is the information form/card to be filled by the donor with all demographic details in order to trace the donor in case of any requirement.

The following information must be included

1. Date of donation
2. Name of the donor
3. Father's / husband's name
4. Age (date of birth)
5. Gender
6. Occupation
7. Address and telephone No. of residence / place of work
8. Blood group, if known
9. Date of last donation
10. Previous donor reaction, if any
11. Consent for inclusion in emergency panel
12. Previous deferral from donation (and its reasons)

(i). Donor Consent

Consent of the donor should be taken for phlebotomy and screening tests for various transfusion- transmitted diseases. Consent should also be taken for revealing the results of screening tests.

Understand that:

- (a) Blood donation is a totally voluntary act and no inducement or remuneration has been offered
- (b) Donation of blood/components is a medical procedure and that by donating Voluntarily, I accept the risk associated with this procedure.
- (c) My donated blood, blood and plasma recovered from my donated blood may be sent for plasma fractionation for preparation of plasma derived medicinal products, all of which may be used for larger patient population and not just this blood bank.
- (d) My blood will be tested for Hepatitis B, Hepatitis C, Malaria parasite, HIV/AIDS and syphilis addition to any other screening tests required ensuring blood safety.
- (e) I would like to be informed about any abnormal test results done on my donated blood: Yes/No

(ii). Directed donation

The donors for directed donation are also selected as above and are informed, that his/her donation may be used in the general pool if not transfused to the designated recipient. Consent is taken for the same in the register / form.

Directed donation from family members is discouraged and when necessary, the blood should be irradiated before transfusion to the family member for prevention of Transfusion – Associated Graft vs. Host Disease.

(iii). Pre-deposit Autologous donation (Ref: DGHS Technical Manual)

The donors for Pre-deposit Autologous donation are accepted after receiving a written request from the treating/concerned doctor that the donor can withstand the stress of donation. Donor selection criteria can be relaxed in case of autologous donors. The blood bank physician may still refuse to accept the donor if he/she doesn't find the donor fit for donation. Autologous Blood donation unit is not taken into the general inventory as relaxation in donation selection criteria may deem it unfit for other patients. However mandatory testing for infectious disease markers is carried out and any unit found reactive is discarded after notifying the concerned physician.

For Autologous Donation : Minimum hemoglobin level should be 11 g/dL or hematocrit \geq 34% There is no upper and lower age limit and no weight restrictions. However, the volume should not exceed 9ml/kg body weight.

(iv). Plateletpheresis (Ref: DGHS Technical Manual)

General screening procedure and the prerequisites are the same as for routine whole blood donation along with following special requirements for serial apheresis program.

- a. Donor should meet all the acceptable criteria.
- b. Age of the donor should be between 18 to 50 years.
- c. The weight of the donor should be more than 60kg.
- d. The results of the TTI screening tests should be non-reactive.
- e. The pre-procedure platelet count should be more than 150,000/ μ L. Donor with less than 1, 50,000/ μ L pre-procedure count is deferred irrespective of fulfilling other criteria.
- f. Donor should not have taken aspirin or any other platelet inhibitor in last 72 hours.
- g. The donor should not be fasting prior to the procedure, however should refrain from oily/spicy food, also citrus fruits or juices.
- h. Donor should have a prominent and easily accessible antecubital vein in at least one of the arms.

Note: *If the donor is on other anti-platelet drug which has a longer platelet inhibitory effect, he/she should be deferred for longer periods accordingly.*

(v). Donation Interval

The minimum time gap between two blood donations should be **12 weeks/3 months**. Whole blood donation must be deferred for at least **72 hours** after plateletpheresis. In case of re-infusion failure, donor should not donate whole blood **for 12 weeks**.

(vi). Private Interview

A detailed sexual history should be taken. Positive history should be recorded in a notebook and kept confidential. It is important to ask donors about their high risk behaviour. Allow sufficient time for discussion in the private cubicle. Try and identify result-seeking donors and refer them to ICTC (Integrated Counselling and Testing Centre). Reassure the donor that strict confidentiality is maintained.

Pre donation counselling

Donors should be given information on the blood donation process and the procedures used to identify their suitability as donors. They should be encouraged to self-exclude or self-defer.

Donor self-exclusion

The decision by a donor to not donate blood because of either being engaged in risky behaviour or the state of his/her own health.

Self-deferral

The decision by the donor to wait until an unsuitable condition resolves. Privacy and confidentiality should be maintained during donor selection.

(vii). Donor acceptance / rejection

Depending on the criteria of donor selection, the donors are accepted or deferred.

When donors are deferred either temporarily or permanently, the reasons for deferral should be explained and appropriate documentation should be done. Temporarily deferred donors should be encouraged to come for donation once the deferral period is over.

6 Blood Collection Procedure

(i). Materials required in the blood collection area.

• Demethylated spirit	• Oxygen cylinder with accessories
• Betadine	• Rubber gloves
• Isopropyl swab	• First aid tray
• Cotton/Gauze swabs	• Tube stripper
• Pilot Tubes: Plain and EDTA	• Electronic tube sealer
• Puncture proof container	• Needle destroyer
• Artery forceps, Scissors	• Blood collecting bags
• Bp Apparatus (non-mercury) / Tourniquet	• Discard Jar with 1% Sodium Hypochlorite
• Blood bag mixer / Comfortable donor couch or chair	• Adhesive plaster

(ii). Selection of blood bag for blood collection

There are variations in the volume of blood to be collected and the type of anticoagulant-preservative solution to be used. Different types of blood bags in use are:

- Single
- Double
- Triple (with or without additive solution)
- Quadruple (with additive solution)
- Penta bags

The approved anticoagulant-preservatives solutions used in the blood bag with the shelf life of whole blood/PRBC

- Acid-citrate-dextrose solution (ACD) : 21 days
- Citrate-phosphate-dextrose solution (CPD) : 28 days
- Citrate-phosphate-dextrose-dextrose solution (CP2D) : 21 days
- Citrate-phosphate-dextrose-adenine solution (CPDA). : 35 days

Whole blood is obtained from human blood donors by venesection. During donation, blood is collected into a sterile, disposable, plastic pack which contains an anticoagulant-preservative solution. This solution usually contains citrate, phosphate, dextrose and often adenine (CPDA). Their individual functions are.

Solutions	Functions
C Sodium Citrate	Binds with calcium ions in blood in exchange for the sodium salt so that the blood does not clot
P Phosphate	Supports metabolism of the red cells during storage to ensure they release oxygen readily at tissue level
D Dextrose	Maintains the red cell membrane to increase storage life
A Adenine	Provides energy source

During storage, metabolism continues in the red cells and platelets, while some plasma proteins lose their biological activity.

The biochemical and metabolic effects of storage on whole blood are

• Reduction in the pH (blood becomes more acidic)
• Rise in plasma potassium concentration (extracellular K ⁺)
• Progressive reduction in the red cell content of 2,3 diphosphoglycerate
• (2,3 DPG) may reduce the release of oxygen at tissue level until it is restored
• Loss of all platelet function in whole blood within 48 hours of donation
• Reduction in Factor VIII to 10–20% of normal within 48 hours of donation. Coagulation factors such as VII and IX are relatively stable in storage

Additive solutions (AS) : used to enhance the shelf life of RBC to 42 days. Most commonly used additive solution is SAGM which contains Saline, Adenine, Glucose and Mannitol. A donation of 450 ml of whole blood requires 100 ml of AS for extending the shelf life to 42 days.



Figure 3: Single Blood Bag



Figure 4: Double Blood Bag



Figure 5: Triple Blood Bag System



Figure 6: Quadruple Blood Bag System



Figure 7: Penta Blood Bag System

(Photos/images source 3-7: Transfusion Medicine Centre, NIMHANS, Bangalore)

Table 1.4: Recommended blood bags for various components

DONOR		COMPONENTS	BAGS	
Weight	Aspirin intake	Prepared	Type	Qty. (ml) of Blood
>50kg	No	PRBC+FFP+PC	Triple or Quadruple	450ml
>50kg	Yes	PRBC+FFP	Double	350/450ml
45-50g	No	Whole blood/ PRBC+FFP	Single/Double	350ml
45-50kg	Yes	Whole blood/ PRBC+FFP	Single/Double	350ml

PRBC: Packed Red Blood Cells

FFP: Fresh Frozen Plasma

PC: Platelet Concentrate

- Before use, check the expiry date of the bag and look for any puncture or discoloration of the bag visually. If so, reject the bag and do not use.
- Use single bag when;
 1. Components are not to be separated from that unit.
 2. When autologous blood is collected for patients e.g. elective surgery.
 3. Therapeutic phlebotomy is being performed on a patient.

(iii). Collection of blood

- Receive donor cordially in the blood collection area as he/she should feel that the staff is caring, concerned and committed.
- After a thorough screening, the donor is asked to lie down on the donor couch in a well-lit and air-conditioned environment.
- Identification of donor must be re-checked by asking him/her to tell their name and tally it with the card, blood bag and sample tubes (pilot tubes) and registration number.

Preparation of phlebotomy site

- Select prominent vein (preferably central) in the antecubital area, apply BP cuff, and inflate till 40 to 60 mm Hg. Alternately, tourniquet can be used. Lower margins of the tourniquet should be one and half inches above the antecubital area to avoid contamination.
- The area should be disinfected with two disinfectants – Iodine based and methylated spirit or isopropyl alcohol or 2% chlorhexidine to prepare the site for phlebotomy.
- Position donor's arm in naturally extended position and provide handgrip and request the donor to squeeze firmly.
- Do not use betadine if donor is allergic to iodine. Start disinfection of the skin of about an area of 5 cm of diameter from the centre outwards in a circular motion.
- Allow this solution to dry for 30 seconds. If the phlebotomy site is touched after cleaning, repeat the skin disinfection procedure.



Figure 8: Cleaning Phlebotomy site with Povidone Iodine

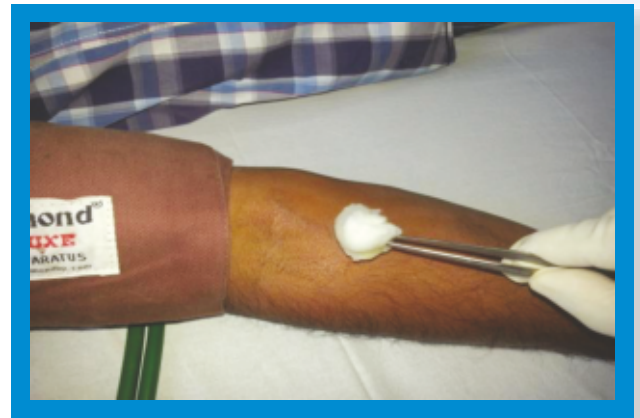


Figure 9: Cleaning Phlebotomy site with Isopropyl Alcohol

(Picture source : Transfusion medicine centre, NIMHANS, Bangalore)

- Set the blood collection monitor for desired volume and place the bag on it.
- Clamp the bleed line of the blood bag using plastic clamp to ensure that no air enters the tubing or the bag once the needle cover is removed.
- Keep the bevel of the needle upward and the shaft at an angle of 15 degree with the arm; insert the needle in the vein 1 to 1.5 cm by a bold single sharp prick.
- Monitor filling of blood bag and assure proper mixing with anticoagulant.
- Sign the donor record card after checking the number on the bag, card and tubes and indicate the arm used for phlebotomy.
- Interact with donor to prevent reaction. Allow collection to continue until desired quantity of blood is collected and **NEVER** leave the donor alone.
- Discontinue collection if donor feels uncomfortable or collection time is more than 10 min or if the donor develops haematoma at venipuncture site.

Note: If the collection is not completed, it should be considered as a donated unit and after due testing, should be discarded as under collected.

- In case of venipuncture failure, second venipuncture may be performed if the donor gives consent to do so. **NEVER** use same arm or bag for a second venipuncture.
- Seal tubing by tube sealer and cut it 8 to 10 inches away from venipuncture end and take samples in pilot tubes.
- Before separating collected unit of whole blood from donor's couch, re-inspect the blood bag for any defects and recheck donor's registration number and name on blood bag, processing tubes, records and donor slip that is to be issued in case of Relative donor / Directed donor / Autologous donor.
- Remove sphygmomanometer cuff/tourniquet.
- Apply pressure by folding the arm at phlebotomy site so that swab may not fall. Affix adhesive tape after verifying that there is no ooze at the venipuncture site and stable clot has formed.
- Rest and refreshment is to be given to donor and observe the donor for at least 15-20 minutes post donation. If he/she feels well, then he/sh.e may be allowed to leave. The first time donor, obese donor or female donor should be given extra rest and may be escorted to refreshment area.

Observe the donor

- To prevent adverse reactions like giddiness, ask the donor not to get up from the chair for 8 - 10 minutes even if he/ she feel perfectly all right.
- Observe for another 10 minutes in the refreshment area while donor has refreshment (a packet of biscuit and a tetra pack fruit drink).
- Inspect the venipuncture site before the donor leaves the donor room. Apply an adhesive tape only after oozing stops. If there is persistent oozing at the site of venipuncture, apply pressure with a dry, sterile cotton swab. If there is haematoma, apply Thrombophob ointment gently over the area after 5 minutes. Inform the donor about the expected change in skin colour. If the pain persists, ask him/her to apply ice and the donor must contact the blood bank personnel.
- Issue the donor replacement/voluntary donor card and ensure that the donor puts his/her signatures on the donor record register.
- Thank the donor and encourage him/her to become a regular voluntary blood donor.
- Ask the donor to write his comments/suggestions in the donor refreshment register.

7

Post Donation Care

Instruct the donors regarding the following:

- Take more fluids for next 4 hours
- Do not smoke or drive for next half an hour
- Do not drink alcohol for next 6 hours
- If bleeding occurs from phlebotomy site, raise the arm and apply pressure on the venipuncture site.
- If the donor is feeling dizzy, make him/her lie down with legs slightly raised above the head level and if symptoms still persist consult nearest doctor, blood bank doctor or clinician.
- Remove the adhesive band after 5-6 hours.
- Do not apply any medication on venipuncture site on your own.
- Avoid lifting heavy weight or strenuous exercise to prevent bruising or bleeding from venipuncture site.
- No extra/special diet is needed. However, iron rich foods may be advised like green leafy vegetables, jaggery, dates, meat (for non-vegetarians), etc.
- Rest and refreshment should be given to all. Thank the donor for their valuable contribution and ask them to repeat the same for this noble cause. A thank you card goes a long way for the donor to come back as a repeat donor.

8

Donor Reaction and its Prevention

Definition: Untoward feeling by blood donor before, during or after blood donation is known as a donor reaction.

Table:1.5 Classification of Donor Adverse Reactions

LOCAL REACTIONS RELATED TO NEEDLE INSERTION
Category
Vessel Injuries Haematoma Arterial puncture Thrombophlebitis
Nerve Injuries Injury of a nerve Injury of a nerve by a haematoma
Other Complications (related to needle insertion) Tendon injury Allergic reaction (local) Infection (local)
RARE, IMPORTANT COMPLICATIONS
Related to Vessel Injury Brachial artery pseudoaneurysm Arteriovenous fistula Compartment syndrome Axillary vein thrombosis
Accidents Accidents related to Vasovagal syncope Other kinds of accidents
Cardiovascular Reactions Angina pectoris Myocardial infarct Acute neurological condition (TIA, stroke)
Related to apheresis procedures Diffuse allergic reaction Anaphylaxis Haemolysis Air Embolus
Death
Other

1. Types of Vasovagal Reactions

- a. Mild Vasovagal Reaction
- b. Moderate Vasovagal reaction
- c. Severe Vasovagal reaction

a. Mild Vasovagal Reaction

Incidence 1-2%

Symptoms: Pallor, perspiration specially on palms, forehead which may be generalized, sighing or yawning, hyperventilation, feeling of warmth or air hunger, dizziness, light headedness, nausea with or without vomiting.

Management:

- Discontinue donation,
- Immediately seal venipuncture site,
- Apply wet towels to donor's forehead and ask donor to cough
- Loosen any tight clothing and ensure clear airway,
- Raise knees with feet flat on the bed/couch,
- Talk to donor to assure that it is nothing serious and he/she will be perfectly alright.
- Never leave the donor alone.

b. Moderate Vasovagal reaction

Symptoms: There is progression of all the symptoms of mild reaction and additional symptoms such as bradycardia, shallow respiration, hypotension (systolic as low as 60mm Hg) and quiet anxiety may be present. There is prolonged recovery. Donor must be protected from injury if unconsciousness occurs.

Management: In addition to treatment as in mild reaction, administration of 95% O₂ and 5% CO₂ may be helpful in case of fainting with hyperventilation. If possible, remove the donor from the general donor area to another room or use a screen to prevent sympathetic fainting. In case facility for monitored administration of 95% O₂ and 5% CO₂ is not available, rebreathing into a paper bag is helpful.

c. Severe Vasovagal Reaction

Symptoms: All symptoms of mild or moderate reaction along with any or all of the following: incontinence of urine or faeces (it's very rare), convulsions - focal or generalized.

Management: Injury to donor must be prevented and a padded tongue blade needs to be placed between the teeth if convulsions are prolonged.

Do not give anything orally during reaction. If there is no improvement within 30 minutes, shift the donor to emergency medical ward and administer 300-500 ml normal saline with or without dextrose.

After recovery post donation, instructions must be given to the donor depending upon the severity of the reaction. Documentation of such reactions must be made on the donor card/donor register and should be explained to the donor. The donor should be called the next morning or evening for follow up and for reassurance.

Observation is very essential. Keep donor under observation in rest room. If need be, assistance should be provided to accompany the donor to his destination to avoid any injury or accident.

2. **Tetany / Muscular spasm / Twitching:** These are usually due to hyperventilation in an apprehensive donor. Ask the donor to breathe in a paper bag to counteract the effect of hyperventilation by increasing amount of carbon dioxide, which provides prompt relief. Do not give oxygen. Give Inj calcium Gluconate slowly over a period of 10 minutes if tetany persists
3. **Haematoma:** Release the tourniquet/pressure cuff immediately. Apply pressure on the venepuncture site and withdraw the needle from the vein. Raise the arm above the head for a few minutes. Apply Thrombophob ointment gently around the phlebotomy site after about 5 minutes. Advise the donor to apply ice if there is pain and inform about the expected change in skin colour.
4. **Convulsions:** Keep the head tilted to the side; prevent the tongue bite; keep the airway patent by inserting a tongue blade or gauze between the teeth
5. **Eczematous reactions of the skin around venepuncture site:** Apply steroid ointment.
6. **Delayed syncope:** These may occur as late as 30 minutes to 1 hour after donation, usually after the donor has left the blood bank. Permanently defer any donor who gives history of such attacks more than twice.
7. **Accidental puncture of Artery :** This is uncommon but you should be able to recognise as a very fast flow of bright red blood. You should discontinue the donation and apply hard pressure after withdrawal of the needle for at least 15 minutes Apply a pressure bandage to be kept for 4-6 hrs Reassure the donor and explain for unsuccessful venepuncture and apologise.
8. **Problems with blood flow :** Occasionally venepuncture is unsuccessful or vein may develop spasm after venepuncture so that blood flow is not maintained. If so do not fiddle with the needle as it can cause haematoma. Remove the needle and discard the unit as it will be contaminated. Reassure the donor and explain for unsuccessful venepuncture and apologise. Record the information.

Table 1.6 Materials required during an emergency in the post donation period

<p>i. Oral medication</p> <ul style="list-style-type: none"> ▶ Analgesic tablets(Paracetamol), Calcium and Vitamin C tablets <p>ii. Injection</p> <ul style="list-style-type: none"> ▶ Inj. Adrenaline ▶ Inj. Atropine ▶ Inj. Avil ▶ Inj. Dexamethasone ▶ Inj. Hydrocortisone ▶ Inj. Phenargan ▶ Inj. Furosemide (Lasix) ▶ Inj. Dilantin ▶ Inj. Perinorm ▶ Inj. Deriphylline ▶ Inj. Soda-bi-carbonate ▶ Inj. KCL ▶ Inj. Calcium Gluconate ▶ Inj. Dopamine ▶ Inj. Rantac ▶ Inj. Diazepam ▶ Dextrose 25% 100 ml ▶ Normal Saline 500 ml ▶ Inj. Noradranaline ▶ Inj. Mefetine ▶ Inj. Diclofenac ▶ Inj. Vitamin K ▶ Inj. Tramadol ▶ Inj. Lignocaine 2% ▶ Inj. Midazolam 	<p>iii. Antiseptics</p> <ul style="list-style-type: none"> ▶ Betadin, Tincture Benzoine <p>iv. Miscellaneous</p> <ul style="list-style-type: none"> ▶ Laryngoscope ▶ Laryngoscope Blade ▶ Ambu Bag with Mask ▶ Reusable oxygen reservoir ▶ Torch ▶ Airway ▶ BT – Set ▶ IV – Set ▶ Syringe ▶ E.T. 7.5mm, 7.0mm ▶ Three-way stop cock ▶ Ventimask ▶ Veinflow – 18 G, 20 G ▶ Stilette ▶ Oxygen cylinder with key ▶ Artery forceps ▶ Kidney tray (Plastic) ▶ Emesis basin ▶ Scissors ▶ Adhesive Plaster ▶ Anti Histaminic Cream ▶ Heparin and Benzyl ▶ Nicotinate ointment ▶ Smelling Salt-Spirit of Ammonia ▶ Tongue Depressor ▶ Oxygen Cylinder ▶ Infusion Set ▶ Paper Bag
---	---

1.3 ORGANIZATION OF BLOOD DONATION CAMPS

Blood donation camp can be organized by

1. A licensed designated Regional Blood Transfusion Centre
2. A licensed Government blood bank
3. The Indian Red Cross Society
4. Licensed registered voluntary or Charitable Trust Organization recognized by State Blood Transfusion Council (SBTC)

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 Licence Approving Authority for the purpose.
- (ii) The designated Regional Blood Transfusion Centre, 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 Licence Approving Authority.
- (iii) *Definition of RBTC is under revision.*

1 Procedure for Organizing a Blood Donation Camp

The organizers or motivators are requested to furnish the following information in the confirmation letter:

- a. Exact venue of the camp
- b. Number of donors (approximately)
- c. Time of the camp
- d. Refreshment for donors i.e. whether it will be arranged by the organizers or department will reimburse for the same.

Name of organization : _____
Contact person : _____
Complete address with : _____
Tel./Fax/Mobile : _____
Phone no. : _____
Email : _____

Table 1.7 Required number of beds according to the expected number of donors

No. of Donors	No. of Couches/Beds
50-100 donors	6 to 8
100-200	8 to 10
200-300	10 to 14
Above 300 donors	14 to 16

Furniture

- Big tables - as per number of donor beds (2 tent house tables will be required for one bed).
- Folding donor couches for mobile camps.
- Chairs with arms or sofa sets for donors in the refreshment area.

Apparatus and equipments required for blood donation camp

1. BP apparatus (non-mercury)
2. Stethoscope
3. Blood bags (Single - 350, Double-350/450, Triple-350/450, and Quadruple-350/450)
4. Donor questionnaire, Registration cards and other essential stationery
5. Weighing device for blood donors
6. Weighing device for blood bags
7. Blood collection monitor
8. Beaker, pipette and tips
9. Tourniquet
10. Bed sheets, draw sheets, blankets/mattress, pillows, Macintosh and arm rests
11. Lancet, blades
12. Footsteps, hand grip balls
13. Portable Hb meter with cuvettes /copper sulphate working solution.
14. Glass tube/ Vaccum tube for pilot sample collection.
15. Big racks for test tubes.
16. Rubber bands.
17. Spirit, betadine, cotton roll.
18. Medicated adhesive tape, micropore or leucoplast roll.
19. Gel packs (coolants), anti-coagulant solution.
20. Plastic waste basket and colour coded plastic bags for waste disposal
21. Donor thank you cards.

22. Emergency medical kit
23. Insulated blood bag container's (Transport boxes) with provision for storage temperature between 2 to 10 degrees centigrade.
24. Certificates of appreciation and honour and donor badges.
25. IEC materials, Banners and post donation standee.
26. Documentation registers, other forms and formats.
27. Soap dispensers.

Space

It should be a well-lit and ventilated hall. This area can be further divided according to the instructions of the doctor in charge as;

- a) Waiting area for donors
- b) Donation area
- c) Post donation care area

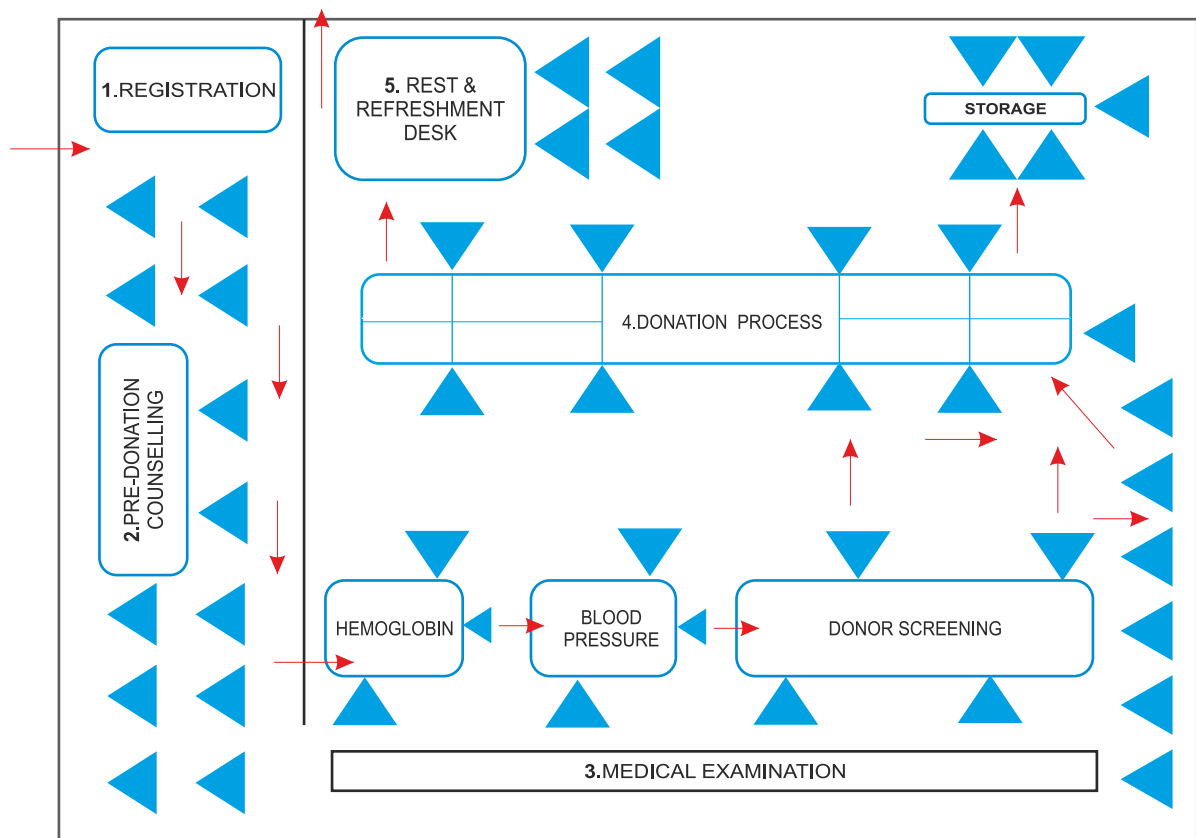


Figure No: 10 Sample layout of blood donation camp

Refreshment

Light snacks and fluids depending upon geographical preferences.

Post-donation care

Post-donation care and refreshment is the same as mentioned in donor room procedures.

Blood Mobile

The blood mobile is one of the modern methods of mobile blood collection facility funded through the third phase of National AIDS Control Programme (NACP) and the National Blood Transfusion Council (NBTC) of India. The concept was to reach the blood donor with the blood collection facility in order to minimise the time spent on travel to the nearest blood donation centre or outdoor blood donation camp/drive. The blood mobile was designed with the concept of stationing it for blood collection at prominent public places and rotate the schedule in a manner that regular repeat voluntary blood donors know that the blood mobile will be stationed for collection near his/her workplace/housing, and could thus plan his/her blood donation in advance.



Figure: 11 Internal panoramic view of blood mobile

2 Storage


The collected blood units are stored in transport boxes during the camp and transportation from the venue of camps back to the blood bank.

3 Records concerning Blood Donation Camp

1. Registration cards/forms
2. Statement of Expenditure
3. Report proforma of staff
 - Donor deferrals register.
 - Donor reactions register.
 - Hemoglobin registers.
 - Other forms and formats mandated by SBTC
 - Register for remarks, suggestions to blood bank by organizers or donors

Choose the Best Option:


1. Age of a blood donor should be between:
 - a. 18 to 65 years
 - b. 18 to 58 years
 - c. 16 to 60 years
 - d. 16 to 58 years
2. The minimum weight of a blood donor should be:
 - a. 40 kg
 - b. 45 kg
 - c. 50 kg
 - d. 55 kg
3. A donor is NOT permanently deferred if he suffers from which of the following disease:
 - a. Hepatitis B
 - b. Hepatitis A
 - c. Cancer
 - d. Heart Disease
4. Hemoglobin of a blood donor should be more than:
 - a. 11.5 gm/dL
 - b. 12 gm/dL
 - c. 12.5 gm/dL
 - d. 13 gm/dL
5. Which type of donor is not accepted:
 - a. Voluntary donor
 - b. Professional donor
 - c. Replacement donor
 - d. Directed donor

- 
6. If a donor is taking aspirin, the blood is collected for preparation of following components:
 - a. PRBC + FFP
 - b. PRBC + PRP
 - c. PRBC + FFP + PC
 - d. Any of the above

 7. The donor phlebotomy site should be inspected for :
 - a. Scar mark
 - b. Tattoo
 - c. Any active lesion
 - d. All of the above

 8. The temperature at which blood is stored is:
 - a. 2° to 6°C
 - b. 2° to 24°C
 - c. 2° to 8°C
 - d. Below -30°C

 9. In the copper sulphate method for hemoglobin estimation, which of the following inference is correct:
 - a. If the drop of blood sinks, the Hb is > 12.5 gm/dL
 - b. If the drop of blood floats the, Hb is < 12.5 gm/dL
 - c. Both of above
 - d. None of the above

 10. The specific gravity of copper sulphate working solution for estimation of Hb should be:
 - a. 1.053
 - b. 1.050
 - c. 1.052
 - d. Not sure
- 

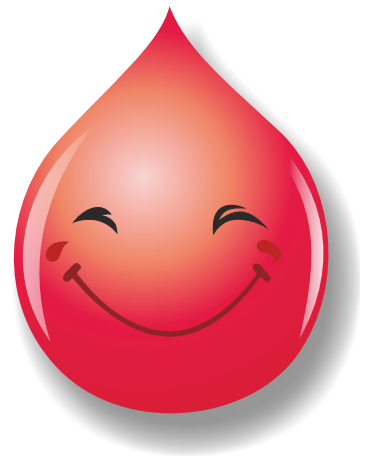
NOTES:

IMMUNOHAEMATOLOGY

Learning Objectives

When you have completed this chapter you should be able to:

- Describe the basic red cell serology
- Describe out ABO grouping and compatibility testing
- Describe about the serological discrepancy and its resolution



2.1 BASICS OF RED CELL SEROLOGY

1 Antigen

An antigen is a substance which when introduced into the body can elicit an immune response.

2 Antibodies or Immuno-globulins

These are group serum proteins, produced in response to antigenic stimulation. There are five classes of immunoglobulin's designated as IgM, IgG, IgA, IgD and IgE. Of these only IgM and IgG are mainly involved in blood bank serology laboratory.

Table 2.1: Characteristics of Antibodies or Immunoglobulins

Characteristics	IgG	IgM	IgA
Heavy chain type	Gamma (γ)	Mu (μ)	Alpha (α)
Biological half-life (days)	23	5	6
Placental transfer	Yes	No	No
Serological behavior	Incomplete	Complete	Complete
Complement fixation	Yes (IgG3 mostly)	Yes	No
Direct agglutination in saline	Usually No	Yes	Yes
React optimally at	37°C	20-24°C	37°C

Naturally occurring antibodies

These antibodies are present without any specific red cell antigenic stimulus. e.g. in a newborn ABO antibodies start appearing in the serum by 3-4 months due to cross-reactivity of ABO antigens with the naturally occurring bacteria, viruses and pollen grains present in the environment. They are usually IgM in nature and are present in the individuals lacking that antigen on their red cells. The latter is known as "Landsteiner's Law".

Immune antibodies

They are usually IgG and are due to immunization either due to pregnancy or transfusion







3 Types of antigen antibody reaction

- a. Agglutination
- b. Sensitization
- c. Neutralization (Inhibition)
- d. Haemolysis

Agglutination

- Most common reaction observed in blood group serology.
- It is due to antibody mediated clumping of particles (e.g. RBCs) that express the corresponding antigens on their surface.
- Different from precipitation where formation of insoluble complexes is due to reaction of soluble antigen and the antibody molecules.
- Agglutination occurs in two stages
 - i. Sensitization – Attachment of antibody on the surface of red cells without bringing about agglutination
 - ii. Agglutination – Due to lattice formation of the sensitized cells.

Table 2.2: Tube Agglutination grading chart

Scale			Macroscopically Observed Findings
0-4	0-12		
4	12		One solid agglutinate, no free red cells detected
3	10		One or two large agglutinates, a strong reaction
2	8		Medium size agglutinates, clear background
1	5		Small agglutinates, with a lot of free red cells
+/-	3		Weak granularity in the red cell suspension
0	0		No agglutinates, an even red cell suspension

Ref:(Denise M. Harmenings, Modern Blood Banking & Transfusion Practices, 5thEd.)

Haemolysis

It is the rupture of the red cells with release of intracellular hemoglobin due to formation of membrane attack complex (MAC) e.g. ABO antibodies, some Lewis and Kidd antibodies.



4

Factors Affecting Antigen Antibody Reactions

a) Antigen antibody ratio

Usually 2 volumes of serum and 1 volume of 2-5% red cell suspension in normal saline are used for optimum reaction whereas equal volume of serum and 2-3% suspension of red cells in LISS is used. Excess of either antibody or antigen may lead to unbound immunoglobulin (pro-zone effect) or a surplus of antigen binding sites (post-zone effect) respectively.

b) pH

Most antibodies react best at pH 6.5 - 7.5 except anti M and anti N which react best at pH 6.5.

c) Temperature

IgM antibodies react optimally at or below 22°C whereas IgG reacts best at 37°C.

d) Type of immunoglobulin

IgM being large molecules can easily bridge the distance between two red cells of 25 nm and are therefore direct or complete antibodies e.g. Anti-ABO, -Le^a, -Le^b, -M, -N, -P. **IgG** has only two binding sites per molecule with maximum distance that they can stretch being only 14 nm, therefore can bring about only sensitization on RBCs e.g. anti- D, -K, -Fy, -S, -s.

e) Incubation time

The time needed to reach equilibrium varies for different blood group antibodies. Usually 60-90 minutes incubation at 37°C is adequate to detect clinically significant weak antibodies using antiglobulin reagent. Various potentiators e.g. LISS and PEG can decrease the incubation time to 10 -15 minutes.

f) Freshness of serum and red cells

Some antibodies (e.g. Jk) can be detected only in the presence of complement which gets deteriorated on storage or by anticoagulants which chelates Ca²⁺ ions. Therefore serum that is not being used immediately should be stored at -20°C or lower.

g) Zeta potential

RBCs repel each other due to negative charge impacted by sialic acid moieties on the membrane. This repulsive force is known as zeta potential.

h) Centrifugation

Centrifugation brings the sensitized antigens close together and thereby facilitates agglutination.

i) Antibody Potentiators or Enhancement Media

They are commercially available reagents that enhance the detection of IgG antibodies by reducing zeta potential of red cell membrane and promote agglutination.

Table 2.3: Reagents and action of potentiators

Reagents	Action
22% Albumin	<ul style="list-style-type: none"> Increases dielectric constant and decreases zeta potential
LISS (Low-Ionic Strength Saline)	<ul style="list-style-type: none"> Decreases the ionic strength of medium (0.03 M) Increases the rate of antibody uptake and therefore decreases the incubation time.
Enzymes (e.g. papain, ficin, bromelain)	<ul style="list-style-type: none"> Removes sialic acid moieties, therefore decreases zeta potential. Remove proteins thereby increasing interfacial tension. Causes spicule formation therefore increases potential number of contact points. <p>Advantage: Increases reactivity to Rh, Jk, P1, Le and I antibodies</p> <p>Disadvantage: Removes certain antigens e.g. M, N, S, Fya and Fyb.</p>
Positively charged molecules (e.g. polybrene, PEG)	<ul style="list-style-type: none"> Neutralization of sialic acid moieties thereby decreasing zeta potential. Removes water of hydration Increases antibody uptake because it is used as low ionic strength solution.
AHG	<ul style="list-style-type: none"> Cross links sensitized cells to give visible agglutination.

2.2 ABO AND RH BLOOD GROUP TYPING

1 Introduction

ABO blood group system is most important in transfusion medicine because of the naturally occurring anti-A and anti-B antibodies in persons who lack the corresponding antigens on RBCs.

Blood Group	Antigens on RBCs	Antibody in serum/plasma
A	A	Anti-B
B	B	Anti-A
O	H (no A and B)	Anti-A and Anti-B
AB	A and B	Neither anti-A nor Anti-B
O _h (Bombay)	No H, A or B	Anti H, anti-A and anti-B

Thereby incompatibility between the donor and the recipient's blood can lead to serious outcomes such as HTR.

Bombay group

Another important blood group is the Bombay blood group. The main features of this blood group is the absence of A, B and H antigens in red cells and the presence of antibodies anti-A, anti-B and anti-H in their plasma. The phenotype of this group is O_h .

This can be observed in vitro by the lack of reaction of Bombay type red cells with anti-sera A, B, AB and H, whereas the serum of a person with O group does not react with anti-A, -B, -AB but reacts very strongly with anti-H antiserum. This indicates a clear differentiation between O and O_h individuals. Bombay (O_h) group individuals can be transfused only O_h blood.

2

Blood Grouping Principles - ABO Grouping

- (i). **Forward grouping:** Cell / Forward / Direct grouping is based on an agglutination reaction between A and B antigens present on RBCs with commercial anti-A and anti-B antisera respectively.
- (ii). **Reverse grouping:** Serum / Reverse grouping is based on an agglutination reaction between naturally occurring anti-A and anti-B antibodies in serum / plasma with reagent A or B red cells respectively.
- (iii). Blood group A is further divided into A_1 and A_2 based on reaction with anti- A_1 lectin (*Dolichos biflorus*). Based on this, the ABO blood group system can be divided into six subtypes A_1 , A_2 , B, A_1B , A_2B and O.

General Precautions

- Freshly collected clotted sample (3-5ml) should be accepted in a vial bearing a gum pasted, tamper proof paper, label marked with the patient's identification details (Name and Registration No.) and they should tally with the same on the requisition form. In case of any discrepancy, ask for fresh sample.
- Do not use haemolysed sample. Use antisera as instructed by the manufacturer.
- Check the specificity of the reagent antisera (anti-A, anti-B, anti-AB and A_1 lectin) daily before use with known positive and negative reagent RBCs.
- Use clean labeled test tubes/slides.
- Serum should always be added before adding cells.
- Keep the ratio between cells and serum 1:2 to avoid discrepant results.

Materials required

• Test tubes (10 x 75 mm) or slides.	• Reagent cells (A, B and O cells) and antisera (anti-A, anti-B, anti-AB, anti- A_1)
• Metallic / Perspex rack.	• Normal saline washed cells and sera
• Marker pens / pencils.	• Centrifuge
• 100 ml glass beakers: 2	• Normal saline (NS)
• Pipettes	• Microscope
• View box	• Magnifying glass
• Tissue paper rolls	• Tube stripper and cutter

(i) Cell / Forward Grouping

Preparation of red cell suspension:

Wash RBCs 3 times with normal saline and finally re-suspend to 2-5% for tube techniques respectively.

Tube technique

1. Label two test tubes as anti-A and anti-B
2. Add 2 drops of reagent antisera to each tube respectively
3. Add 1 drop of NS washed test cells (2-5% suspension) to each tube
4. Mix the contents of each tube by gentle shaking and spin after 5-10 minutes at 1000 rpm for 1 minute.
5. Look for agglutination / haemolysis
6. Record the result immediately.

(ii) Serum/Reverse Grouping

Preparation of reagent A, B and O RBC:

Label 3 test tubes as A, B and O cells. Make a pool of 3 known donor cells of each group and wash 3 times with NS. Make 2-5% cell suspension in NS.

Method

1. Label three tubes as A, B and O cells
2. Add 2 drops of test sera in each tube
3. Add 1 drop of reagent red cells in each corresponding tubes
4. Mix and centrifuge at 1000 rpm for 1 minute
5. Record the result immediately

(iii) Preparation of O Rh (D) Positive Sensitized Red Cells (Check cells for Antiglobulin Test)

Reagents required:

1. Human polyspecific anti-D or Human IgG + Monoclonal IgM or Monoclonal IgG + IgM anti-D Serum
2. O Rh (D) positive cells

Procedure:

1. Select a dilution of polyspecific anti-D serum that coats the O Rh (D) positive washed red cells at 37 °C in vitro but that does not agglutinate them. One has to determine by experience to what extent the anti-D serum should be diluted to give sensitized cells (no agglutination).
2. Add 3-5% washed red cells suspension of O Rh (D) positive cells equal to the volume of diluted anti-D serum.
3. Mix, incubate at 37 °C for 30-45 minutes.
4. Look for agglutination. If there is agglutination, the procedure is repeated by taking more diluted anti-D serum.

- If there is no agglutination, wash the cells three times with a large volume of normal saline manually or by automatic cell washer. Make a 5% suspension of sensitized cells in saline.
- Add 2 drops of polyspecific AHG serum to 1 drop of the 3-5% sensitized and washed red cells.
- Mix, spin immediately at 1000 rpm for 1 minutes.
- Cells should show +2 agglutination, if there is no agglutination the whole procedure is repeated by taking less diluted anti-D serum.

Table 2.4: Interpretation of forward and reverse grouping results

Anti-A	Anti-B	Anti-AB	Lectin A1	Lectin H	Anti-D	A1 Cells	B Cells	O Cells	Auto-Control	Blood Group
3 to 4+	-	3 to 4+	3 to 4+	2 to 3+	3 to 4+	-	3 to 4+	-	-	A ₁ +
3 to 4+	-	3 to 4+	3 to 4+	2 to 3+	-	-	3 to 4+	-	-	A ₁ -
3 to 4+	-	3 to 4+	-	3 to 4+	3 to 4+	-	3 to 4+	-	-	A ₂ +
3 to 4+	-	3 to 4+	-	3 to 4+	3 to 4+	1 to 2+*	3 to 4+	-	-	A ₂ +*
3 to 4+	-	3 to 4+	-	3 to 4+	-	-	3 to 4+	-	-	A ₂ -
-	3 to 4+	3 to 4+	-	3 to 4+	3 to 4+	3 to 4+	-	-	-	B +
-	3 to 4+	3 to 4+	-	3 to 4+	-	3 to 4+	-	-	-	B -
3 to 4+	3 to 4+	3 to 4+	3 to 4+	1 to 2+	3 to 4+	-	-	-	-	A ₁ B +
3 to 4+	3 to 4+	3 to 4+	3 to 4+	1 to 2+	-	-	-	-	-	A ₁ B -
3 to 4+	3 to 4+	3 to 4+	-	1 to 2+	3 to 4+	-	-	-	-	A ₂ B +
3 to 4+	3 to 4+	3 to 4+	-	1 to 2+	-	-	-	-	-	A ₂ B -
3 to 4+	3 to 4+	3 to 4+	-	1 to 2+	3 to 4+	1 to 2+*	-	-	-	A ₂ B+*
-	-	-	-	3 to 4+	3 to 4+	3 to 4+	3 to 4+	-	-	O +
-	-	-	-	3 to 4+	-	3 to 4+	3 to 4+	-	-	O -
-	-	-	-	-	3 to 4+	3 to 4+	3 to 4+	2 to 3+	-	O _h +
-	-	-	-	-	-	3 to 4+	3 to 4+	2 to 3+	-	O _h -

* With anti-A₂ antibodies symbol for negatives

3 Blood Grouping - Rh Grouping

Rh blood group system is second in importance after ABO in transfusion practice because of its high immunogenicity. Common Rh antigens are D, C, E, c and e. An individual is designated Rh positive or negative on the basis of the presence or absence of Rh (D) antigen. Approximately (92-95%) of the Indian population is Rh (D) positive.

(i). Tube technique for Rh D grouping:

(a) Anti - D antisera

Reagents

1. Anti- D antisera (Monoclonal IgM or blend of IgM and IgG)
2. Controls: known Rh positive and negative cells

Procedure

1. Prepare 2-5% washed cell suspension of test and control cells
2. Label three tubes as positive control, negative control and test
3. Add 2 drops of anti- D in each tube
4. Add 1 drop of cell suspension in respective tubes
5. Mix and centrifuge at 1000 rpm for 1 minute
6. Re-suspend gently and look for agglutination
7. Grading of agglutination is same as that of ABO system
8. Phenotyping (CDE/ce) may be done for all D negative blood donors.

(b) Extended Phenotyping

Reagents

1. Anti- C, anti- c, anti- E and anti- e antisera (monoclonal)
2. 2-5% suspension of washed test cells
3. Known positive and negative controls

Procedure

1. Label the test tubes as anti- C, anti- c, anti- E and anti- e along with positive and negative controls
2. Add 2 drops of the respective antisera in their labeled tubes
3. Add 1 drop of test as well as control cells in the respective tubes
4. Mix and centrifuge at 1000 rpm for 1 minute
5. Re-suspend gently and look for agglutination
6. Record the results immediately

(ii). Weak D Phenotype

Weak variant of D antigen is detected only by indirect antiglobulin test.

Procedure

1. Take 1 drop of anti- D (blend of IgG and IgM) in a labeled test tube
2. Add 1 drop 2-5% test cells which test D negative on routine testing with anti-D.
3. Incubate for 20-30 minutes at 37°C
4. Wash the cells thrice with NS
5. Decant the last supernatant, wash and add 2 drops of poly-specific Coombs sera.
6. Centrifuge at 1000 rpm for 1 minute
7. Gently re-suspend and look for agglutination

Interpretation:

If Indirect Antiglobulin Test (IAT) is positive, the donor is weak D

Positive: The corresponding blood unit should be labeled as RhD positive. Also, the donor should be advised to receive RhD negative blood in case he/she requires transfusion in future.

2.3 ABO GROUPING DISCREPANCIES

Anomalous results in blood group testing i.e. where forward and reverse grouping fail to tally with each other. It can be due to:-

- Technical discrepancy
- Clinical Discrepancy

1 Technical Discrepancy

1. Clerical errors
2. Missed identification of blood specimen
3. Mixing of blood samples
4. Contaminated reagents or not following manufacturer's instructions
5. Non-calibrated centrifuges
6. Cell suspension either too light or too heavy
7. Contaminated or dirty glass wares

2 Clinical Discrepancy

In this the problem lies with the patient. To solve this type of discrepancy essential information regarding patient's age, diagnosis, transfusion history, H/o medication and pregnancy must be taken into account.

ABO blood group discrepancies are mainly divided into 4 major groups.

(i). Group I Discrepancy

This type of discrepancy is most common compared to the other groups. It is mainly seen in reverse grouping due to weak / missing antibodies. Some of the common conditions associated with this type of discrepancy are:-

1. Newborns
2. Elderly patients
3. Patient with leukemia or lymphoma
4. Patients on immunosuppressive drugs
5. Patients with immunodeficiency diseases
6. Patients with bone marrow transplant

Resolution

For newborns, only forward grouping is done till 4 months of age.

These discrepancies can be solved by enhancing the serum grouping reaction. This can be achieved by incubating the cell serum mixture at low temperatures (4°C for 15-30 minutes) or by prolonging incubation at room temperature (½ hr - 1 hr at 22°C)

(ii). Group II Discrepancy

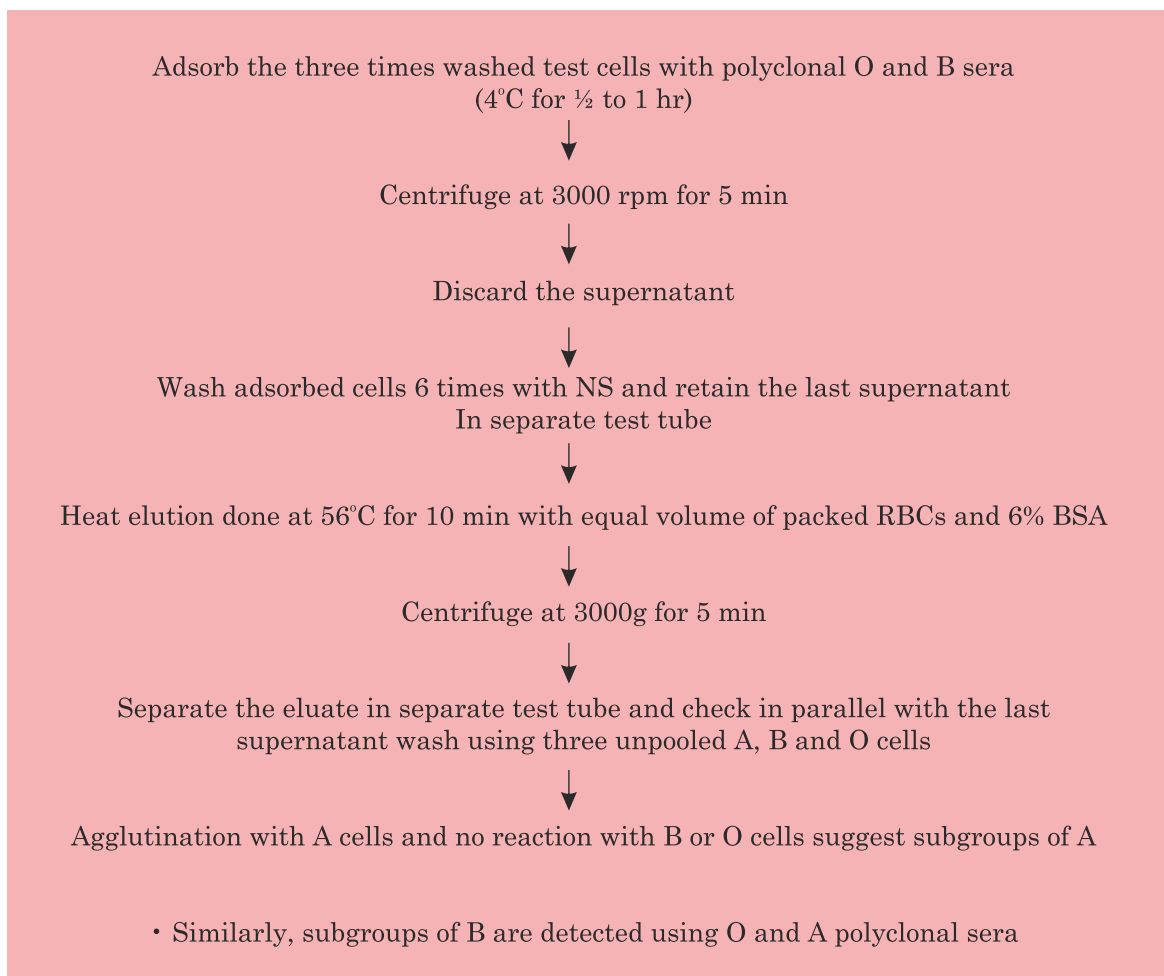
This is due to missing or weak antigens. This type of discrepancy is least common. The causes are:

1. Subgroups of A or B
2. Leukemia and Lymphoma
3. Excess antigen of blood group soluble substances
4. Acquired A or B antigens.

Resolution

Subgroups of A or B can be solved by:

- Repeating blood grouping by using washed cells
- Use of anti-AB antisera and anti-A1 lectin
- Adsorption and Elution technique (see chart below)



(iii). Group III Discrepancy

This is due to proteins or plasma abnormalities. The causes in this group are:

1. Elevated levels of plasma globulins as seen in cases of Multiple Myeloma, Waldenstrom's macroglobulinemia and Hodgkin's lymphoma
2. Elevated levels of fibrinogen
3. Use of plasma expanders such as dextran
4. Wharton's jelly in cord blood samples

Resolution

- The main problem is due to Rouleaux formation, which is resolved by washing the cells with normal saline 6-8 times, and confirming it with microscopic examination. 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 reexamined for agglutination. In difficult cases, hyaluronidase treatment of the test sample is recommended.

(iv). Group IV Discrepancy

1. Polyagglutination: this is due to exposure of hidden erythrocytes antigens (T antigen in bacterial or viral infections)
2. Patient with cold auto antibodies
3. A₂ or A₂B individual with anti-A1 antibodies
4. Naturally occurring or irregular antibodies reacting at room temperature
5. Cis-AB

Resolution

Polyagglutination

- Symptoms suggestive of infection
- Auto control negative
- DAT negative
- Use of various lectins (Glycine soja, Arachis hypogea)

Cold auto agglutination

1. Warm saline washes (at 37°C - 40°C) of auto agglutinated cells
2. Pre-warming of sera and reagent cells (at 37°C)
3. Performing the test at 37°C

Most of the auto agglutinins are removed by the above techniques; if not Dithiothreitol (DTT) 0.01M can be used to make cells free (equal quantity of 0.01 M DTT and washed packed test cells at 37°C for 15 minutes)

3

Other Clinically Significant Antibodies to Minor Blood Group System Antigens

Most clinically significant antibodies react at body temperature (37°C), are IgG isotype, and are capable of destroying transfused antigen-positive RBCs and causing transfusion reactions of various severities. For example: Anti-K (Kell), anti-Fy_a, -Fy_b (Duffy), anti-Jk_a, -Jk_b (Kidd), anti-N, -S, -s antibodies.

2.4 ANTI GLOBULIN TEST

The antiglobulin test, formerly known as Coombs test was first described by Cambridge immunologists Robin Coombs, Arthur Mourant and Rob Race in 1945 for detecting incomplete antibodies in serum

Principle

- Antibodies and complement molecules are globulins
- Anti-human globulin (AHG) reacts with immunoglobulin bound to the RBCs or free in the serum
- AHG reacts with Fc portion of human antibodies
- AHG helps in lattice formation by cross linking sensitized RBCs.

Applications

Table 2.5 Direct Antiglobulin Test (DAT) (Used to detect in-vivo sensitization of RBCs)

Diagnosis	Detection
Haemolytic disease of Fetus & Newborn (HDFN)	Maternal antibodies coating fetal RBCs
Haemolytic transfusion reaction (HTR)	Recipient antibody coating donor RBCs
Auto immune Haemolytic anemia (AIHA)	Auto antibodies coating self RBCs

Table 2.6: Indirect Antiglobulin Test (IAT) (Used to detect in-vivo sensitization of RBCs)

Application	Detection
1. Compatibility testing	Recipient antibodies with donors cells
2. Antibody screening	Recipient antibodies with screening cells
3. Antibody identification	For antibody specificity
4. Antibody titration	Rh antibody titre
5. Red cell phenotype	Weak D test, K, Jk, Fy antigens

1

Anti-Human Globulin reagent (AHG)

Two types of anti- human globulin reagents are available:

- Polyspecific AHG reagent: It usually contains anti-IgG and anti-C3d but may also contain anti-C3b, C4b and C3d.
- Monospecific AHG reagent : It contains either of these i.e. anti-IgG, -IgM, -IgA or complement antibodies.

Procedure

- Add 2 drop of test serum to a test tube
 - Add 1 drop of 5% suspension of pooled O + red cells
 - Incubate at 37°C for 60 minutes
 - Wash the red cells 6 times with normal saline
 - Add 1 drop of AHG reagent to red cell button
 - Look for agglutination
 - Negative results should be confirmed by adding 1 drop of IgG coated cells(check cells)
- The addition of IgG coated cells to negative AHG is required for the validity of the test.**

Control cells for AHG

1. Positive Control – O Rh (D) positive sensitized cells
2. Negative Control – O Rh (D) negative cells

2

Sources of Error in Anti-globulin Test

(i) False positive results

- Agglutination of red cells before adding AHG reagent
- Over centrifugation
- Dirty glassware
- Leaching of silica in saline from glassware
- AHG containing anti-human species antibody
- Cold auto-antibodies in sample

(ii) False negative results

- Inadequate washing of cells
- Delay or interruption of washing procedure
- Failure to add AHG reagent
- Under centrifugation
- Too heavy or too light red cell suspension
- Pro-zone effect
- Insufficient incubation
- AHG reagent that has lost potency

2.5 COMPATIBILITY TESTING (CROSS MATCHING)

Compatibility tests are done to ensure that particular unit of blood may be safely transfused to a patient. Normally group specific blood, ABO and Rh (D), as that of the patient is selected. However in certain situation, because of the non-availability of group specific blood, group O blood (red cells) is selected for A or B patient and A or B blood for an AB patient as explained earlier.

Compatibility testing (cross match) includes

- Major cross match (Compatibility) – Recipient's serum is cross matched with donor's red cells for IgM and IgG antibodies compatibility.
- Minor cross match (Compatibility) – Donor's serum is cross matched with recipient's red cells for IgM and IgG antibodies compatibility. If donor's serum has been screened for irregular antibodies with OR, R, and OR R cells of antibody screening panel and found negative, minor cross-match can be avoided.

If incompatibility is not detected in cross matching then, it is likely that the donor blood transfused into patient will survive normally.

Finding of incompatibility indicates that transfusion of such blood is paternally dangerous and further steps should be taken to identify the antibody

1 Major Compatibility Tests

It is done both for IgM and IgG antibodies

Compatibility Test for IgM Antibody/ Antibodies

Requirement

1. Recipient's serum.
2. Donor's red cells taken from the tube attached to the bag.

Saline technique

Saline technique is designed to detect compatibility of IgM antibody (ies) in patient's serum against antigens on donor's red cells.

Method

1. Label one tube for each donor sample to be tested.
2. Put two drops of patient's serum in labelled tube.
3. Add one drop of 2-4% of saline suspended red cells of donor.
4. Mix and incubate for 5–10 min. (spin method) or incubate for 30-60 min (sedimentation method) at RT.
5. Centrifuge at 1000 rpm for 1 min. in spin method (after 5–10 min. incubation), centrifugation is optional in sedimentation method.
6. Read the result, observe for hemolysis and agglutination.
7. Negative result should be confirmed under microscope.

Note: In emergency, spin technique is acceptable.

Saline technique is inadequate as a complete compatibility test because it is inadequate to detect clinically significant IgG antibodies.

Interpretation

Agglutination or hemolysis indicates a positive result (incompatible)

Compatibility Test for IgG Antibody/ Antibodies

Anti-Human Globulin Test (IAT)

Indirect anti human globulin test (IAT) is the most important and widely used serologically procedure in modern blood banking to test the IgG compatibility between recipient's serum and donor's cells. The majority of incomplete antibodies are IgG and are detected by AHG test.

Method:

1. Put two drops of patient's serum in a labelled tube.
2. Add one drop of 2-4% saline suspended red cells of donor.
3. Incubate for 30-60 min at 37°C
4. Centrifuge at 1000 rpm for 1 min, check for hemolysis / agglutination
5. If there is no hemolysis / agglutination, wash the cells three times with normal saline.
6. Perform IAT Test
 - Add two drops of polyspecific AHG serum to washed cells
 - Centrifuge at 1000 rpm for 1 minute
 - See for agglutination
7. Add IgG coated red cells to negative AHG test.
8. Centrifuge and check for agglutination – if there is no agglutination test is invalid.

Interpretation

Hemolysis or agglutination at any stage indicates incompatibility.

Note: Cross-match can be done by two tubes technique for IgM and IgG separately as described above or by one tubes in which donor' cell and the patient's serum after step 5 in saline technique is incubated at 37°C for 20-30 minutes and then do IAT.

In major – cross for IgG antibodies albumin or enzyme or LISS can be used with IAT to increase sensitivity. For techniques see chapter of Antiglobulin Test.

2 Minor Compatibility Tests

- If donor's serum has not been screened for irregular antibodies with OR, R1 and OR, R2 cells of screening panel, and found negative, it is advisable to do minor cross matching.
- In minor cross – match donor's serum is cross-matched with recipient's red cells for both IgM and IgG antibodies compatibility.

Method

Method is same as that of major cross-match except in minor cross-match donor's serum is tested against patient's cells both for IgM and IgG antibodies compatibility.

Compatibility Testing in Emergencies

- On the request of the clinician blood can be issued in emergency after ABO and Rh (D) typing followed by cross match by immediate spin tube technique for IgM compatibility.
- In extreme cases, where there is no time to take sample and to test it, O, Rh (D) negative blood, preferably red cells, may be given on the request of the clinician.
- Donor's unit that has not been tested or partially tested for compatibility for IgM and IgG antibodies against patient's serum, should be clearly labelled 'Uncrossed-match Blood'.
- It is advisable to complete the routine cross-matching both for IgM and IgG compatibility, after issue of blood with incomplete or without compatibility test.

Massive Transfusion

- When massive transfusions are given, that is when the number of units transfused in a 24 hours period exceeds the recipient's blood volume, compatibility testing may be reduced to checking the ABO and Rh (D) types of the transfused units. If the patient has known allo-antibody reactive at 37°C, the blood should be negative for the relevant antigen, as much as possible.
- After the emergency has been dealt with, if antibodies are detected in pre-transfusion sample, and in case further transfusion is necessary compatible blood is selected.
- Donor's units that have not been tested or have been partially tested against the patient's serum, should be clearly labelled "uncross matched blood".

1. Open-heart surgery

- In open-heart cases, all the procedures are the same as given above in compatibility testing. In addition, make a pool of donor cells [i.e. 5 or 6 in number] in separate tube and from these pooled cells, take 1 drop of 3-5% cell suspension and add 2 drops of the patient's serum (ratio 1:2) in four different tubes. These tubes are kept at various temperatures, viz: 4°C, RT, and 37°C.
- Wash these incubated cells three times with normal saline; after centrifugation decant the supernatant saline and then add polyspecific Coombs sera (AHG). Centrifuge and see for agglutination. If there is no agglutination, 'the blood units are compatible.'
- Blood unit taken for compatibility testing should be as fresh as possible (within 5-7 days of storage). In case of rare blood groups (e.g. Rh-negative patients), 7-10 day old blood may be issued with appropriate component (PC and FFP) supplementation

2. Multiple Myeloma

In cases of multiple myeloma, wash the patient's cells 6-8 times in normal saline and perform cell grouping from these washed cells and for reverse grouping, serum is diluted with normal saline in the ratio of 1:4.

Compatibility test: The procedure is same as given in routine compatibility testing, but patient's cells washed 6-8 times and diluted serum are taken to perform Major Crossmatch, Minor crossmatch and AHG phase compatibility testing.

3. Severe anemia

In case of severe anemia (Hemoglobin < 7 gm/dL), always ask for EDTA sample along with clotted sample. Perform cell grouping from EDTA sample. Rest of the procedure is the same as given in routine cross match testing.

4. Cancer cases

- Always perform cell grouping from washed cells in known cases of cancer patients.
- Compatibility test for cancer cases is same as given in routine compatibility test.

In case of packed red blood cells, supplementation with components (PC, FFP) may be required.

4

Compatibility test in Autoimmune Hemolytic Anaemia (AIHA)

Autoimmune Hemolytic anemia is due to production of antibodies directed against patient's own red cell antigens and hence there is a decrease in the life span of red blood cells. It is classified into four categories:

1. Warm autoimmune Hemolytic anemia
2. Cold Agglutinin syndrome
3. Paroxysmal cold Hemoglobinuria (PCH)
4. Drug Induced Autoimmune Hemolytic anemia

(I). Blood grouping in AIHA

ABO Typing: There is no difficulty in performing direct grouping for ABO system or for any other blood group system in which the antibodies are saline acting. For Phenotyping, other blood group systems such as Kell, Kidd, Duffy, perform DCT on the patient's cells, which should be negative. If the DCT is positive, treat patient's cells with chloroquine di-phosphate or ZZAP (1% activated cysteine papain + DTT) reagent to remove the auto antibodies.

Rh Typing: If the cells are heavily coated with auto antibodies, either cold or warm-reactive, it may cause discrepant Rh typing. IgM-coated cells are treated with sulfhydryl reagents [such as 2-ME (mercapto-ethanol) or DTT (Dithiothreitol)] to resolve spontaneous agglutination. Also, IgG antibodies can be dissociated from the cells by treatment with ZZAP or chloroquine di-phosphate (as in case of ABO grouping) or any other elution method that leave red cells intact for subsequent typing. 6% bovine serum albumin control should be kept along with above tests.

(ii). Detection of Alloantibodies in AIHA

Allo-antibodies are suspected along with auto antibodies in-patient with AIHA, if-

1. Indirect Coomb's test is stronger than Direct Coomb's Test (DCT)
2. If there is difference in strength of reaction, use panel of cells for characterization of the antibody.

Compatibility test:

The compatibility test depends upon the specificity of the antibody. If a non-specific antibody is present, use a different panel of cells (donors), by carrying out cross matching using titration method (as given in Rh section). Issue least incompatible blood (means using different panel of cells) for which titre is lowest.

For cold auto-antibodies, the compatibility is done both at 4°C and at 37°C by means of titration method. (Issue the least incompatible blood).

5

Compatibility test in new-borns

Pre – Transfusion Testing of Neonates (In less than 4 months old infant)

Procedure:

- Group ABO and Rh (D) group of infant cells should be determined by cell grouping only.
- Direct AHG test on baby's cells is performed.
- Maternal serum should be screened for any irregular antibody, if mother's blood sample is available.
- If the antibody screening and DAT is negative in mother's blood, and no evidence of HDN, cross – matched blood of the same ABO and Rh (D) type as that of the infant using mother's serum (if ABO compatible and sample is available) or baby's serum. This is to exclude the possibility that incomplete antibody from the mother may be in baby's serum in the absence of the antigen on the baby's red cells to that antibody.
- If the antibody screening is positive, the direct AHG is positive or HDN is present, the donor's blood must be cross – matched against maternal serum. The neonate's serum can also be used (if mother's blood sample is not available).
- When group O blood needs to be given to an infant who is group A and / or B, then red cell concentrates of units with low titre anti – A or anti – B should be used. It is a good practice to use packed cells re-suspended in one third volume of AB plasma (or A plasma or B plasma as appropriate).

Double Volume Exchange Transfusion (DVET) in newborns

- Cases of neonatal jaundice for DVET require both new-born and mother's blood sample.
- For transfusion purpose, all the paediatric patients are considered as neonates for the

first three to four months of their life, as they do not have any naturally occurring antibodies (i.e., Anti - A or Anti - B) in their serum/plasma. Therefore for compatibility testing, the mother's sample should accompany the neonatal sample to look for any irregular antibodies transferred from mother to baby.

- Mother's sample is very essential besides the baby's sample. Perform the ABO and Rh grouping of baby as well as of mother and do reverse grouping of only the mother's sample.

Rule of Thumb:

Give ABO and Rh compatible blood to baby i.e. which must be compatible with both baby and mother

Choice of blood for Double Volume Exchange Transfusion

1. ABO group of the baby and Rh type of mother
2. Blood group compatible to both baby and mother

Table 2.7: Choice of blood for Double Volume Exchange Transfusion

Baby's Blood Group	Mother's Blood Group	1 st Choice	2 nd Choice
O	O	O	-
O	A	O	-
O	B	O	-
O	AB	O	-
A	O	O	-
A	A	A	O
A	B	O	-
A	AB	A	O
B	O	O	-
B	A	O	-
B	B	B	O
B	AB	B	O
AB	O	O	-
AB	A	A	O
AB	B	B	O
AB	AB	AB	A,B,O
Rh Positive	Rh Negative	Rh Negative	-
Rh Negative	Rh Positive	Rh Negative	-

1. Blood should be cross matched against mother's serum by Indirect Coombs Test (IAT/ICT) i.e. AHG phase testing
2. If mother's blood is not available, or group is not known, give O Rh negative Packed RBCs only.
3. Blood used should not be more than 5 to 7 days old.
4. Compatible plasma or AB plasma should be issued for reconstitution.

Table 2.8: Choice of Blood / Blood Components

For Packed Red Blood Cells(PRBCs)						
Donor						
Recipient	A	B	O	AB	Rh Positive	Rh Negative
A	Option-1		Option -2			
B		Option-1	Option -2			
O			Option-1 (the only option)			
AB	Option-2	Option -3	Option -4	Option-1		Option -2
Rh Positive					Option-1	
Rh Negative						Option-1 *

2.6. QUALITY CONTROL OF IMMUNOHEMATOLOGY REAGENTS

(DGHS Technical Manual)

Quality control is to be done for the following reagents:

1. Antisera - anti-A, anti-B, anti-A, B, anti D, anti human globulin (AHG)
2. Lectins e.g. anti-A1, anti H
3. Albumin
4. Enzymes
5. Saline
6. Reagent red cells
7. Normal saline/Low ionic strength solution (LISS)

1 General instructions for use of Reagents

1. They should be stored at a prescribed temperature in a safe and hygienic place in a proper manner.
2. All supplies should be sterile, pyrogen free, safe, pure and potent.
3. The oldest lot should be used first.
4. All reagents should be used in a manner consistent with the instructions provided by the manufacturer.
5. Representative samples of each lot of reagents should be tested regularly for appearance, specificity, avidity, reactivity and potency.
 - a) **Appearance:** No turbidity, precipitates, particles or gel formation by visual inspection.
 - b) **Specificity:** Clear-cut reactions with red cells having corresponding antigen(s), and no reaction with negative controls.
 - c) **Avidity:** It is the time taken for earliest appearance of macroscopic agglutination with 50% red cells suspension in homologous serum/normal saline using slide technique.

- d) **Reactivity:** No immune haemolysis or prozone phenomenon, as the final end point result should be as agglutinations or no agglutinations.
- e) **Potency:** Undiluted serum should give 3+ to 4+ agglutination in tube with 2-5% red cells suspension at room temperature. The potency is determined by the titre of the antisera.

2

Quality control of antisera (reagent) by titre estimation [e.g. anti-A]

1. Put 2 drops of normal saline in each tube from tube No. 1 to 10.
2. Now add 2 drops of anti-A sera to the first tube and wipe off the excess serum from pipette with gauze piece.
3. Mix the mixture thoroughly with the help of pipette and transfer 2 drops of mixture to the second tube and continue this procedure up to tube No. 10. From the last tube discard 2 drops.
4. Further add 1 drop of 5% saline cell suspension of pooled A positive cells in each tube from tube No.1 to tube No.10
5. Keep all the tubes at room temperature.
6. Centrifuge the tubes at 1000 rpm for 1 minute.
7. Take out all the tubes from the racks and see for agglutination against white light on view box.

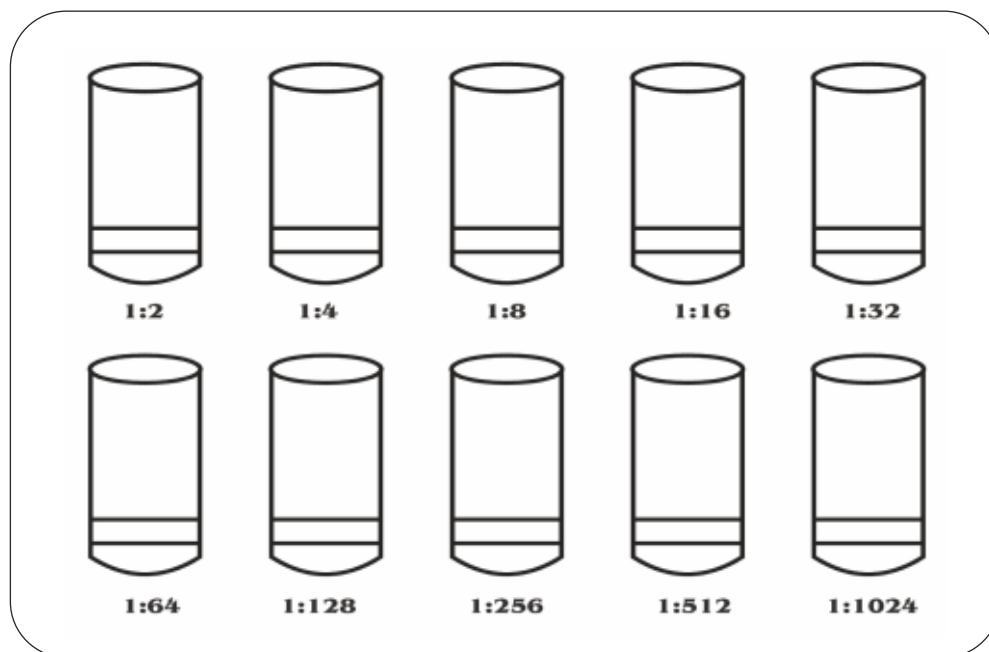


Figure 12: Quality control of antisera by time estimation

Important

- The pipette used for serum and saline should be the same for accurate titration.
- There should not be any serum in the cells otherwise it will neutralize the action of AHG.

3

ABO typing Reagent Antisera

All ABO typing reagent antisera should be tested as per the following criteria of Quality control.

Table 2.9: Quality control of ABO reagents

Parameter	Quality requirements	Frequency of control
Appearance	No turbidity, precipitate, particle or gel formation by visual inspection	Daily
Specificity	Clear cut reactions with red cells having corresponding antigens and no reaction with negative control	Daily and with each new lot

Table 2.10: Quality Control of Anti-A, Anti-B and Anti-AB (Monoclonal antisera) - Frequency of control

Parameter	Quality Requirements	Frequency of Control
Appearance	No haemolysis, Precipitate, Particles or gel formation by visual inspection	Daily
Specificity	Capability to bind to specific antigen – Clear cut reactions with red cells having corresponding antigens & no reaction with negative control	Daily & with each new lot
Avidity	Rapidity with which it binds to antigen – Macroscopic agglutination with 50% red cell suspension using slide test – TIME (IN SECONDS)	Daily & with each new lot
Reactivity	No immune haemolysis, Rouleaux formation or prozone phenomenon	Each new lot
Potency	Undiluted serum should give 3 + reaction in test tube using 3% RBC suspension at RT	Each new lot

Table 2.11: Quality Control of Anti-A, Anti-B and Anti-AB (Monoclonal antisera) - Titre & Intensity

Antisera	Type of red cell suspension (2-5%)	Avidity time (seconds)	Titre	Intensity
Anti - A	A ₁	3 - 4	1 : 256	++++
	A ₂	5 - 6	1 : 128	++ to +++
	A ₂ B	5 - 6	1 : 64	+++
	O and B	-	-	-
Anti - B	B	3 - 4	1 : 256	++++
	A ₁ B	5 - 6	1 : 128	++++ to +++++
	O and A ₁	-	-	-
Anti - AB	A ₁	3 - 4	1 : 256	++++
	B	3 - 4	1 : 256	++++
	A ₂	5 - 6	1 : 128	+++

4 Anti D

- Each new vial of anti-D should be checked for its specificity and avidity by known Rh positive and known Rh-negative cells.
- Each new batch titre must be done to know its potency.
- Positive and negative controls must be put for validation of results.

Table 2.12: Quality control of anti D

Parameter	Quality requirements	Frequency of control
Appearance	No turbidity, precipitation or gelling	Each day
Reactivity	No Haemolysis, rouleaux formation or prozone phenomena	Each new lot
Specificity	Reaction with O positive (R ₁ r) cells and no reaction with O negative (rr) cells	Each day and each new lot

Table 2.13: Avidity, intensity and titre of anti D

Anti D with R ₁ r cells*	Avidity	Intensity	Titre (Immediate spin)	Titre after Incubation
IgM monoclonal	5-10 sec	+++	1:64 - 1:128	1:128 - 1:256 (at RT)
IgM + IgG blend monoclonal	10-20 sec	+++	1:32 - 1:64	1:128 - 1:256 (at 37°C)

*Negative control with rr cells should be run in parallel

5 Extended Rh Phenotyping Sera

Anti-E, anti-C, anti-e, anti-c should also be checked with known positive and negative panel of cells for its specificity and avidity.

6 Anti Human Globulin (AHG) Serum (formerly called as Coombs' reagent)

- Each vial of every new batch must be checked for its specificity and sensitivity using IgG coated cells.
- Each test must have positive and negative controls.
- To check nonspecific reactions, non-sensitized O, A, B cells should be used.
- All negative AHG test should be confirmed by addition of IgG coated cells in the test result: IgG coated cells should give positive agglutination.

Quality Control of Coombs Sera or Anti-human Globulin

1. Put 2 drops of normal saline in each tube from tube No. 1 to 10.
2. Now add 2 drops sera to the first tube from a known ICT positive patient, wipe off the excess serum from pipette with gauze piece.
3. Mix the mixture thoroughly with the help of pipette and transfer 2 drops of mixture to the second tube and continue this procedure up to tube No. 10. From the last tube discard 2 drops. Thus prepare serial dilutions as described earlier.
4. Further add 1 drop of 5% saline cell suspension of O positive cells to each test tube from test tube No.1 to test tube No.10.
5. Incubate at 37°C in water bath for 60-90 minutes.
6. Take out all the tubes from water bath and wash with normal saline 3 times and then add Coombs sera.

Table 2.14: Quality control of anti-human globulin (AHG)

Parameter	Quality requirements	Frequency of testing
Appearance	No turbidity, precipitation or gelling	Each day
Reactivity	No Haemolysis, rouleaux formation or prozone phenomena	Each new lot
Specificity	No agglutination of unsensitized red cells	Each new lot
	Agglutination of red cells sensitized with anti D having IgG	
	Agglutination of red cells sensitized with complement binding Ab	
	Agglutination of red cells coated with C3d or C3b but no agglutination with C4 coated cells	

- Anti-IgG activity – check by titration with IgG (anti- D) sensitized O cells (Titre- 1:64).
- Complement activity – check with red cells coated with C3b/C3d or sensitised with complement binding anti-Le^a (Titre- 1:4).

7 Reagent Red Cells

- The red cells used are pooled A cells, B cells and O cells.
- The cells need to be washed (at least thrice) in saline to remove serum, plasma, hemolysed cells, small clots which may lead to false positive reactions.
- The supernatant of last wash should be clear.
- 2-5% cell suspension - conventional tube technique
- 30-40% for slide technique

Table 2.15: Quality control of reagents red cells

Known red cells	Anti-A	Anti-B	Anti-D
A	4+	Neg	-
B	Neg	4+	-
O positive	Neg	Neg	4+
O negative	Neg	Neg	Neg

Parameter	Quality requirements	Frequency of testing
Appearance	No Haemolysis in supernatant	Daily
Reactivity	Clear cut reactions with known antisera	Daily

8 Enzymes

- Papain, bromelin, trypsin, ficin may be used, but papain is commonly used.
- Lowe's papain (cystine activated); one or two stage technique is adopted.
- Test with positive and negative controls should be performed, as it is suitable.

Table 2.16: Quality control of enzymes

Parameter	Quality requirements	Frequency of testing
Reactivity	No reactivity and haemolysis with inert AB serum	Each day
Potency	2+ to 3+ agglutination of red cells sensitized with weak anti D IgG type	Each lot

9 Bovine Serum Albumin

Table 2.17: Quality control of bovine serum albumin

Parameter	Quality requirements	Frequency of testing
Appearance	No precipitation or gelling	Each day
Reactivity	No agglutination of unsensitized red cells	Each new lot
	No Haemolytic activity	Each new lot
	No prozone phenomena	Each new lot
	≥ 98% Albumin by serum electrophoresis	Each new lot
Specificity	IgG anti D should give a titre of 32 to 64 with R ₁ r red cells	Each new lot

Choose the Best Option:

1. For ABO blood grouping, which of the following statement is true:
 - a. Only forward grouping is required
 - b. Only reverse grouping is required
 - c. Both forward and reverse grouping are required
 - d. Not sure of the answer


2. Which is true regarding the IgM antibodies:
 - a. Can be detected at room temperature
 - b. Cannot fix complement
 - c. Best reactivity is at 37°C
 - d. All of the above

3. Donor typed as O+ve on forward/ cell grouping will show which of the following results:
 - a. Anti-A - 4+, Anti-B - 4+, Anti D - 4+
 - b. Anti-A - Neg, Anti-B - 4+, Anti D - 4 Neg
 - c. Anti-A - Neg, Anti-B - Neg, Anti D - 4+
 - d. Anti-A - 4+, Anti-B - Neg, Anti D - Neg


4. For Rh (D) typing on donor units, including weak D typing which anti- D antisera is required:
 - a. IgM monoclonal anti- D
 - b. IgG monoclonal anti D
 - c. IgM + IgG monoclonal anti- D
 - d. d. Not sure of the answer

5. Immediate spin cross match technique detects the following EXCEPT:
 - a. IgM antibodies
 - b. IgG antibodies
 - c. ABO incompatibility
 - d. Saline reacting antibodies

6. During daily quality control check of anti-A antisera, which of the following statements is true:
 - a. A1 Cells -4+, B Cells - Neg, O Cells - Neg
 - b. A1 Cells - Neg, B Cells - 4+, O Cell - Neg
 - c. A1 Cells - 1+, B Cell - Neg, O Cells - Neg
 - d. A1 Cells - 4+, B Cell - Neg, O Cells - 4+

- 
7. The desired titre of monoclonal Anti-A antisera as quality control criteria is:
 - a. 1:128
 - b. 1:256
 - c. 1:64
 - d. 1:32

 8. Which of the following statement is false about strength of agglutination:
 - a. 4+ - A Single large clump
 - b. 3+ - Multiple large size clumps with turbid background
 - c. 2+ - Medium Size clumps with clear background
 - d. 1+ - Small size clumps with turbid background

 9. If A+ve blood is not available in the stock which of the following options are correct:
 - a. A → B → AB → O whole blood
 - b. A → AB → O → B packed red cells
 - c. A → O whole blood
 - d. A → O packed red cells
 - e. Not sure of the answer
- 

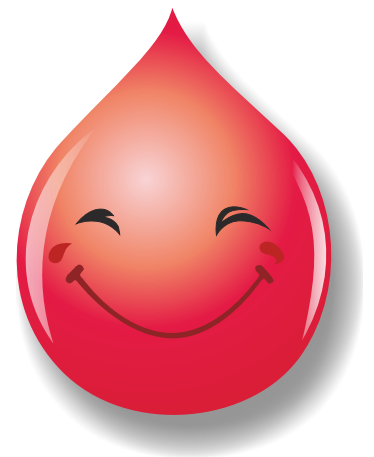
NOTES:

TRANSFUSION TRANSMISSIBLE INFECTIONS

Learning Objectives

When you have completed this chapter you should be able to:

- Recognize the transfusion- transmissible infections (TTIs)
- Know the characteristics of TTIs



Introduction

Screening of donated blood for TTIs represents an important strategy for blood safety and availability. The first line of defense in providing a safe blood supply and minimizing the risk of transfusion-transmissible infections, is to collect blood from well-selected, repeat voluntary non-remunerated blood donors. The prevalence of TTI is generally much lower in voluntary donors than among replacement donors. A lower prevalence of TTIs in the donor population reduces the rate of discarded blood and hence results in improved efficiency and use of resources.

Transfusion-transmissible infections

The microbial agents of importance in blood transfusion services are those organisms that are transmissible by blood and can cause morbidity and mortality in recipients. In order to be transmissible by blood, the infectious agent or infection usually has the following characteristics:

3.1 CHARACTERISTICS OF TRANSFUSION TRANSMISSIBLE PATHOGENS

1. Cause mild or asymptomatic infections.
2. If symptomatic, would have a long incubation period eg. Months (HBV, HCV) or even years (HIV) prior to development of symptoms.
3. Might cause a “carrier state” of infection.
4. Might cause a “latent state” of infection in host cells by incorporating their own DNA in the host’s DNA (HIV, HTLV, and CMV).
5. Would be stable under the storage conditions at which blood and components are stored.
6. Would be present in blood components either in plasma or cells

Following tables show various organisms that can be transmitted by blood transfusion and their characteristics, respectively.

Table 3.1 Transfusion Transmitted Agents

Sr. No	Microbial Agent Type	Name
1	Virus (Enveloped)	HIV-1, HIV-2, HTLV – 1, HTLV-2, HHV-6, HHV – 8, EBV, HBV, HCV, HEV, GBV-C , CMV, West Nile Virus
2	Virus(Non- Enveloped)	HAV, Parvovirus B 19, TTV, enteroviruses
3	Bacteria (Endogenous)	<i>Treponema pallidum</i> (Syphilis), <i>Borrelia burgdorferi</i> (Lyme disease), <i>Brucella melitensis</i> (Brucellosis), <i>Yersinia enterocolitica</i> .
4	Bacteria (Exogenous- environmental/ skin commensals)	<i>Staphylococcus</i> sp., <i>Pseudomonas fluorescens</i> , <i>Salmonella enteritidis</i> , <i>Citrobacter freundii</i> , <i>Serratia marcescens</i> , <i>Enterobacter cloacae</i> , <i>Flavobacterium</i> sp., <i>Serratia</i> sp., <i>Coxiella burnetti</i> , <i>Rickettsia rickettsii</i> .
5	Protozoa	<i>Plasmodium</i> sp., <i>Babesia microti</i> , <i>B. divergens</i> , <i>Trypanosoma cruzi</i> , <i>Leishmania</i> sp., <i>Toxoplasma gondii</i> ,
6	Prions	Variant Creutzfeldt – Jakob Disease (vCJD)

New infections are called **'incident' infections**. The term incidence describes the frequency of new infections in a defined population within a defined period of time. The incidence tells us what is happening now.

The term **prevalence** describes the proportion of a population who, at a given time, has evidence of the infection. The prevalence of an infectious agent, such as HIV antibody, can tell us what has already happened.

The term **window period** describes the period when there is viraemia but no detectable evidence of antibodies. Blood taken in this period is usually infectious, but the virus cannot be detected by current screening tests as ELISA for detection of antibody to HIV.

Some infections such as HIV, hepatitis B and hepatitis C remain present in the blood; this is often called a **'chronic carrier state'**. The blood of individuals who are chronic carriers will transmit the infection to others.

As per the Drugs and Cosmetics Act, 1940 of India, current mandatory tests for screening of blood donation in India include

- Anti-HIV-1 and 2 antibodies
- Anti-HCV antibodies
- Hepatitis B Surface Antigen (HBsAg)
- Serological test for syphilis
- Malaria parasite

3.2 HUMAN IMMUNODEFICIENCY VIRUS

HIV is a retrovirus, an enveloped RNA virus, which is transmissible by the parenteral route. It is found in blood and other body fluids. Once in the bloodstream, the virus primarily infects and replicates in lymphocytes. The viral nucleic acid persists by integrating into the host cell DNA. A number of different groups and subtypes have been identified with some significant antigenic differences. HIV-1 group M is responsible for more than 99% of the infections worldwide. The prevalence of HIV-2 is mainly restricted to countries in West Africa. The appearance of antibody marks the onset and persistence of infection, but not immunity.

As HIV can be present in the bloodstream in high concentrations and is stable at the temperatures at which blood and components are stored, the virus may be present in any donated blood from an HIV-infected individual. Infectivity estimates for the transfusion of infected blood products are much higher (around 95%) than for other modes of HIV transmission owing to the much larger viral dose per exposure than for other routes.

HIV is transmitted through sexual contact, sharing of HIV contaminated needles and/or syringes, transfusion of blood/ components, and nosocomial exposure to HIV contaminated blood or bodily fluids. It can also be passed vertically from a mother to her infant.

1

Screening

The methods used to identify the presence of HIV employ the following screening targets:

- Serological markers:
 - ♦ Anti-HIV-1 & anti-HIV-2
 - ♦ HIV p24 antigen (p24 Ag)
- Viral nucleic acid: HIV RNA.

The assay should be capable of detecting subtypes specific to the country or region.

Using the present anti-HIV ELISA tests, antibody to HIV becomes detectable approximately 21 days after exposure to infection.

Newer technologies such as HIV-1 p24 antigen test and HIV nucleic acid amplification testing (HIV NAT) can significantly reduce the window period.

The test selected for donated blood should be a combined HIV-1/HIV-2 assay, which is highly sensitive and specific. All serum/plasma should be tested with one ELISA or simple/rapid assay. Units of blood yielding reactive or indeterminate test results must be considered as probably infected with HIV and should be discarded using universal safety precautions. The non-reactive units may be taken on blood inventory for use.

3.3 HEPATITIS 'B' VIRUS

Hepatitis B virus (HBV) is a member of the hepadnavirus group and is an enveloped DNA virus. HBV is transmissible by the parenteral route and may be found in blood and other body fluids. Once in the bloodstream, the virus travels to the liver where it replicates in hepatocytes. HBV is highly prevalent in certain parts of the world such as the Far East and Africa (Ref: AABB Technical Manual).

In recently infected individuals, viral DNA is normally present, although not always at high levels. Chronically infected individuals may either be infectious (viral DNA present) or non-infectious with HBV, but does not in itself distinguish between recent and chronic infections. The distinction between acute and chronic infection is not relevant to blood screening. All HBsAg positive donations should be considered to be at high risk of transmitting HBV and should not be released for transfusion.

The serology of HBV is complex. A number of different serological markers develop during the course of infection, including hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (anti-HBc). In addition, HBV DNA can be detected in the majority of cases, although in HBsAg negative phases of infection, the DNA levels are generally relatively low

and the viraemia may be transient. Hepatitis B surface antigen is the prime marker used in blood screening programs. It normally appears within three weeks after the first appearance of HBV DNA and their levels rise rapidly. The presence of HBsAg may indicate current or chronic infection and thus potential infectivity. Most blood transfusion services screen donated blood for HBsAg using sensitive immunoassays.

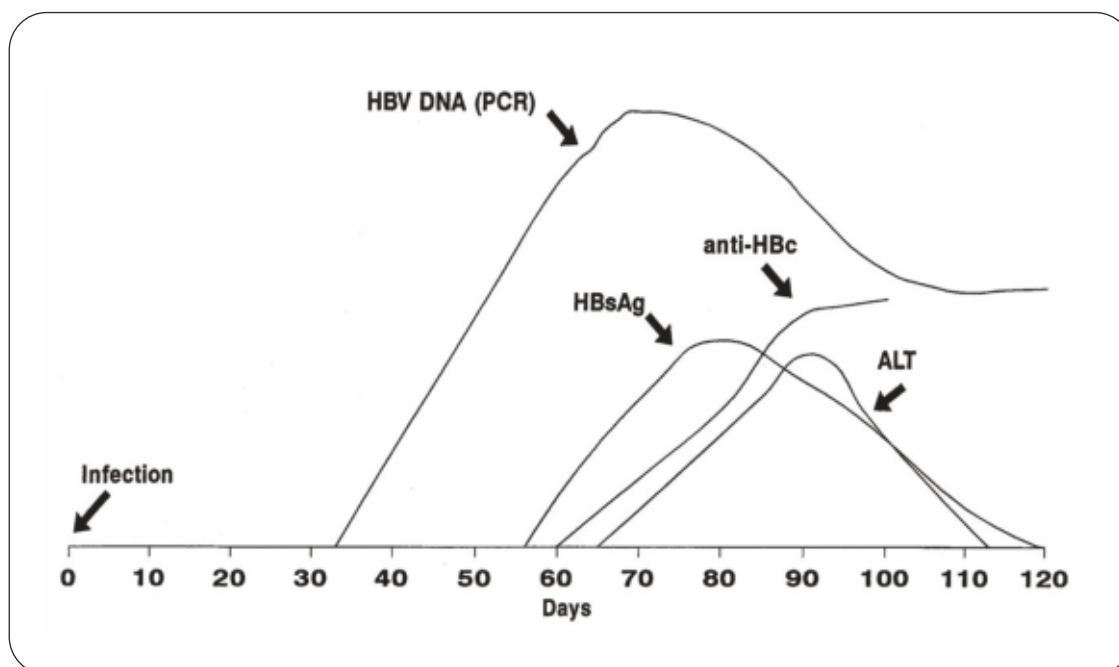


Figure 13: Appearance of serological markers in HBV infection

1 Screening

The methods used to identify the presence of HBV employ the following screening targets:

- Serological markers:
 - ♦ Hepatitis B surface antigen
 - ♦ Hepatitis B core antibody, in some situations
- Viral nucleic acid: HBV DNA.

3.4 HEPATITIS 'C' VIRUS

Hepatitis C virus (HCV) is a member of the flavivirus group and is an enveloped RNA virus. It is transmissible by the parenteral route and may be found in blood and other body fluids. Once in the bloodstream, the virus travels to the liver where it replicates in hepatocytes, resulting in a similar picture to that seen with HBV infection.

In recently infected individuals, virus is normally present. Screening for both HCV antigen and antibody does not in itself distinguish between recent and chronic infection. All HCV antigen-antibody reactive donations should be considered to be at high risk of transmission of HCV and should not be used for clinical use.

1

Screening

The methods used to identify the presence of HCV employ the following screening targets:

- Serological markers:
 - ♦ HCV antibody
 - ♦ HCV antigen
- Viral nucleic acid: HCV RNA.

HCV antibody becomes detectable approximately 30 to 60 days after infection. Viral antigen normally appears between 0 and 20 days after viral RNA first appears. Antibody is generated and can be detected between 10 and 40 days after antigen is first detected. Until recently, anti-HCV has been the prime serological marker for blood screening programs. However, HCV antigen can be detected in the peripheral blood earlier than antibody in the course of early infection. HCV antigen assays, both antigen only and combined antigen-antibody, have been commercially available for a number of years. These have been introduced in some countries to improve the overall effectiveness of serological HCV screening.

3.5 SYPHILIS

Syphilis is caused by the spirochete *Treponema pallidum*. It is transmissible by the parenteral route and may be found in blood and other body fluids. Once in the bloodstream, the bacteria spread throughout the body. A primary lesion, chancre, usually occurs about three weeks after exposure, although the duration may be shorter in cases of transfusion-transmitted infection where the organism enters the bloodstream directly. Syphilis is endemic in many parts of the world.

The treponemes are relatively fragile, in particular being heat-sensitive, storage below +20°C for more than 72 hours results in irreparable damage to the organism such that it is no longer infectious. Thus, although clearly potentially infectious, the risk of transmission through blood and blood components stored below +20°C is very low. Blood components stored at higher temperatures (above +20°C), such as platelet concentrates, present a significantly higher risk of transmitting syphilis.

1

Screening

The methods used to identify the presence of syphilis employ the following screening targets:

- Non-specific, non-treponemal markers: antibody to lipoidal antigen (reagin)
- Specific treponemal antibodies.

Specific assays commonly used for blood screening are Treponema Pallidum Haemagglutination Assays (TPHA) and enzyme immunoassays (EIAs). These detect specific treponemal antibodies and thus identify donations from anyone who has ever been infected with syphilis.

Non-specific assays such as Venereal Diseases Research Laboratory (VDRL) and rapid plasma reagin (RPR) tests identify those individuals who may have been more recently infected. They detect antibodies to cardiolipin or lipoidal antigen (reagin); the plasma levels of these antibodies rise significantly in active infection due to the cellular damage. The sensitivity of these assays is lower than specific assays and the test results may not always be positive, even when the infection is recent.

3.6 MALARIA

Malaria is caused by parasites of the Plasmodium species. There are four main types that infect humans: *P. falciparum*, *P. vivax*, *P. malaria* and *P. ovale*. Malaria is primarily transmitted to humans through the bite of the female anopheles mosquito. Although primarily transmitted by mosquitoes, malaria is readily transmitted by blood transfusion through donations collected from asymptomatic, parasitaemic donors. The parasite is released into the bloodstream during its lifecycle and will therefore be present in blood donated by infected individuals. The parasites are stable in plasma and whole blood for at least 14 days when stored at +4°C.

1

Screening

There are a number of potential targets for malaria screening and the selection of screening method may depend on whether it is endemic in the country or not. The methods used to identify the presence of malaria employ the following screening targets:

- Direct detection of parasite by thick film

Detection of Malaria

Diagnosis of malaria can be done either microscopically or macroscopically.

a) Microscopic Examination: It includes examination of thin and thick blood films. However, microscopic examination of blood film is not suitable for screening large number of blood donations as it is difficult to find parasites in blood films in short time especially if density of parasites is less than 100 per microliter of blood. However, the demonstration of parasites within the red cell is considered the gold standard for diagnosis in suspected cases.

b) Macroscopic methods include:

- a. tests for malaria antibody
- b. tests for malaria antigen
- c. parasitic metabolic products.

Detection of malaria antigen remains the method of choice for detection of malaria in blood donors. It is based on the chromatographic immunoassay principle.

The malaria antigen test contains a membrane strip, which is pre-coated with two monoclonal antibodies as two separate lines across a test strip. The sample is added into the sample well after which assay buffer is added. The test results are read after 20 minutes. Presence of only the control band indicates negative result. Absence of control band indicates an invalid test. Interpretation of the strip for identification of different species of Plasmodium, must be done as per the manufacturer’s instructions.

No blood or blood product should be released for transfusion until all required tests are shown to be non reactive or negative.

3.7 GENERALISED COURSE OF TTI

For persistent TTIs, the course of infection is broadly represented in the figure below. As seen in the figure, there is a “window period” of infection prior to formation of detectable antibody during which conventional ELISA for antibody may be negative.

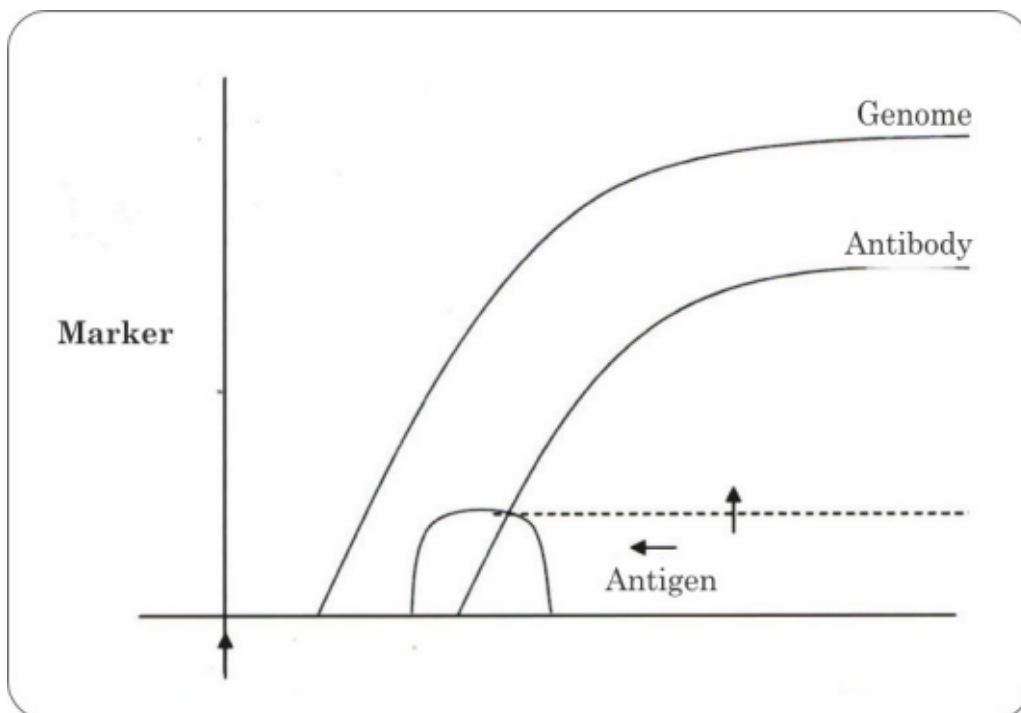


Figure 14: Generalized course of Transfusion Transmissible Infections (TTIs)

1 Residual Risk of Transfusion - Transmitted Infections:

Despite various measures taken to protect blood supply, there still exists residual risk for transmission of pathogens after screening. This is due to following reasons:

1. Window period of donation
2. Absence of seroconversion
3. Genetic variability in the virus strain
4. Laboratory errors

Window period of donation: The window period is the time interval between the donor becoming infectious and the infection being detected by a laboratory test. During this period, the particular screening marker is not yet detectable in a recently infected individual, even though the individual may be infectious. This period is dependent upon the type of lab test used: in general antibody appears only after viral antigens are detectable in the blood. Nucleic acid, as part of the native infectious agent itself, is the first detectable target to appear, followed within a few days by antigen, and subsequently by antibody as the immune response develops.

One or a combination of markers of infection can be used to detect a particular infection during the screening process. Various assay systems developed for blood screening detect:

- Antibodies that indicate an immune response to the infectious agent
- Antigens that are produced by the infectious agent and indicate the presence of that agent
- Nucleic acid (RNA/DNA) of the infectious agent.

Table 3.2: TTIS with various methods of detection

Virus	Screening marker*	Assay	Comments
HIV	Anti HIV 1&2	Immunoassay	<ul style="list-style-type: none"> • Essential for effective HIV screening; screening for HIV antibody is recommended as minimum standard for blood safety • Currently the most efficacious assays are combination antigen-antibody assays • Specific detection of antibodies to both HIV - 1 & HIV - 2 is essential.
	HIV p24 antigen	Immunoassay	<ul style="list-style-type: none"> • First serological marker of HIV infection • A valuable target for donation screening although viral antigen is neutralized by antibody • Screening for HIV antigen only is not appropriate as levels fall as specific antibody levels rise • HIV antigen may be detected at the same time or very soon after first detection of RNA • Currently the most sensitive HIV serological assays combine detection of both antigen (p24 antigen) and antibody (anti - HIV-1 & -2). These assays are considered to be the most effective for the serological screening of donations

	HIV RNA	Nucleic Acid Amplification technology	<ul style="list-style-type: none"> • First circulating marker of HIV infection but the window between the detection of HIV RNA and HIV p24 antigen may be short • Screening for HIV RNA has been implemented in a number of countries • Value of RNA screening is related to serological screening performed and incidence of infection in donors • Evaluate increased safety vs costs and logistics
Hepatitis B	HBsAg	Immunoassay	<ul style="list-style-type: none"> • First serological marker of HBV infection • Significant quantities of HBsAg produced and released into circulation, the majority not associated with viral nucleic acid • Essential for effective HBV screening; screening for HBsAg recommended as minimum standard for blood safety
	Anti-HBc	Immunoassay	<ul style="list-style-type: none"> • Used as an additional marker in some countries to identify resolving infections when HBsAg has declined below detectable levels but HBV DNA may still be present. • May be the only circulating marker of infection at that point • Assays may lack specificity and specific confirmation is not available • Anti-HBs levels should be determined for all anti-HBc reactive donations to identify resolved infections • Accepted in many countries that donations that are both anti-HBc reactive and have an anti-HBs level is above 100 mIU / ml are suitable for clinical use
	HBV DNA	Nucleic acid amplification technology	<ul style="list-style-type: none"> • First circulating marker of HBV infection, but with limited usefulness in blood screening unless individual donation testing is performed • Virus is generally low-titre and the window between the detection of HBV DNA and HBsAg is generally very short
Hepatitis C	Anti - HCV	Immunoassay	<ul style="list-style-type: none"> • Currently the most commonly used serological marker of HCV infection • Appears in response to infection, but window period from first appearance of viral RNA may be relatively long • Screening for HCV antibody recommended as minimum standard for blood safety
	HCV antigen	Immunoassay	<ul style="list-style-type: none"> • First serological marker of HCV infection • A valuable target for donation screening although viral antigen is neutralized by antibody • Screening for HCV antigen only is not appropriate as levels fall as specific antibody levels rise • HCV antigen may be detected at the same time or very soon after first detection of HCV RNA • Very limited assay availability • The most sensitive HCV serological assays combine detection of both antigen and antibody. These assays are considered to be the most effective for the serological screening of donations although currently only a limited number of assays are commercially available
	HCV RNA	Nucleic acid amplification	<ul style="list-style-type: none"> • First circulating marker of HCV infection but the window between the detection of HCV RNA and HCV antigen may be short • Screening for HCV RNA has been implemented in a number of countries, primarily for the safety of plasma for fractionation • Value of RNA screening is related to serological screening performed and incidence of infection in donors • Evaluate increased safety versus costs & logistics

Syphilis	Antibody to <i>Treponema pallidum</i>	Particle agglutination (TPHA)	<ul style="list-style-type: none"> • First specific serological marker of syphilis infection • Essential for effective syphilis screening; screening for specific treponemal antibody recommended as minimum standard for blood safety
	Antibody to lipoidal antigen (reagin)	RPR	<ul style="list-style-type: none"> • First serological marker of syphilis infection • Do not detect syphilis specific antibody • Lack sensitivity and specificity

Ref: WHO document “Screening Donated Blood for Transfusion Transmissible Infections” Recommendations, 2010.

3.8 TYPES OF ASSAY

Various types of assay have been developed for use in blood screening over the past three decades. The assays most commonly in use are designed to detect antibodies, antigens or the nucleic acid of the infectious agent. However, not all assays are suitable in all situations and each assay has its limitations which need to be understood and taken into consideration when selecting assays.

The main types of assay used for blood screening are:

- Enzyme immunoassays (EIAs)
- Haemagglutination (HA)/ particle agglutination (PA) assays.
- Rapid/simple single-use assays (rapid tests)

Recently, nucleic acid amplification technology (NAT) assays. – Chemiluminescent immunoassays (CLIAs) are also being used.

In the context of blood screening, appropriate evaluation is required in selecting the type of assay for each TTI, based on critical assay characteristics, such as sensitivity and specificity, as well as cost and ease of use.

1 Enzyme Linked Immuno Sorbent Assay (ELISA)

Enzyme immunoassays are the most commonly used assays for screening donated blood for TTIs. ELISAs are suitable for the screening of large numbers of samples and require a range of specific equipment. These assays may be performed either manually or on automated assay processing systems. ELISAs have different solid phases to immobilize the antigen or antibody. Most commonly, the solid phases used are:

- Base and sides of a polystyrene micro well
- Surface of polystyrene or other material
- Micro – particles
- Strips of nylon or nitro-cellulose membrane, specifically used in Western blots

Types of ELISA

Indirect ELISA (e.g. 3rd generation HCV, anti HBc ELISA)

This type of ELISA uses solid phase antigen to trap specific antibody. Antibody is detected using enzyme conjugated anti human globulin (AHG) that when bound will cause substrate to change colour.

Competitive ELISA (e.g. anti HBc ELISA, syphilis, etc)

To solid phase antigen, test sample (antibody) and enzyme conjugate are added. Both antibodies (one in the test sample and one in enzyme conjugate) compete for solid phase antigen. Negative reaction causes substrate to change colour; positive reaction has no colour change. The concentration of conjugate antibody is such that the smallest amount of test antibody is sufficient to ensure that it binds to antigen in preference to conjugated antibody

Antibody capture ELISA (e.g. HIV ELISA)

Wells are coated with solid phase anti- human globulin. The test serum antibody is added, incubated and then washed. Virus specific enzyme conjugated antigen is then added to identify the captured antibody. Development of colour indicates a positive reaction.

Sandwich ELISA (e.g. 3rd generation HIV, HBsAg assays)

There can either be solid phase antigen – test antibody – conjugated antigen format (as in HIV ELISA) or solid phase antibody – test antigen – conjugated antibody format (as for HBsAg). The main advantage is increased specificity as the number of false positives decrease.

Particle agglutination assays

Particle agglutination assays detect the presence of specific antibody or antigen in a test sample through the agglutination of particles coated with the complementary specific antigen or antibody respectively. Agglutination assays, mainly antibody assays, use a range of particles including red cells (Haemagglutination) and inert particles such as gelatin and latex. This use of inert particles has the advantage of reducing non-specific reactivity against cross reacting red cell antigens. The basic principles of haemagglutination and particle agglutination assays are the same, irrespective of the type of particles used. PA assays are still used extensively for the detection of syphilis antibodies.

PA assays do not involve multiple steps or need wash equipment. In a manual system, they are read visually, the reading of results is dependent on subjective evaluation and no permanent record of the test results can be kept. These tests are suitable in emergency situations for immediate release of blood, in case of plateletpheresis donations and in blood banks with low volume of collection.

2

Rapid/simple single-use assays (rapid tests)

Developed in 1980s, these assays have been proven to be as accurate as ELISA. The testing takes less than 30 minutes however, technical errors are more common due to its simplicity.

Rapid/simple single-use assays are discrete, individual, disposable assays: i.e. they are used once and discarded. These assays exist in a number of different presentations. Many rapid tests are based on a form of immune chromatography in which the added sample flows down an insert strip and reacts with previously immobilized reagents. The sample can be serum, plasma or even whole blood in some cases. Any positive reaction is visualized as a dot or a band appearing on the device strip. Most of the assays also include a control dot or band that is used to validate the results of each individual device, irrespective of the specific test result. These are divided into three main groups-

- Membrane based Enzyme Immuno-assay.
- Immuno-filtration method.
- Immunochromatography.
- Particle-agglutination assay.

Other rapid test formats may be dipsticks in which, antigen is attached on the teeth of comb like devices. Rapid tests are provided in simple-to-use formats that generally require no additional reagents except those supplied in the test kit. They are read visually and give a simple qualitative result within minutes. The reading of results is dependent on subjective evaluation and no permanent record of the original test results can be kept.

The merits and demerits of both ELISA and Rapid assays are compared in the following table

Table 3.3: Comparison of ELISA with Rapid Assay

Characteristic	ELISA	Rapid Assay
Sample	Serum, Plasma	Serum, Plasma, Whole Blood
Testing	Batch	Individual
Time required	>2 hours	< 30 min
Infrastructure	Specialized set up	Minimal
Subjective variation	Minimal	Inter reader variability
Quality Control	Possible	Difficult to perform

3.9 SELECTION OF ASSAYS

Sensitivity and specificity are the key factors to be considered in selecting a specific assay. For the screening of blood donations, assay should be highly sensitive and specific.

Sensitivity (measures the proportion of positives that are correctly identified)

- The ability of an assay/reagent to detect even in very small amounts of sample
- The ability of a test to detect positive cases (the absence of false negatives)
- Probability of an assay detecting all infected individuals

The kit selected should be highly sensitive to ensure that the test result will be reactive in an infected individual. It is the ability of the assay to detect weakest possible positive sample.

Specificity (measures the proportion of negatives that are correctly identified)

- The degree of false reactivity associated with an assay/reagent
- The ability of the test to identify all negatives correctly (produces no false positives)


SENSITIVITY	Detects infected Individuals	$\frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}} \times 100$
SPECIFICITY	Detects uninfected Individuals	$\frac{\text{True negatives}}{\text{True Negatives} + \text{False positives}} \times 100$

The kit selected should have high specificity to ensure that a test result will be non reactive in an individual who is not infected. It is the ability of the assay not to detect false or nonspecific positives.

The use of rapid assays is generally not recommended for blood screening as they are designed for the immediate and rapid testing of small numbers of samples. These assays are performed using manual techniques; the results therefore have to be transcribed by staff and there is a lack of permanent records and traceability. As a result, they may have limited use in laboratories where through-put is medium to high. They may also be appropriate when a laboratory needs to screen specific donations on an emergency basis for immediate release of products due to a critically low blood inventory or when rare blood is required urgently.

3.10 BLOOD SCREENING PROCESS

The screening of donated blood and the quarantine of blood and blood components represent critical processes that should be followed to ensure that blood units are safe. Based on the screening results, they should either be released for clinical use or be discarded. Laboratory screening for TTIs should be performed on blood samples collected at the time of donation. All blood samples, donations and components should be correctly labelled to ensure correct



identification throughout the screening process. The BTS should also have appropriate systems for linking all test results to the correct donations and donors so that donor records can be reviewed each time they come to donate. These systems will ensure that the correct results are linked to each donation and prevent errors resulting in the transfusion of an unsafe unit.

Quality control of Assays

Continuous measures that are taken to ensure that for each assay test is working accurately as per the limits of the test so as to produce valid and acceptable results. This indicates

- The test is valid
- All test conditions for that run have been met
- All test results for that run are reliable

The Essential components for quality control that must be performed during every assay are as follows

- Each test run must include one full set of controls that yield results within the limits of standards for acceptability and validity
- Follow strictly the incubation time, reagent concentration, temperature range mentioned on the test insert
- Use kits within their shelf life
- Test is invalid if the minimum no of controls are not in acceptable range

Controls

There are two categories of controls

1. Internal/Kit controls
2. External controls

Internal/Kit controls

- Set of controls provided with the kit- both positive as well as negative
- Should be used with the same batch only
- These do not detect any minor deterioration of the kit

External controls These can be procured from National reference centres, commercially as control panels and can be prepared in house from reactive/non reactive blood bags

- Set of controls included from outside
- Positive samples -borderline positive and negative
- Should be used with the same batch only
- These detect any minor error in the assay performance

Protocol for preparing in house External control

Select a high titre Sero Reactive serum/plasma



Retest the sample with another kit



Heat inactivate the sample at 56°C for 30 minutes



Recalcify plasma to obtain serum*



Make serial doubling dilutions with sero non reactive serum



Test run the sample in triplicates in three different wells at different spaces



Record OD values of controls and triplicate samples of each dilution and calculate the cut off on the basis of control supplied with kit.
Calculate the Elisa Ratio (ER) which is calculated as $\text{Sample OD/Cut off OD}$



Select the sample with dilution with ER between 1.5-2 as this is the borderline Reactive



Prepare External control aliquots of the dilution selected above



Can be Stored at -20°C for one year



Once thawed can be stored at $2-8^{\circ}\text{C}$ for one week



The external controls should be put for each run to ensure validity of the test results An external control with mean ± 2 SD means the test is valid

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK											
B	NC											
C	NC											
D	PC											
E	PC											
F	PC											
G	S1											
H	S2											

NC-Negative control; PC-Positive control; S-Sample.

*Recalcification of plasma to obtain serum

- Prepare 10X recalcification solution (0.25Mcacl₂,6H₂O55g and .08MMgcl₂6H₂O,16g dissolved in 100ml distilled water)
- Autoclave at 121°C for 20 minutes at 15lb pressure
- Add 1.5 ml of recalcification solution to one unit of blood/250ml of plasma
- Incubate at 37°C for 30-60 min
- Store overnight at 4°C
- Centrifuge at 1500rpm for 20 min
- Separate serum aseptically and Test for HIV antibodies
- Store serum in convenient small aliquots for future use

ELISA Testing

1. Remove reagents from the refrigerator 30 minutes prior to testing. Mix the reagents gently by inverting the vials without foaming.
2. Bring the samples to room temperature before testing.
3. Arrange all samples to be tested serially in ascending order in which they are to be tested in a test tube rack.
4. Place the plate in front of the test tube rack
5. Make entries in the work sheet and map the plate
6. Follow the instructions as per kit insert for ELISA testing

Cut off O.D. is automatically calculated

Interpretation: of absorbance values:

- (i) The samples below the cut off are considered non-reactive.
- (ii) Equal to cut off are considered initially reactive
- (iii) Above cut off are considered Reactive
- (iv) Sample close to cut off value 10% below cut off (grey zone)
- (v) Samples with grey zone results are repeated in one well.

Validation of the test

- Check the validity of the Blank (if used) as well as negative and positive control absorbance value as per pack insert of the kit.
- Examine absorbance values of the controls before the sample results can be interpreted. If the run fails to meet the criteria as per package insert consider the test as invalid and repeat the whole test again.

Trouble shooting in ELISA testing

In spite of being vigilant and following the SOP, many a times a test run fails. The reason may be apparent in some cases but no in others. Troubleshooting refers to measures undertaken to determine why a run has failed.

General approach to trouble shooting

Factors responsible for some common general problems/errors

1. Specimen : lysed blood, lipaemia, deterioration, volume not sufficient.
2. Clerical/transcription : wrong name/ID no/wrong result typed, labeling etc.
3. Kit related : conjugate gone bad, storage not proper, inter-lot variations etc.
4. Laboratory variations : due to different kit, different SOP, different technique. This kind of variation may be inter-laboratory as well as intra-laboratory. So, results are non-reproducible.
5. Environmental conditions: higher temperature, humidity.
6. Equipment problems: ELISA reader, washer, calibration of pipettes, calibration of refrigerator etc.
7. Technical errors: carelessness, not following SOP, pipetting errors, worksheets not maintained.
8. Borderline reactor/grey zone reactors.
9. Quality control not practiced.

Methods for identifications of some common general problems

1. Review protocol with the technician who ran the test, carefully checking for procedural omissions or changes.
2. Double check component expiration dates.
3. Verify that all physical parameters of the assay were followed and met (e.g. times and temperatures).
4. Confirm that the support equipment such as pipettes, plate washing and reading systems, etc. are working properly. Verify that the preventive maintenance and servicing procedures have been performed.
5. Check the wavelength of the plate reader.
6. Investigate the calibration of the pipettes. If the assay calls for 10 ml of specimen, and the pipette delivers 9 ml this constitutes a 10% error and may be sufficient to cause the controls to fall outside the acceptable limits. Proper calibration of pipettes is essential for accurate results.
7. Check the quality of the distilled water.
8. Check that reagents are not contaminated and were prepared and stored properly. There are several situations in which characteristic appearances in a test system may indicate problems. Examples include;
 - a) If the substrate tablets appear “Orange” when they should appear “yellow to white” – indicates that the tablets have been contaminated or have deteriorated, and thus cannot be used.
 - b) If the reagent “indicator cells” or “carrier particles” in an agglutination assay appear clumped or otherwise heterogenous – this reagent may have been contaminated or may have deteriorated. Most package inserts describe the appearance of the reagents contained within the kit, and thus ensure comparison of the actual appearance with what is expected.

Specific assay problems : Some examples and their possible solutions

1. All wells in an ELISA are some colour in each row. This could be either due to conjugate going bad, not working or substrate going bad or washer problems. Try another kit from same lot to pinpoint the cause of error.
2. The plate visually reads O.K. i.e. different wells have different colours but the result on spectrophotometer shows – almost identical ELISA values. This is due to error in detection and/or reference wavelength of the spectrophotometer/ELISA reader. Correct the wavelength and then read.
3. Negative control strip has an unexpected band. Due to contamination between wells/reaction has been allowed to develop too long. Repeat procedure with same controls plus negative control from another kit/lot number to identify errors.

Variations in test results

The test results produced by the same laboratory (reproducibility) and or by a different laboratory on the same specimen may vary. This can be due to an error at any step right from collection of specimen to testing and final reporting of the result.

The reasons for inaccurate results may be on account of :

1. Specimen problems.
2. Clerical errors
3. Kit dependent problems.
4. Technologists dependent errors
5. Equipment problems.
6. Environmental problems and their influence on the test result
7. Non repeatable (Reproducible) results.

Table: 3.4 Specimen problems

Sl. No.	Errors	Resolution
1.	Insufficient volume for repeating the test in case of reactive result.	Collect 3-5 ml of blood depending upon the objective of testing and strategy of testing.
2.	Haemolysis can cause false positive or false negative result in ELISA and background noise on spot test.	Repeat sample. In case it is not possible, record "sample haemolysed" in the result.
3.	Lipaemia causes pipetting error, otherwise it causes no error in the test result.	Buy widebore pipettes and pipette tips. Record "sample lipaemic" in the result.
4.	Bacterial contamination breakdowns antibody and will affect the borderline reactive result.	Refrigerate the samples for short storage (one week), store at - 200 C for long storage (>one week).
5.	Frozen/thawed sample. This may affect the borderline positive samples	Avoid freeze thawing as far as possible till the test is performed.
6.	Aliquoting errors	All aliquots should be labelled. A uniform method of storing samples/aliquots should be followed in the laboratory. Organize the specimen in racks and leave a tube space empty between aliquots made from different specimens.

Table 3.5: Clerical errors

Sl. No.	Errors	Resolution
1.	Logging in specimen : may be mixing in names, wrong history, wrong number etc.	Set up the log books/record properly; collect second sample from the same patient and perform testing.
2.	Result print out : the well number on worksheet and the print out not matched properly (transcription error).	Transcribe the result from the print out sheet to the worksheet carefully. Repeat the test.
3.	Lipaemia causes pipetting error, otherwise it causes no error in the test result.	Supervisory review/vigilance of results by a second person will correct the error.
4.	Reporting to the wrong person or result communicated without post test counseling and confidentiality.	Report result to the correct person after post test counseling and maintaining the confidentiality. Reporting should be as per the "National HIV Testing Policy".

Table 3.6: Kit Dependent errors

Sl. No.	Errors	Resolution
1.	Used after expiration date.	Use before expiration date.
2.	Mixing of reagents from different kits/lot numbers	Use reagent for the kits for which they are made. Do not mix reagents from different.
3.	Performance characteristics (sensitivity, specificity and delta values) not satisfactory.	Do not use unsatisfactory kits.
4.	Deterioration/contamination of one or more component or reagents of the kit. (faulty transport/storage).	Do not use contaminated deteriorated (control values different from those mentioned in package insert) kits. Storage at the optimal temperatures and maintain cold chain during transport of kits.
5.	Intra-lot and inter-lot variation in kit performance due to faulty manufacturing practices.	Random monitoring of performance characteristics of the kits to ensure the quality.

Table : 3.7 Technologist dependent errors

Sl. No.	Errors	Resolution
1.	Dilutional errors	Carefully calculate the volumes of all components required for the test and perform dilutions of reconstituted components accordingly to get the desired volumes.
2.	Scratching the coated antigen from the well.	Do not touch the plate well while adding the sample.
3.	Inconsistent technique for test and controls particularly quality control samples.	Treat each sample as well as controls in exactly the same way.
4.	Mixing reagents from different lots of kits.	Use reagents for the kits for which they are prepared for use. Do not mix reagents.
5.	Mixing of samples	The worksheets should be meticulously prepared indicating which specimen goes into which well. The internal quality control samples should be randomly placed for each run.
6.	Do not follow the SOP	Each step of SOP must be diligently followed.
7.	Wrong pipetting due to carelessness.	Supervision, vigilance and training for accurate pipetting.

Table 3.8: Equipment based errors

Sl. No.	Errors	Resolution
1.	Use of inappropriate pipette tips	Use appropriate volume pipette tips to deliver accurate volumes.
2.	Equipment not maintained as per requirement.	Maintain and calibrate the equipments as per the requirement (Refer to chapter on equipment maintenance and calibration).
3.	Washer not working satisfactorily.	The washer should be placed under annual maintenance control for optimal working.
4.	Use of wrong U.V. filters	Use the U.V. filters for reading results as per the directions in package insert.
5.	Improper reader	ELISA reader must be placed under maintenance for accurate results.
6.	Refrigerator/deep freeze not working to optimum level.	Calibrate temperature to ensure that right temperatures are maintained in refrigerator/deep freeze.

Table 3.9: Environment dependent errors

Sl. No.	Errors	Resolution
1.	High and low temperature can affect the enzyme reaction and the result	Calibration of incubators to maintain the right temperatures is very important.
2.	Drying of plates	Maintain humidity so that the plates do not dry.

Table 3.10: Non repeatable / Non-results

Sl. No.	Causes	Resolution
1.	Mislabelling of specimen	Test 2nd sample collected from the same patient.
2.	Specimen deterioration	Parallel testing of the sample with stored sample. Repeat testing.
3.	Borderline reactors.	Follow up and repeat testing.
4.	Carelessness of the technologist	Vigilance in the laboratory. Training of personnel.
5.	SOP not changed with the change in kit.	Review SOP and change if required particularly if new type of kits are purchased.

Every step of quality control has to be practiced every day to ensure that the procedures and results are accurate and no false positive or false negative results are generated by the TTI laboratory for ensuring Blood Safety.

3.11 PREVENTIVE STRATEGIES FOR TRANSFUSION TRANSMISSIBLE INFECTIONS

A variety of strategies have evolved in recent years in an attempt to decrease the morbidity and mortality associated with TTI. These strategies are summarized in the following box.

- I) Careful donor selection.
 - a. Repeat voluntary blood donors
 - b. Education counselling and retention of these donors
 - c. Improvement in the blood donor screening criteria
- II) Reduce the risk of product contamination
 - a. Improved venipuncture site disinfection
 - b. Removal of first aliquot of the donor blood by using bags with diversion pouch.
- III) Improved blood component processing and storage.
 - a. Optimizing storage temperature
 - b. Universal leukocyte reduction
- IV.) Improved pre- transfusion blood testing
 - a. Sensitive and specific serological testing
 - b. NAT test
 - c. Visual inspection of component before use
- V.) Reducing recipient exposure to blood donor
 - a. Optimizing transfusion indications
 - b. Increased use of single donor products
- VI.) Pathogen inactivation

Choose the Best Option

1. Answer in true or false:
According to Drugs & Cosmetics Act 1940, Ministry of Health and Family Welfare, Govt. of India, all blood and blood components should be screened for various transfusion-transmitted infections such as:
 - a. HIV p 24 antigen T/F
 - b. Hepatitis B surface antigen T/F
 - c. Anti-HBc antibody T/F
 - d. Hepatitis B surface antibody T/F
 - e. Anti- HIV I/II antibody T/F
 - f. Anti- hepatitis C virus antibody T/F
 - g. VDRL/RPR test T/F
 - h. Malaria parasite T/F
2. ELISA test stands for
 - a. Enzyme Linked Immuno Solution Assay
 - b. Enzyme Linked Immuno Sorbent Assay
 - c. Enzyme Linked Immuno Savlon Assay
 - d. All of the above
3. Correct sequence of the adding sample and reagents in an ELISA test is;
 - a. Sample → conjugate → substrate → stop solution
 - b. Conjugate → sample → substrate → stop solution
 - c. Sample → substrate → conjugate → stop solution
 - d. Not sure of the sequence
4. Reactive sample on an ELISA test is:
 - a. OD of the test is less than cut off value
 - b. OD of the test is above the cut off value
 - c. OD of the test is at and above the cut off value
 - d. None of the above
5. Comb AIDS rapid test for HIV is based on
 - a. Dot blot technique
 - b. Line Immunoassay
 - c. Flow through technique
 - d. Lateral flow technique
6. The end result of VDRL/RPR test for syphilis is:
 - a. Agglutination
 - b. Flocculation
 - c. Precipitation
 - d. All of the above

NOTES:

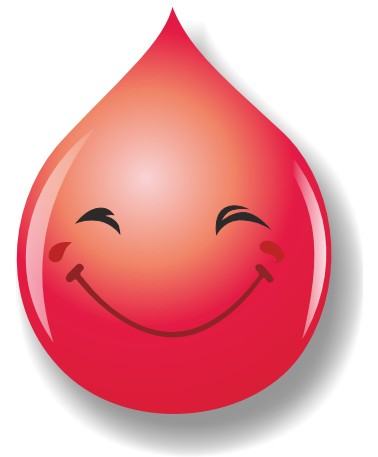
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BIOSAFETY

Learning Objectives

When you have completed this chapter you should be able to:

- Define Bio-safety and occupational hazards
- Describe the modes of exposure to blood borne pathogens.
- Understand universal precaution methods



Definition

Biosafety is the use of laboratory practices, procedures and equipments for safety when working with potentially infectious microorganisms.

4.1 RISK TO HEALTHCARE WORKERS (HCW)

Blood is the most important source of HIV, HBV and HCV infections. The risk depends upon the following factors.

- Prevalence of infected individuals in the population.
- Types of exposure, e.g. exposure of intact skin, breached skin, mucous membrane or needle stick injury.
- Quantity of blood to which HCWs are exposed e.g. more in case of hollow bore.
- The relative infectivity and concentration of the virus; HIV the risk is 0.05 to 0.3% (viral load 10 to 100 viral particles per ml plasma) HCV the risk is 3.0 to 10% (viral load is 10000 to 100000 viral particles per ml) HBV the risk is 10 to 30% (viral load is 10,000,000 viral particles per ml).
- Number of exposures.

Table 4.1: Modes of exposure to blood borne pathogens in the laboratory

Procedure	HCW at Risk	Source/Modes of Transmission
Collection of blood	Laboratory technical staff / phlebotomist	<ul style="list-style-type: none"> • Needle stick injury • Broken specimen container • Blood contamination of hand with skin lesion/breach
Transfer of Specimen	Laboratory Technician , phlebotomist and support staff and transport worker	<ul style="list-style-type: none"> • Contaminated exterior of the container/requisition slip • Broken container • Spill/splash of specimen
Processing of Specimen	Laboratory Technical Staff	<ul style="list-style-type: none"> • Puncture of skin • Contamination of skin/mucous membrane from contaminated work surface • Spills/splashes of specimen • Broken specimen container • Faulty techniques • Perforated gloves
Cleaning / Washing	Laboratory support staff	<ul style="list-style-type: none"> • Puncture of skin • Contamination of skin from contaminated glass ware • Spills/splashes • Contaminated work surface
Disposal of Waste specimen transport/ Mailing	Laboratory support staff, transport, postal staff	<ul style="list-style-type: none"> • Contact with infectious waste specially sharps, broken containers • Leaking container or packaging.

4.2 GOOD LABORATORY PRACTICES

As a general rule all samples should be treated as though they are potentially infectious

- Have a Biohazard sign displayed on the doors of the rooms where infectious agents are handled.
- Entry to laboratory working area should be only for laboratory person
- Doors to the laboratory should be kept closed.
- No smoking, eating, or drinking is allowed in laboratory area

Preparation of hypochlorite solution

Available chlorine concentration (labelled on bottle)	0.1% for general purpose and wiping working area	1% for spillage
Sodium hypochlorite (available chlorine 4%)	2.5 ml sodium hypochlorite + 97.5 ml water	25 ml sodium hypochlorite + 75 ml water
Sodium hypochlorite (available chlorine 5%)	2.0 ml sodium hypochlorite + 98 ml water	20 ml sodium hypochlorite + 80 ml water

1 General Laboratory Hygiene

The work surfaces should be cleaned with a disinfectant before and after any procedures are performed. The equipments are cleaned with disinfectant at the end of each working day. 1% sodium hypochlorite and isopropyl alcohol are used as effective disinfectants.

The staff should avoid eating and drinking in the labs. There should be restricted access to the labs and only authorized staff should be present.

2 Universal Precautions

Universal (standard) precautions should be used consistently by all HCWs. These include barrier protection / personal protective equipment like gloves, lab coats, occlusive bandages on skin abrasions or cuts, plastic aprons for staff, clean reusable items and disposing waste. Gloves should be of good quality, well-fitting and disposable. One should avoid touching exposed body parts like face with gloved hands and take precaution not to touch the door handles and computers with gloved hands. If gloves get torn while working, they should be removed immediately and hands washed with soap and running water. The protective lab coats and aprons should be removed while leaving the laboratory.



3

Safe Handling of Specimens and Sharps

All samples should be collected in screw capped containers and capped securely to prevent spills and leaks. Disposable syringes and needles should be used for sample collection. Used needles must be disposed into a blue coloured “Puncture proof container”. Used needles should not be recapped. A hub cutter to be used to cut the needle hub from syringe. Used syringes should be discarded into the Red coloured disposal bins. Samples (pilot tubes) from donors should be placed in racks in upright position and transported to labs in leak-proof plastic or rigid thermocol containers.

4

Management of Blood Spills

Blood spills should immediately be covered with absorbent paper sheets or gauze sponges to contain the spread of blood, gloves must be worn while handling blood spills. It should then be covered with 1% sodium hypochlorite for at least 30 minutes. The paper towels / gauze should be disposed as biohazard waste. Any broken glass pieces should be swept with dustpan and brush and disposed as sharps. The spill should be immediately reported.

5

Hepatitis B Vaccination for all Laboratory Staff

All blood bank staff must be vaccinated for Hepatitis B with three doses (0, 1 and 6 months). Revaccination should be considered for health care providers working in high risk environment once in 10 years. Previously vaccinated health care providers need to check the Hepatitis B antibody titre and revaccinate if the titres are less than 10 IU.

6

Post Exposure Prophylaxis (PEP)

An exposure that may place a HCW at risk of contracting blood-borne infection includes a percutaneous injury which may be through mucous membrane contact, prolonged contact with intact skin or immediate contact with breached skin. The exposure must be reported to the laboratory supervisor immediately and the staff Health services contacted and treated as an emergency. The exposed site should be washed immediately. Blood should be collected for testing after written informed consent.

(i) PEP for HBV

Table : 4.2 PEP for HBV

Recommendations for Hepatitis B prophylaxis following per cutaneous or per mucosal exposure.

Exposed Person	Treatment when source is found to be		
	HBsAg – positive	HBsAg–negative	Source not tested or unknown
Unvaccinated	HBIG x 1* and initiate HB vaccine	Initiate HB vaccine	Initiate HB vaccine
Previously vaccinated known responder	Test for anti-HBs titre 1. If adequate, no treatment 2. If inadequate, HB vaccine booster dose	No treatment	No treatment
Previously vaccinated known non-responder	HBIG x 2 or HBIG x 1 plus 1 dose of HB vaccine	No treatment	If known high-risk source, may treat as if source were HBsAg-positive
Response unknown	Test for anti-HBs 1. If inadequate HBIG x 1 plus HB vaccine booster dose 2. If adequate, no treatment	No treatment	Test for anti-HBs 1. If inadequate @, HB vaccine booster dose 2. If adequate, no treatment

*HBIG dose 0.06 ml/kg IM

@ Adequate anti-HBsAg is > 10 SRU by RIA or positive by EIA.

(ii) PEP for HCV

There is no vaccination and no recommended chemoprophylaxis. Only follow-up testing for sero-conversion is recommended.

(iii) PEP for HIV

Table 4.3: HIV Post-Exposure Prophylaxis Evaluation

Exposed	Status of Source (see below)			HIV status Negative
	HIV + and low Risk	HIV + and high Risk	HIV status unknown	
Mucous membrane / non-intact skin; small volume (drops)	Consider 2-drug PEP	2-drug PEP	Consider 2-drug PEP	No PEP is required if the source blood is confirmed HIV negative
Mucous membrane / non-intact skin; large volume (major blood splash)	2-drug PEP	3-drug PEP	Consider 2-drug PEP	
Percutaneous exposure: not severe solid needle, superficial	2-drug PEP	3-drug PEP	Consider 2-drug PEP	
Percutaneous exposure: severe large bore hollow needle, deep injury, visible blood in device, needle in patient artery/vein	3-drug PEP	3-drug PEP	Consider 2-drug PEP	

Low risk HIV exposure is said to occur when the exposure source is asymptomatic and has normal CD4 counts. High risk HIV exposure is said to occur when the source has advanced AIDS, high viral load or low CD4 counts.

Table 4.4: Post-Exposure Chemoprophylaxis

Basic regimen: 2 drugs (NRTIs) (4 weeks therapy)	Zidovudine (AZT/ZDV) – 300mg twice/day is used for all types of exposure + Lamivudine (3TC) – 150 mg twice a day is added to increase the effectiveness of ZDV and to prevent resistance to ZDV
Expanded Regimen: 3 drugs (2 NRTIs + PI) (4 weeks therapy)	Basic Regimen (AZT/ZDV + 3TC) + Nelfinavir 750 mg three times daily or any other boosted protease inhibitor. (for higher risk categories – consult expert)

In case the HIV source person is on antiretroviral therapy, the ART physician must be consulted to know if there is any drug resistance .

(Refer updated PEP guidelines from NACO website)

Follow-up in case of PEP

As mentioned, the first blood sample is collected immediately after exposure. Subsequent blood samples are collected at 6 weeks, 12 weeks and 6 months to assess the seroconversion if any. During this period all standard precautions to avoid HIV transmission should be observed.

4.3 BIOMEDICAL WASTE MANAGEMENT

Biomedical waste means any waste, which is generated during diagnosis, treatment or immunization of human beings or animals or in research activities pertaining thereto or in the production or testing of biological and including categories mentioned in Schedule I of the Biomedical Waste (Management and Handling) Rules, 1998, (Draft 2011) as published by the Ministry of Environment and Forests.

Table 4.5: Categories of Bio-Medical Waste

Option	Waste Category	Treatment and disposal
Category 1	Human anatomical waste	Incineration/deep burial
Category 2	Animal waste	Incineration/deep burial
Category 3	Microbiology and Biotechnology waste	Local autoclaving / microwaving / Incineration
Category 4	Waste sharps	Disinfection (autoclaving / microwaving and mutilation / shredding) Final disposal through authorized common BMW Treatment facility or secure landfill or concrete waste sharp pit.
Category 5	Discarded Medicines and Cytotoxic drugs	Incineration / destruction and drug disposal in secured and fills
Category 6	Soiled waste (items contaminated with blood and body fluids)	Incineration / autoclaving / microwaving
Category 7	Infectious Solid waste (Tubings, catheters, intravenous sets etc.)	Disinfection by chemical treatment autoclaving / microwaving and mutilation / shredding
Category 8	Chemical Waste	Disinfect chemically and discharge into drains

The Blood bank waste handling is an essential job which needs to be under supervision .
Broad guidelines for waste segregation are to be followed.

Table: 4.6 Segregation and Disposal

Sl. No.	Area	Type of Waste generated	Segregation	Treatment of waste Disposal
1.	Donor Screening area	Swabs	Yellow Bags	Incineration
		Lancets	Puncture proof Container with Hypochlorite	Landfill/Safe pit
		Micro pipette tip	Red Bag	Shredder
		Gloves	Red Bag	Shredder
2.	Blood collection room	Swabs	Yellow Bag	Incineration
		Bag Needle	Needle destroyer (Puncture proof container)	Landfill/Safe pit
		Blood Bag tubing	Red Bag	Shredder
		Gloves	Red Bag	Shredder
		Wrappers(All Labs)	Black Bag	Municipal garbage
3.	Component lab	Blood bag tubing	Red Bag	Shredder
		Apheresis Kits	Red Bag	Shredder
		Gloves	Red Bag	Shredder
4.	TTI Lab	Sample tubes (Glass)	Hypochlorite solution	Recycle
		Sample tubes (Plastic)	Red Bag	Shredder
		Discarded plates	Red Bag	Shredder
		Washer waste	Hypochlorite solution	Drain in sewer line
		Chemicals reagents, kits Controls	Hypochlorite solution	Drain in sewer line
		Reactive Bags	Autoclave/Red Bag	Shredder
		Broken Sample tubes (Glass)	Hypochlorite solution	Land fill
		Gloves	Red bag	Shredder
5.	Serology Lab	Sample Vials (Glass)	Hypochlorite solution	Recycle/Land fill
		Blood Bag tubing	Red Bag	Shredder



Figure 15: Universal Bio Hazard Symbol



Figure 16: Colour Coded Bins



Figure 17: Red Bin with Infected Waste Discard



Figure 18: Black Bin with General Waste Discard

Ref: (Picture source: Transfusion Medicine centre, NIMHANS, Bangalore)

4.4 DISINFECTION

Disinfection is the reduction in the number of pathogenic microbes so that the material/object/surface becomes safe for handling. Sodium hypochlorite is the most frequently used general lab disinfectant. Its advantages are that it is affordable, easily available and has virucidal and bactericidal action. Its limitations are that the solution gradually loses its strength, hence requires daily preparation of fresh 1% solution from stock solution (generally available as 4%) and secondly it corrodes metals. Sodium hypochlorite should be poured in plastic jars or bins for use in labs.

Disinfection of glassware and plastic ware is achieved by immersing in 1% sodium hypochlorite for at least 30 minutes. Subsequently glassware can be placed in hot air oven at 160°C for 1 hour.

4.5 AUTOCLAVING

In this process saturated steam under pressure is used to decontaminate the infectious material. The main factors influencing quality of steam disinfection are temperature, time and presence of saturated steam. A pressure of 15 psi at 121°C for 45 to 60 minutes is required for adequate microbial destruction. The quality of this procedure is checked with strips or vials of *B. stearothermophilus*, at least once a month. Records of autoclaved blood units and quality control must be maintained.

Autoclave should be fitted with pressure and temperature indicator. Bio safety practices should be in accordance with the Biomedical Waste Management and Handling Rules, 1998 (Draft 2011).

4.6 DISPOSAL OF BLOOD AND LABORATORY MATERIAL

Nurses play an important role in disposal of blood and laboratory material. They should strictly follow all biomedical waste management rules and regulations in the disposal of blood and laboratory material. They can place ready reckoner for all other staff in following the standard protocols.

- Blood bank staff should know the methods of disposal of blood bags. Method of disposal of blood bags should comply with the requirements of Biomedical Waste Rules of the Ministry of Environment and Forests and local pollution control board. Needles should be burnt using electric needle destroyers or soaked in hypochlorite solution or discarded in a puncture proof container of a non-chlorinated plastic. These should then be sent for deep burial or incineration.
- The spill on table tops/sinks should be covered with filter papers or plain cloth and soaked with 1% hypochlorite solution for at least 30 minutes and later swabbed and discarded into red bag.
- Hypochlorite – detergent solution 0.5-1.0 per cent solution of hypochlorite is the best general purpose disinfectant if contact is maintained for at least 30 minutes. (Except for metallic equipment which could be autoclaved or put in cidex). Disposal by Sterilisation: Autoclaving for 30 minutes at 121°C and 15p.s.i. (68.5 cm Hg) is the method of choice. Validation with use of biological indicator (*B. stereothermophilus*) should be done at least once a month.

Choose the Best Option:

1. The general rule regarding handling of blood and blood products is that:
 - a. All samples should be treated as potentially infectious
 - b. Reactive sample to be treated as potentially infectious
 - c. Samples from patients to be treated as potentially infectious
 - d. Sample for donors to be treated as potentially infectious

2. Yellow plastic bags are used for disposal of the following bio-medical waste in the blood bank:
 - a. Cotton swabs/tissue paper contaminated with blood/serum/plasma
 - b. Paper/general waste
 - c. Needles/lancets
 - d. All the above

3. Barrier protection does not includes:
 - a. Gloves
 - b. Hepatitis B vaccination
 - c. Masks
 - d. Occlusive bandages

4. To maintain general laboratory hygiene the following should be enforced:
 - a. Restricted entry to work areas
 - b. Avoid eating, drinking, smoking in the laboratory
 - c. Avoid mouth pipetting
 - d. All of the above

5. Which of the following is NOT an advantage of sodium hypochlorite:
 - a. Bactericidal
 - b. Virucidal
 - c. Affordable
 - d. Stable at room temperature

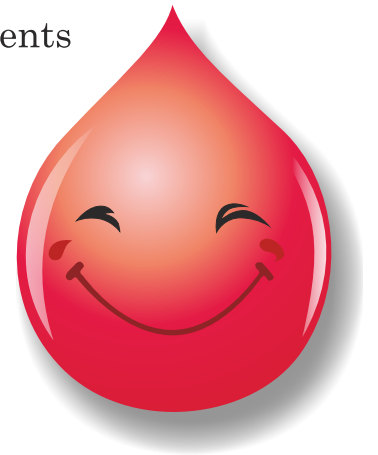
NOTES:

BLOOD COMPONENTS

Learning Objectives

When you have completed this chapter you should be able to:

- Describe constituents of blood and its components
- Carry out component preparation
- Carry out quality control of blood /blood components



5.1 CONSTITUENTS OF BLOOD

Blood is composed of plasma in which the following highly specialized cells are suspended

- Red blood cells (erythrocytes)
- White blood cells (leucocytes)
- Platelets

Plasma contains proteins, chemical substances, coagulation factors and numerous metabolic substances. It is capable of clotting.

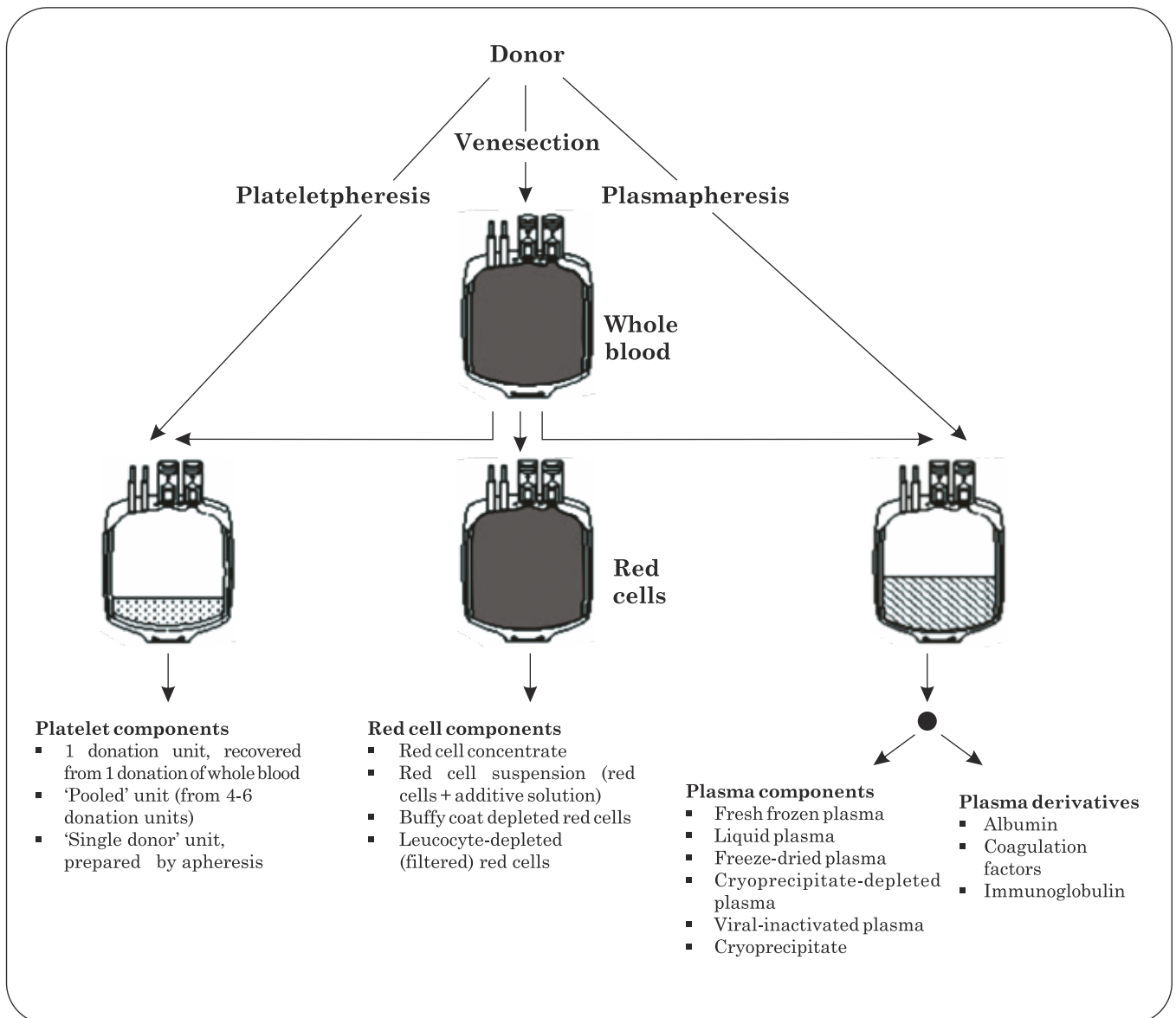


Figure 19: Constituents of Blood

The volume occupied by both cells and plasma in the vascular system is called the total blood volume.

- In an adult, this is approximately 7% of body weight or 70 ml/kg. For example, a 60 kg man would have a blood volume of 70 x 60, which is 4200 ml.
- As children have higher water content, the blood volume is calculated to be 8% of body weight or 80ml/kg.
- It is higher still in the neonate and is calculated to be 85–90 ml/kg

Red blood cells

Red blood cells (erythrocytes) are produced in the bone marrow under the controlling influence of the renal hormone erythropoietin. After entering the bloodstream, they have a life-span of approximately 120 days before being broken down in the reticuloendothelial system. The red cells contain the iron-containing pigment hemoglobin, whose primary function is to store and transport oxygen. Red cells are the most numerous of the cells in the blood and normally occupy about 45% of the total blood volume.

Hemoglobin is usually measured in grams per decilitre (g/dL) or grams per millilitre (g/100 ml) of blood. In adult males, a typical level would be approximately 14 g/dL and in adult females 13 g/dL.

White blood cells

White blood cells (leucocytes) are a family of cells consisting of:

- Granulocytes
- Lymphocytes
- Monocytes

They are produced in the bone marrow and lymphatic tissue. Their principal role in the blood is to identify, destroy and remove any foreign material that has entered the body. These cells are therefore important in fighting infection and in developing resistance to infection in response to natural exposure or immunization. White cells occupy less than 1% of the total blood volume.

Platelets

Platelets are small fragments of cells (megakaryocytes), which are produced in the bone marrow and contain enzymes and other biologically active substances. Their function is to respond to any vascular wall damage by gathering together at the site of injury to form an initial temporary platelet plug and releasing their contents into the blood.

The released contents of platelets are largely responsible for the subsequent coagulation process by activating the blood clotting mechanism that results in the permanent deposition of a fibrin clot at the site of the damage, preventing further bleeding.