HANDBOOK ON

Component Preparation for BCSU

2015

NACO

NBTC
राष्ट्रीय रक्त संचरण परिषद
National Blood Transfusion Council

Ministry of Health and Family Welfare
Government of India

भानु प्रताप शर्मा
सचिव
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सत्यमेव जयते

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Government of India
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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.

18/4/15

(B.P. Sharma)

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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)

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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 indentified 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) 29/8/15

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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.

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Acknowledgement

The Training Module for Blood Bank Medical Officers and Laboratory Technicians has been developed by Blood Transfusion Services Division, NACO and National Blood Transfusion Council, Ministry of Health and Family Welfare under the guidance and active leadership of Shri. Lov Verma, I.A.S, Ex-Secretary (Health) and Shri. B.P. Sharma, I.A.S, Secretary (Health).

The constant encouragement of Shri. N.S.Kang, Additional Secretary NACO and Shri. K.B. Agarwal, IAS, Joint Secretary, NACO have greatly helped in undertaking this important activity.

A special thanks to Dr. Shobini Rajan, Assistant Director General (BTS); Dr Harprit Singh, National Programme Officer (BTS) and Dr. Shanoo Mishra, Programme Officer Quality (BTS), NACO and other team members for their constant effort and hard work in preparing the module.

It is commendable to note that a comprehensive set of document has been reviewed with the coordinated and concerted efforts of various organizations and individuals from the Apex Training Institutes, PGI Chandigarh, KEM Mumbai and CMC Vellore. A detailed list of contributors is included within this document. My heartfelt thanks to all for their expertise and time spared towards technical review.

I extend my sincere thanks to the U.S. Centers for Disease Control and Prevention-Division of Global HIV/AIDS (CDC-DGHA), India and Christian Medical Association of India (CMAI) for providing technical assistance and support for the preparation of this set of modules.

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ACRONYMS

3TC	Lamivudine
ACD	Acid Citrate Dextrose
AIDS	Acquired Immuno Deficiency Syndrome
AMC	Annual Maintenance Contract
Aph	Apheresis
AZT/ZDV	Zidovudine
BTS	Blood Transfusion Services
CD4	Cluster of Differentiation
CDC	Centers For Disease Control and Prevention (United States)
CDP/CPP	Cryo Deficient Plasma/ Cryo Poor Plasma
CMC	Comprehensive Maintenance Contract
CMV	CytomegaloVirus
CMAI	Christian Medical Association of India
CRYO	Cryoprecipitate
CO_2	Carbon-di-oxide
$CuSO_4$	Copper Sulphate
DAT	Direct Anti-globulin Test
D&CA	Drugs and Cosmetics Act, 1940
DGHS	Directorate General of Health Services
DHTR	Delayed Haemolytic Transfusion Reaction
DIC	Disseminated intravascular coagulation
EBV	Epstein Barr Virus
EDTA	Ethylene Diamine Tetra acetic Acid
EIA	Enzyme Immuno Assay
ELISA	Enzyme-Linked Immuno Sorbent Assay
FFP	Fresh Frozen Plasma
FIFO	First In First Out
FNHTR	Febrile Non-haemolytic transfusion reactions
GA	General Anaesthesia
Hb	Hemoglobin
HBIG	Hepatitis Immunoglobulin
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B Virus
Hct	Hematocrit
HCV	Hepatitis C Virus
HCW	Health Care Worker
HDN	Haemolytic Disease of the Newborn
HDV	Hepatitis D Virus
Hg	Mercury

HIV	Human Immunodeficiency Virus
HTC	Hospital Transfusion Committee
HTR	HaemolyticTransfusion Reaction
IAT	Indirect Antiglobulin Test
ICTC	Integrated Counselling and Testing Centre
ICU	Intensive Care Unit
IH	Immunohematology
IV	IntraVenous
LPRBC	Leukocyte Poor Red Blood Cells
MSW	Medical Social Worker
NACO	National AIDS Control Organization
NBTC	National Blood Transfusion Council
NCPE	Non Cardiogenic Pulmonary Edema
NRTI	Nucleoside Reverse Transcriptase Inhibitor
O_2	Oxygen
OT	Operation Theatre
PC	Platelet Concentrate
PEP	Post Exposure Prophylaxis
PRBC	Packed Red Blood cells
PRO	Public Relation Officer
PRP	Platelet Rich Plasma
PTP	Post Transfusion Purpura
QMS	Quality Management System
RBCs	Red Blood Cells
RIA	Radio-Immuno-Assay
RPR	Rapid Plasma Reagin
SAGM	Sodium chloride, Adenine, Glucose Mannitol
SBTC	State Blood Transfusion Council
SOP	Standard Operating Procedure
Sp.gr	Specific Gravity
TA-GVHD	Transfusion Associated Graft Versus Host Disease
TPHA	*treponema pallidum* Haemagglutination Assay
TRALI	Transfusion Related Acute Lung Injury
TTIs	Transfusion Transmitted Infections
VDRL	Venereal Disease Research Laboratory
WB	Whole Blood

LIST OF TABLE

CHAPTER 1

BLOOD COMPONENTS PREPARATION AND THEIR USES

1.1 Introduction:

Effective blood transfusion therapy depends upon the availability of different blood components. The blood components, used separately or in combinations, can meet the transfusion need of most patients and minimize the risks of blood transfusion therapy.

A blood donor donates whole blood, from which different components are prepared. The ability to separate various components from whole blood is desirable for the following reasons:

1. Separation of blood into blood components allows optimal survival of its each constituent. For example, after 24 hours storage of whole blood at 2-6°C, it has few viable platelets and decreased levels of labile coagulation factors, such as factor V and VIII, while after component separation, the platelet concentrate can be stored for 5 days at 22°C in platelet storage satellite bag and levels of Factors V and VIII present in the FFP can be maintained at optimum levels for upto 1 year when stored at- 30°C or below.
2. Component preparation allows transfusion of the only specific blood component that is required by the patient.
3. Transfusion of only the specific constituent of the blood needed avoids the use of unnecessary component, which could be contraindicated in a patient. For example, because of the risk of hypervolemia, an elderly anemic patient in congestive heart failure may not easily tolerate the transfusion of two units of whole blood, while the same patient can be transfused two units of red blood cells easily.
4. By using blood components, several patients can be treated with the blood from one donor, leading to optimal use of every unit of donated blood.
5. Use of blood components, supplements blood supply - adds to blood inventory.

Blood components such as red cells, platelets, and plasma are prepared from whole blood donations. These components have tightly regulated preparation and different storage requirements.
Blood group compatibility between the component and the patient is considered during product selection and issue. Each component carries the same risk of hepatitis, human immunodeficiency virus (HIV) transmission as the original unit of whole blood.
In contrast, plasma derivatives (fractions) such as albumin, immunoglobulins coagulation factor concentrates, etc. are prepared from large pools of donor derived plasma. They plasma derivatives have more flexible storage requirements and are given without regard to ABO compatibility.
Depending on the manufacturing process, derivatives may carry a decreased risk of hepatitis and HIV transmission as compared with components. The blood plasma components given below can be prepared in blood bank by conventional methods (e.g. centrifugation, freezing and thawing) for therapeutic use.

1.2 Blood Components (Cellular and Plasma) and Plasma Derivatives (Fractions):

1.2.1 Cellular components

A. Whole blood derived

- Red cell concentrate/packed red blood cell (PRBC)
- Leucocytes-Reduced packed Red cells/Leuco depleted Packed Red Cells
- Platelet Concentrate/ random donor platelets (RDP)
- Leucocytes-Reduced Platelet Concentrate
- Granulocyte concentrate

B. Apheresis derived

- Erythrocytapheresis
- Platelet Apheresis/ single donor platelets (SDP)
- Granulocytes Apheresis
- Peripheral blood stem cells

1.2.2 Plasma components

- Fresh frozen plasma
- Single donor plasma
- Cryoprecipitate
- Cryo-poor plasma

1.2.3 Plasma derivatives

- Albumin 5% & 20%
- Factor VIII concentrate
- Factor IX concentrate
- Prothrombin complex concentrate
- Intravenous Immunoglobulins
- Fibrinogen
- Other coagulation factors, immunoglobulins and proteins for clinical use

Preparation of blood components is possible due to:

- Different specific gravity of cellular components
- Refrigerated centrifuge
- Multiple Plastic packs system

1.2.4 Specific gravity of Components

Table 1.1: Different specific gravity of cellular components

Component	Specific Gravity
Whole blood	Whole blood 1.053
Red Cells	1.08 – 1.09
Platelet	1.03 – 1. 04
Plasma	1.02 – 1. 03

Due to different specific gravity of cellular components, they can be separated by centrifuging at different centrifugal force (in g) for different time.

1.2.5 Centrifugation for blood component preparation

Refrigerated centrifuge rotor speed and duration of spin are critical in preparing components by centrifugation. The three major variables that affect the recovery of cells from WB by differential centrifugation are rotor size, centrifuge speed, and duration of centrifugation. Each centrifuge should be calibrated for optimum speeds and times of spin for the preparation of each component. Centrifuges used for separation are calibrated to produce highest product yield in the shortest time at the lowest possible spin so as to cause the least trauma to each product and at the same time maintaining optimal temperature for component viability

All current methods for separation and preparation of the three major blood components— RBCs, platelets, and plasma—rely on one or more centrifugation steps. Based on the requirement for

preparation of platelets from WB, two primary methods are available first preparation from buffy coats and second preparation from PRP.

1. PRP METHOD:

Preparation of platelets from PRP begins with a **soft spin** of the WB, followed by separation and **hard spin** of the PRP. In a unit of blood, the centrifuged products settle in layers, starting from bottom: red blood cells, white blood cells, and platelet rich plasma. After separating platelet-rich plasma (PRP) from the red cells, the PRP is centrifuged at a heavy spin for a longer time. This time platelets settle to the bottom of the bag. The plasma is transferred into another satellite bag. Platelet pellet obtained, is held undisturbed for 30 to 60 minutes before being resuspended. Once platelets are resuspended, they are stored at room temperature (between 20^0 to 24^0 C) with continuous agitation during storage as per FDA requirements

2. BUFFY COAT METHOD:

Compared to the PRP preparation method, the first step in buffy coat method is high g-force centrifugation followed by a low g-force step. A buffy coat layer is obtained between the red cells and the plasma which appear after primary centrifugation. The red cell and the plasma are transferred to their respective bags and sealed. The satellite bag containing the buffy coat along with some plasma and red cells is the hanged and allowed to rest. Followed by a soft spin centrifugation, the supernatant platelet is separated and drawn off in a platelet bag and the remaining buffy coat is discarded after separation. The buffy coat method yields more plasma, greater red cell loss, better initial white blood cell (WBC) reduction before filtration, and moderate reduction in viable bacteria in the platelets.

1.2.6 Equipment

1. Refrigerated blood bank centrifuge
2. Blood bank refrigerator
3. Freezer –20º C or below
4. Platelet agitator
5. Weighing scale for balancing centrifuge cups
6. Tube sealer
7. Plasma Defroster or Water Bath
8. Plasma expresser (manual)
9. Component Separator
10. Stripper/ Cutter
11. Aluminum canisters for cryoprecipitate
12. Stripper, cutter

1.2.7 Consumable items

1. Sealing rings, Rubber Bands
2. Plastic boxes for storing fresh frozen plasma in freezer
3. Double, Triple and quadruple blood bags
4. Appropriate sticker type labels and instructions for each component
5. Gloves

1.3 Precautions to be observed in preparing components:

1.3.1 In collection of blood:

- Proper selection of donor
- Clean and aseptic venepuncture site to minimize bacterial contamination

- Clean venepuncture with minimum tissue trauma and free flow of blood
- The flow of blood should be uninterrupted and continuous. If any unit takes more than eight minutes to draw, it is not suitable for preparation of platelet concentrate, fresh frozen plasma or cryoprecipitate.
- A correct amount of blood proportionate to anticoagulant should be collected in primary bag that has satellite bags attached with integral tubing.
- Monitor the collection of blood with Automatic Mixer/Scale which is used for collecting the desired amount of blood and mixing the blood with the anticoagulant.
- If platelets are to be harvested, the blood bag should be kept at room temperature (20-24°C) until platelets are separated. Platelets should be separated **within 6-8 hours** from the time of collection of blood.
- Triple packs system with two attached bags makes it possible to make red cells, platelet concentrate and fresh frozen plasma. While quad packs system with three attached bags are used for preparing red cells, platelet concentrate, cryoprecipitate (factor VIII) and Cryo- poor plasma. Double bags are used for making red cells and plasma only.

1.3.2 In centrifugation

- Opposing cups with blood bag and satellite bags must be equal in weight otherwise excessive eccentric loads placed on rotor of centrifuge cause irregular wear and tear and eventual breakage.
- The bags should be so placed that its broad side faces the outside wall of the cup.
- Rubber discs should be used for balancing.
- Plastic over-wears for bags may be used (optional).
- Correct speed of centrifugation and time must be maintained as they are the most critical factors in component preparation.
- Observe for any abnormal vibration till the required speed is attained, if there is any, stop the centrifuge and check the weight of the opposite cups with bags. Centrifuges used for separation are calibrated to produce highest product yield in the shortest time at the lowest possible spin so as to cause the least trauma to each product and at the same time maintaining optimal temperature for component viability. In a unit of blood, the centrifuged products settle in layers, starting from bottom: red blood cells, white blood cells, and platelet rich plasma. After separating platelet-rich plasma (PRP) from the red cells, the PRP is centrifuged at a heavy spin for a longer time. This time platelets settle to the bottom of the bag. The plasma is transferred into another satellite bag.

1.4 Preparation of blood components

Whole blood contains 450 ± 10% or 350 ± 10% of donor blood plus anticoagulant solution. The name of the anticoagulant is used with the name of the product e.g. CPDA-1 Whole Blood. Whole blood has a hematocrit of 30-40 per cent. Stored blood has no functional platelets and no labile coagulation factors V and VIII.

1.4.1 Preparation of red blood cell concentrates (packed red cells)

Red blood cells (packed red cells) are prepared by removing most of the plasma from a unit of whole blood. Red cells have higher specific gravity than plasma, the red cells settle in the lower portion of bag due to the gravitational settling (sedimentation) or centrifugation. The plasma is transferred into a satellite bag.

Red blood cells preparations are:

a) **Sedimented red cells**: They have a PCV of 60-70 percent, 30 per cent of plasma and all original Leucocytes and platelets.

b) **Centrifuged red cells**: They have a PCV of 70- 80 percent, 10-20 percent of plasma and all original Leucocytes and platelets,

c) **Red cells with additive (Adsol or SAG-M)**: They have PCV of 50-60 percent, minimum plasma and all Leucocytes and platelets.

a) Sedimentation:

The blood after collection is kept upright in refrigerator at 2-6°C, the red cells settle down and the clear supernatant plasma is transferred into a satellite bag.

b) Centrifugation:

1. Collect appropriate volume of donor blood in CPDA double or triple bag.
2. Store at 2-6°C till processed.
3. Place bags in the buckets of refrigerated centrifuge and balance the opposite bags accurately.
4. Centrifuge at heavy spin (5000 x g) for 5 minutes at 2-6°C.
5. Express approximately 3/4 of the plasma into the satellite bag.
6. Double seal the tube between primary and satellite bags with plasma. Separate the satellite bag with plasma and keep at -30°C or below.
7. Keep the red cells at 2-6°C.

c) Red Cells in Additive Solution (Adsol/SAGM):

1. Collect the appropriate volume of donor blood in primary bag of additive system, consisting of a primary bag containing anticoagulant solution CPD or CP2D attached with at least two satellite bags, one of which is empty and another contains 100 ml of additive solution e.g. Adsol or SAGM.
2. Store at 2-6°C till processed.
3. Centrifuge at heavy spin as above.
4. Remove most of the supernatant plasma in the empty satellite bag.
5. Add the additive solution to the red cells.
6. Keep the red cells at 2-6°C and plasma at -30°C or below.

1.4.2 Preparation of Leucocyte-Reduced Blood Components

Leucocytes in blood components can cause:

- Non-hemolytic febrile transfusion reaction (NHFTR)
- Human Leucocyte antigen (HLA) alloimmunizaion
- Transmission of Leucotropic viruses [Cytomegalovirus (CMV), Epstein- Barr virus (EBV) and human T-cell lymphotropic virus type 1 (HTLV-1)]
- Transfusion Associated Graft Versus Host Disease (TAGVHD)
- Transfusion Related Acute Lung Injury (TRALI)
- Transfusion related Immunosuppression

Reducing the Leucocytes content to less than 5×10^8 in one unit of RBCs prevents most of the non-hemolytic febrile transfusion reactions (NHFTR). For other complications such as preventing transmission of CMV or alloimmunization to HLA antigens, Leucocytes content must be reduced to less than 5×10^6 in a unit of RBCs.

Leucocyte reduction has been used with considerable success to prevent FNHTR but at times post storage Leuco-reduction is not much effective as cytokines generated by Leucocytes during storage can cause FNHTR.

Cytokines are generated by Leucocytes, even at 2-6°C but to a much greater extent at 20- 24°C. Levels of interleukin one alpha plus beta (IL- lα and IL-1β), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) rise sharply in units stored with Leucocytes compared with similar units stored after Leuco-reduction (pre storage Leuco-reduction). Cytokine levels rise in direct proportion to the

number of Leucocytes. Hence Leuco-reduction before storage (pre-storage Leucoreduction) in blood bank is much better than the Leuco-reduction after storage (post-storage Leucoreduction) at the bed side of the patient to eliminate FNHTR. Donor's lymphocytes engrafting in the recipient and reacting against host antigens may cause GVHD.

The reaction between Leucocyte antigens and antibodies can result in Leuco-agglutination, aggregates of white cells being trapped in the microcirculation of the lungs, causing pulmonary edema, that is transfusion related acute lung injury (TRALI). In most of the cases of TRALI, Leucocytes antibodies in previously sensitized transfusion recipient react with Leucocytes in transfused blood or plasma, or reverse to it that is, Leucocyte antibodies in blood or plasma react with the recipient's Leucocytes forming Leucocyte aggregates which are trapped in the microcirculation of lungs causing TRALI.

Table 1.2: Approximate Residual Leucocytes in Cellular Blood Components

Cellular Blood Components	WBC Count
Fresh whole blood	10^9
Red blood cell concentrate	10^8 - 10^9
Buffy coat-depleted red cells	10^8
Red cells, Leucocyte-reduced by filtration	$<10^7$
Washed red cell concentrate	10^7
Deglycerolized red cells	10^6- 10^7
Platelet concentrate	$\leq 10^7$

a) Methods of the preparation of Leucocyte-Reduced Red cells

1. Centrifugation and removing of buffy coat
2. Filtration
3. Washing of red cells with saline
4. Freezing and thawing of red cells

a.1 Centrifugation and removal of buffy coat:

In the centrifugation method for Leucocyte reduction, the buffy coat layer between the red cells and the plasma which appear after centrifugation is drawn off along with plasma and some red cells into a satellite bag. A variation of this method is to spin the red cells in an inverted position so that red cells can be transferred into a satellite bag, leaving the buffy coat, some red cells and plasma behind in primary bag.

Method

1. The whole blood unit is centrifuged in an upright position at 5000 x g for 5 minutes at 4°C.
2. The supernatant plasma, buffy coat and some red cells are transferred into a satellite bag.
3. Double seal the tubing between the primary bag and the satellite bag, separate them.
4. Keep the red cells at 4°C.

This is one of the easiest and least expensive methods and it can be done in close system, but it is the least efficient. It reduces the Leucocytes level by 70-80% (less than 1 log) and sacrifices 20% of the red cells. It does not meet minimum standards for WBC reduction. Besides its hematocrit is more than 80% which is difficult to transfuse unless some plasma is returned back or additive solution is added. It is also laborious method. Today this method is being replaced by other more efficient techniques.

a.2 Filtration:

Many types of filters are available today that can produce an acceptable Leucocyte-reduced product depending on the requirement.

Micro aggregate filters are polyester or plastic screen filters with a pore size of 20-40 micron, which trap most of the micro aggregates composed of white cells, platelets, and fibrin threads that form in blood after 5-6 days of refrigerated storage. The effectiveness of Micro aggregate filters of Leucoreduction is increased by cooling the unit at 40C for 3-4 hours after centrifugation and before filtration. Filtrations of this type usually give a 1 -2 log reduction (90-92%) of Leucocytes in the unit ($< 5 \times 10^8$) and recover most of the red cells. Such LR-red cell reduces the incidence of NHFTRs only but does not achieve other goals of Leuco-reduction. Newer Leucocyte-reducing filters (third generation) use selective absorption of Leucocytes or Leucocytes and platelets. They are made of polyester or cellulose acetate and will produce a 2 to 4 log (99-99.9%) reduction of the WBCs ($< 5 \times 10^6$). There is very little loss of red cells and the process is quiet easy. They prevent alloimmunization to HLA antigens, CMV transmission, and NHFTRs.

Leucoreduction can be done at three different points.

1. Pre-storage Leucoreduction
2. After storage Leuco-reduction in blood bank, before issue
3. Bed side filtration

Pre-storage Leucoreduction:

Leucocytes begin to disintegrate quickly when stored at 1-6°C. These white cells fragments may initiate an immune response to HLA antigens and carry viral activity. White cells in stored blood may also produce cytokines which may cause NHFTRs. In order to prevent these effects it may be desirable to remove white cells prior to storage. This can be done by using one of two technologies, the sterile tube connection device or the inline filter.

Using the sterile tube connecting device, a bedside LR-filter can be connected to a unit of blood prior to storage and to another sterile bag. The cells can be filtered from the primary bag through attached filter into the attached bag. This retains the original expiry date of the initial product and all red cells, platelets, and plasma.

The second technology is possible with specially designed blood collection bags system with integral (in line) filters incorporated between primary collection bag and a satellite bag.

Within 8 hours after phlebotomy, the blood is passed through this filter into an attached collection bag. The filtered red cells in the bag are Leucocyte reduced and retain the original expiry date.

Leucocytes removal with this system is 99-99.9 per cent (3 log), with > 90 % red cells remaining.

Table 1.3: Impact of Pre-storage Leucoreduction

Results from Pre-storage Leucoreduction	WBC Count
Cytokines production is reduced or eliminated	Decrease in febrile non-hemolytic transfusion reactions
White cells are removed before fragmentation	• Decrease alloimmunization • Reduce exposure to intracellular viruses (e.g. CMV)
Tumor metastases are reduced (in animal study)	• Prevent immunomodulation • Reduce tumor spread

After storage Leucoreduction:

The blood or red cells are filtered to reduce Leucocytes in the blood bank before issue. In laboratory filtration of blood /red cell can be properly standardized and adapted to QC program. However red cells may have disintegrated Leucocytes and cytokines.

Bedside filtration:

Bedside filtration commonly being practiced has been shown to be quiet effective to prevent NHFTRs but they may also have disintegrated Leucocytes and cytokines which may cause FNHTRs. They are not much effective for prevention of HLA alloimmunization.

a.3 Washing of Red Cells for Leucocyte reduction:

Washing of red cells removes Leucocytes, platelets and plasma; it can be done manually or using machine e.g. Automated Cell washing machine.

Manual Method

1. Collect the blood in a double bag and store at 2-4°C, till it is processed.
2. Centrifuge the bag at 5000 x g (heavy spin) for 5 minutes at 2-6°C.
3. Separate the plasma along with buffy coat into a satellite bag/transfer bag.
4. Double seal the tube between primary and satellite bags.
5. Connect the bag with red cells to I.V. saline bottle with bag to bottle connector under laminar flow. Transfer about 250 ml saline in the red cell bag.
6. Clamp the tube of bottle connector (transfer set) and seal the tube of the bag distal to the spike or needle of the transfer set by di-electric sealer. Remove the needle from the tube of the bag and cover the spike/needle with its plastic cover.
7. Mix well red cells and saline. Centrifuge the bag at heavy spin (5000 x g for 5 minutes).
8. Place the bag on the expresser and remove the saline in waste receptacle.
9. Repeat the washing three times (steps 5 to 8).
10. After the final wash add 60-70 ml saline in the red cells and mix.
11. Seal the tubing close to the bag and separate the connector.
12. Keep washed red cells at 2-6°C.
    - Whole process is done under laminar flow.
    - Shelf-life of the washed red cells is 24 hours.
    - All aseptic precautions should be taken.

Semi-automated Method

Leuco-reduction in blood component by removing the buffy coat from whole blood by manual procedure is laborious. This led to the need to automate the preparation of Leuco-depleted components from whole blood to achieve consistency in the quality and reproducibility. Top and Bottom/ Top and top methods are available to produce Leuco-reduced blood components and to harvest Leuco-reduced platelet concentrate from the buffy coat.

In Buffy coat technique, whole blood is centrifuged at relatively high speed. After hard spin, the buffy coat contains both WBCs and platelets. In fact, the law of physics dictates that not only the platelets at the top of bag will fall to the buffy coat but also that the platelets at the bottom of bags will rise to the buffy coat. So the buffy coat is good source of platelets also. The plasma is transferred into a satellite bag and red cells separate into the bag at bottom having SAGM additive solution. The primary bag, containing suspension of 10-20 ml red cells, buffy coat and some plasma, is centrifuged at soft spin and platelets suspended in plasma are expressed into empty satellite bag carefully and slowly.

Automatic Component Extractor

This is an automatic extractor using two parallel pressure plates, one is stationary and other is pneumatically driven that simultaneously expel plasma in an empty satellite bag and red cells into

another bag containing SAGM, an additive solution. The system is designed to leave a specific volume of buffy coat with plasma in the original collection pack. A light transmitter and two photocells control the flow of red cells and plasma into separate bags by the means of two automated clamping devices.

The other system consists of one 600ml bag made of PVC plasticized with DEHP having 63 ml CPD solution with two outlet at the bottom and one at the top. One of the bottom outlets has connecting tubing with needle for the phlebotomy; while the other is connected to a 400 ml bag made of PVC plasticized with DEHP containing 100 ml SAG-M additive solution. The top outlet is connected via a V-piece tubing to two empty 400 ml bags made of PVC plasticized with TOTM, one for the collection of plasma and the other for the collection of platelets to store for 5 days.

Preparation of LR-red cells (Concentrate Method):

1. 450 ± 10% blood is collected in the primary bag of LR bag system having CPD anticoagulant. The anti-coagulated blood is stored at room temperature (22°C) for about 4 hours before further processing.
2. The whole blood is centrifuged at heavy spin for 8 minutes.
3. The centrifuge unit is put in the automatic component extractor and a button is pressed to activate pressure plate.
4. Open the tube connection between the primary bag and plasma transfer pack to allow flow of plasma to one of the satellite bag. Then open the tube connection between primary bag and the bag with SAG-M to allow flow of blood.
5. When the process is complete both top and bottom tubing automatically clamp and the flow of red cells and plasma stop.
6. The bags containing red cells and plasma are sealed with di-electric sealer and separated.
7. Red cell in additive solution is kept at 2-6°C and plasma at -30°c or below.

Preparation of platelet concentrates from Buffy coat:

1. The primary bag with buffy coat and plasma with satellite bag is left hanging for about 2 hours at room temperature (22°C), and then centrifuged at light spin at 22°C.
2. The supernatant plasma with platelets is slowly transferred into the empty bag using conventional extractor.

Washing RBCs:

Washing RBCs in saline to remove the plasma causes small losses of red cells in the bag transfers, as well as loss of the supporting nutrients, glucose, phosphate, and adenine. Because the cells are used soon after washing, generally within 6 to 24 hours, only the glucose loss is physiologically important. There is still a small amount of glucose inside the washed red cells, but it is metabolized quickly especially if the cells are not promptly refrigerated. In the absence of glucose, metabolism stops and the cells are very susceptible to oxidative stress and erythro-apoptotic changes.

sIrradiation

During storage at 4ºC, red cells lose potassium. This potassium collects in the supernatant fluid in the closed storage bag at a rate of about 1 meq/day until equilibrium is reached between intra and extracellular concentrations, usually at about 60 to 70 meq/L depending on the storage hematocrit. Gamma irradiation in doses of 2500 cGy, given to prevent graft-versus-host disease, damages the red cell membranes and increases this rate of potassium loss to approximately 1.5 meq/day; however the

reinfused red cells have normal in-vivo survival. The current FDA regulation that irradiated RBCs expire 28 days after irradiation limits the potential maximum potassium concentration by limiting the period of potassium loss. Nevertheless, care must be taken in all situations when large volumes of older, high-potassium RBC units are used to prime cardiopulmonary bypass, dialysis, or apheresis circuits and then administered at high flow rates into the central circulation.

1.4.3 Preparation of Platelet Concentrate (PC)

Platelet concentrates can be prepared from:

a) Random donor platelet (prepared from whole blood)

b) Single donor platelet prepared by apheresis using various cell separators.

Random Donor Platelet

It is prepared from whole blood kept at room temperature (20-24°C) and within 6 to 8 hours of collection. As mentioned above for preparation of platelets from WB, two primary methods are available:

1. Preparation from buffy coat
2. Preparation from PRP.

In buffy coat method mentioned above, the first step may be a high g-force centrifugation followed by a low g-force step for the buffy coat platelet preparation method (the opposite of the PRP platelet separation method). The platelet prepared by either of the methods are suspended in 40 mL to 70 mL of plasma and stored at 20-22°C under constant agitation in platelet incubator with agitator till used. The shelf life is 3-5 days depending on the type of plastic bags used.

Table 1.4: Contents of 1 unit of RDP prepared from 450 ml blood

Parameter	Quality requirement
Volume	70-90 ml
Platelet count	> 6-9 x 10^{10}
pH	> 6.0
RBC contamination	Traces to 0.5 ml
WBC contamination	> 5.5 to 10^{6}

Calculation of Platelet Yield in the platelet concentrates

Number of platelets in whole blood = Platelet per mm^3 x 1000 x volume of whole blood (ml)

Number of platelets in P.C. = Platelet per mm^3 x 1000 x volume of P.C. (ml)

Calculation

$$\% \text{ of Platelets yield in RDP} = \frac{\text{Number of platelet in PRP X 100}}{\text{Number of platelet in whole blood}}$$

Precaution and storage:

pH should never fall below 6. A decline in pH causes:

- Change in shape of platelets from disc to sphere.
- Pseudopod formation.
- Release of platelet granules.

The above changes are responsible for low recovery and poor survival of platelets in vivo. Maintenance of pH and function of platelets on storage depends upon permeability of storage bag to oxygen and carbon dioxide. Platelets are stored in bags made of standard polyvinylchloride (PVC) with Di-(2)-ethyl hexyl phthalate (DEHP) plasticizer up to 72 hrs at room temperature (20-24°C). New plastic bags made of polyolefin with no plasticizer, or thin walled PVC with Tri - (2-ethylhexyl) trimellitate (TOTM) plasticizer maintain pH level and platelet function up to about 7 days but it is recommended to use them within 5 days from the date of collection of blood.

Agitation during storage helps the exchange of gases, maintenance of pH, and reduces formation of platelets aggregates. Agitator (flat bed) with 1 to 1.5 inch strokes at 70 cycles per minutes at 20-24°C gives good results.

1.4.4 Preparation of Granulocyte Concentrate

Granulocyte concentrate can be prepared by:

a) Single donor unit

b) Leukapheresis by blood cells separator

As the specific gravity of red cells and granulocytes is very similar, the separation of granulocytes by centrifugation is not satisfactory. Leukapheresis is a better method.

Procedure: (Preparation of Granulocytes from donated whole blood).

1. Collect 450 ml of donor blood in 450 ml of CPDA or Adsol SAGM triple packs system and keep at 20-24°C before separating the buffy coat, but in no case more than 6 hrs after collection of blood.
2. Keep the bags in the buckets of refrigerated centrifuged and balance them accurately. Centrifuge the blood bags at 20-24°C at light spin for appropriate time e.g. 2000x g for 3 minutes.
3. Express the supernatant plasma into first satellite bag. Leave about 20 ml of plasma above the cellular layer (buffy coat) in the primary bag. Double seal the tubing between primary bag and satellite bag with plasma and separate it.
4. Express 20 ml of plasma and the upper 20-25 ml of the cellular layer, rich in white cells, into another satellite pack. Double seal the tube and separate.

The yield of white cells by this method is about 1×10^9, of which about half i.e. $0.5\text{-}0.6 \times 10^9$ are granulocytes per 450 ml unit of blood. It is heavily contaminated with platelets and other leucocytes. Granulocytes can be stored at 20-24°C but they should be used as early as possible as and not later than 24 hours from blood collection.

1.4.5 Preparation of Fresh Frozen Plasma (FFP)

FFP is plasma obtained from a single donor either by normal donation or by plasmapheresis and rapidly frozen within 6-8 hours of being collected. It contains all coagulation factors and great care must be taken during collection of blood, freezing and thawing to preserve their activity.

Collection of blood

1. Blood should be collected by a clean, single venepuncture.
2. Flow of blood should be rapid and constant.
3. Total time taken to collect 450 ml of blood should not be more than 8 minutes.

Procedure:

1. Collect appropriate volume of blood in 350-450 ml CPDA double bags systems or 450 ml SAGM/Adsol triple bags system.

2. Store at 4°C or in air-conditioned room till processed but not for more than 6-8 hours.
3. Place bags in the bucket of the refrigerated centrifuge, balance them accurately and centrifuge at heavy spin (5000 x g for 5 minutes) at 4°C.
4. Express approximately four fifth of the plasma into a satellite bag, if blood is collected in CPDA triple /double bag system. Double seal the tube between primary bag and the satellite bag having plasma with metal clips or di-electrical sealer. Separate the satellite bag having plasma. If blood is collected in SAGM or Adsol triple bag system, express all plasma in satellite bag.
5. Label the plasma bag and is rapidly frozen. This should be done as soon as possible after collection, in any case within 6-8 hours. The complete freezing process should be as short as possible and preferably should not take more than one hour. Rapid freezing can be achieved by blast freezer or by spreading the plasma in a thin layer (bags laid flat and not vertical) in freezer at - 70°C or placing the bags protected by a plastic over wrap at - 70°C in ethanol dry ice bath.
6. It has been shown that the most labile coagulation factors are preserved for one year if FFP is kept at -30° C or below. FFP that is stored at–65°C may be stored for longer than 12months, but such storage requires FDA approval. Plasma should be thawed at 30 - 37°C in a water bath or other approved device. While thawing, the component should be placed in a protective plastic overwrap. FFP once thawed has a shelf life of 24 hours at 1 - 6°C. Thawed plasma held longer than 24 hours must be relabelled as Thawed Plasma, and it can be stored for an additional 4 days at 1- 6°C

The formation of cryoprecipitate (containing factor VIIIC, fibrinogen and fibronectin) should be avoided during thawing as it would reduce the expected clotting properties.

Table 1.5: Contents of 1 unit of FFP prepared from 450 ml of whole blood

Plasma	175 - 230 ml
All coagulation Factors	1 IU/ml of each factor(including factors V &VIII)
Fibrinogen	200 - 400 mg

1.4.6 Preparation of Cryo Precipitate (cryo)

Cryoprecipitate are precipitated proteins of plasma, rich in Factor VIII and fibrinogen, obtained from a single unit of fresh plasma (approximately 200 ml) prepared by rapid freezing within 6 hours of collection. It is rich in factor VIII, von-Willebrand factor, fibrinogen, Factor XIII and fibronectin.

Several factors which improve the yield of factors VIII in cryoprecipitate are:

1. Clean, single venepuncture at the first attempt.
2. Rapid flow of blood, donation of blood (450 ml) obtained in less than 8 minutes should be used.
3. Adequate mixing of blood and anticoagulant.
4. Rapid freezing of plasma as soon as possible after collection, in any case within 6-8 hours after collection as done for preparing FFP.
5. Rapid thaw at 4° C in circulating water bath.
6. Use of siphon technique which prevents thawed plasma remaining in contact with the cryoprecipitate.

Procedure

1. Prepare fresh frozen plasma (FFP), as described under FFP, for processing into cryoprecipitate.
2. Freeze the plasma at -70° C in freezer or in ethanol dry bath.
3. Thaw frozen plasma either at 4°C in a cold room (air thaw) or at 4°C in circulating water bath.
   - If FFP is thawed in a cold room, hang the bag in an inverted position with ports lower most and place the second satellite bag on a lower shelf. Observe the pack frequently to make sure the thawed plasma is flowing in to the satellite bag and not accumulating in the primary bag. When 10-15 ml of plasma remains with cryoprecipitate, seal the tubing and separate the bags.
   - If FFP is thawed in 4°C water bath, centrifuge the bag when the plasma is slushy, at 5000 x g for 5 minutes at 4°C. Then supernatant cryo-poor plasma is siphoned out in the satellite bag, leaving 10-15 ml plasma with cryoprecipitate. Seal the tubing and separate the bags. Label bags.
4. Store the bag with cryoprecipitate at -30°C or lower and bag with Cryo-poor plasma in the second satellite bag is stored at -20°C or below. Storage and shelf life of cryoprecipitate is one year at -30°C or below.

Table 1.6: Contents of cryoprecipitate

Plasma	10- 15 ml
Factor VIII	80 - 100 IU
Fibrinogen	150 - 250 mg
von-Willebrand Factor	40 - 70%
Fibronectin	55 mg
Factor XIII	20 - 30% of the original

Reconstituting cryoprecipitate (Thawing and issue of Cryoprecipitate)

Reconstitute cryoprecipitate before issue by placing in an overwrap in a 37°C water bath until the cryoprecipitate has dissolved. Cryoprecipitate should be resuspended thoroughly by gentle kneading. After thawing pool the cryoprecipitate from all thawed bags into one bag under laminar flow by means of bag to bag connector. Wash the empty bags with 10 ml of normal saline to dissolve residual cryoprecipitate and add to pooled cryoprecipitate. Once thawed, cryoprecipitate should be kept at 2-6°C and administered within 4 hours. It should not be frozen. One bag of cryoprecipitate contains on an average 80-120 units of factor VIII in 15-20 ml of plasma.

1.4.7 Preparation of cryoprecipitate-poor-plasma (CPP)

It is a by-product of cryoprecipitate preparation. It lacks labile clotting factors V and VIII and fibrinogen. It contains adequate levels of the stable clotting factors II, VII, IX and X. It is frozen and stored at -20°C or lower temperature for 5 years. This can be sent for Plasma Fractionation.

1.5. Operation and Maintenance of Refrigerated Centrifuge

1. Switch on power supply and the centrifuge switch. Close lid if cover light lights up. Wait for digital display to stabilize.
2. Set the desired program no according to the requirement of component. e.g., (FFP- No. 1, PRP- No.6 for 450 ml bag and No 8 for 350 ml bag , PRC No.-7 for 450 ml bag and 9 for 350 ml bag, PRC Final No. 4) Let the desired temperature attain.

3. Load the centrifuge cups. Always put two bags in one cup, otherwise there is a chance of the bags rupturing. Use a saline/water bag in place of second blood bag if necessary.
4. Load the centrifuge fully. All cups including empty cups should be in place. Close the outer lid.
5. Start the centrifuge by pressing the start button.
6. Observe for any vibration till the required speed is attained, otherwise stop immediately by pressing the stop button.

Maintenance of centrifuges

1. After work wipe the cups with distilled water. Centrifuge should be dried and cover left open.
2. Periodically (once in a month), the head should be removed and cleaned with soap and water to prevent corrosion.

Precautions

1. Opposing cups must be equal in weight otherwise excessive eccentric loads placed on the rotor cause irregular wear and eventual breakage of the rotor shaft.
2. The bags should be placed so that its broad side faces the outside wall of the centrifuge.
3. Weighing sticks, rubber discs or rubber pieces should be used for balancing.
4. Speed of centrifugation must be checked periodically as it is the most critical parameter in component preparation by differential centrifugation.

CHAPTER 2

PRESERVATION, STORAGE AND TRANSPORTATION OF BLOOD

2.1 Introduction

The primary objective of preservation, storage and transportation of blood and blood components is to preserve the viability and function of each relevant constituent, prevent any physical changes of the blood constituent during storage and minimize bacterial growth.

Various anticoagulant-preservative mixtures have been formulated with the ultimate goal of preventing clotting and to provide proper nutrition for cell metabolism during storage. This ensures that blood and blood components are kept therapeutically viable for a stipulated time.

2.2 Objectives

Aim of this section is to acquaint the medical officers, staff nurses and laboratory technician with the various anticoagulants available for preservation of blood and blood components, their mechanism of action and stipulated shelf life for the constituent. In addition, to emphasize the role of adequate and proper storage and transportation of blood to maintain the therapeutic effectiveness of the blood components till the time it is transfused to the recipient.

2.3 Anticoagulation and Preservation

Various anticoagulant-preservative solutions have been formulated for better red cell preservation, are listed below:

Table 2.1: Common anticoagulant-preservative solution for red cell preservation

WHOLE BLOOD	RED CELLS	RED CELLS - FROZEN STATE
• Acid Citrate Dextrose (ACD) • Citrate phosphate Dextrose solution (CPD) • Citrate phosphate dextrose adenine. (CPDA-1) • Heparin • EDTA (Ethylene diamine tetra acetic acid)	• CPD - SAG (Saline, adenine, glucose) • CPD - SAGM (Saline, adenine, glucose, mannitol) • CPD - ADSOL (Adenine, saline, glucose, mannitol)	• High glycerol solution • Low glycerol solution

2.3.1 Preservation of Whole blood

a) Citrate Based Anticoagulants

The important citrate based anticoagulant - preservative solutions used are ACD, CPD, and CPDA (CPDA-1 and CPDA-2).

a.1) Acid citrate dextrose (ACD):

The most widely used ACD solution has the following formula:

Trisodium citrate	22.0g
Citric acid (monohydrate)	8.0 g
Dextrose (monohydrate)	24.6 g
Distilled water to make	upto 1 Litre

For each 100 ml of blood, 15 ml of the ACD solution (pH 5.0) is sufficient (e.g. for 450 ml, 67.5 ml of ACD solution is required).

ACD - Mechanism of Action

Citrate (Calcium chelator)	• **Prevents coagulation** • **Retards glycolysis**
Citrate (Calcium chelator)	• **Improves red cell viability** • **Provides energy for ATP synthesis** • **Decreases rate of hydrolysis of phosphorus**
Citric Acid	• **Prevents glucose caramalization during autoclaving** • **Provides optimal pH with citrate for red cells** •

ACD Solution

- Preserves ATP level
- Helps maintain red cells shape.
- Prevents haemolysis
- Maintains pH

Shelf life of whole blood / red cells in ACD = 21 days

(> 70% transfused cells viable after 24 hours)

a.2) Citrate phosphate dextrose (CPD) :

CPD Solution

- Decreases acidosis

- Improves ATP synthesis

Shelf-life of whole blood in CPD = 28 days

a.3) Citrate Phosphate Dextrose Adenine (CPDA):

Adenine

- Helps maintain high ATP levels
- Blood collected in CDPA
- safe
- well tolerated
- 2,3 DPG levels can be maintained for 12-14days
- shelf-life = 35 days

Table 2.2: Formula for CPD, CPDA-1 and CPDA-2

Constituents	CPD	CPDA-1	CPDA-2
Tri sodium citrate	26.30g	26.30g	26.30g
Citric acid	3.27g	3.27g	3.27g
Sodium di hydrogen phosphate	2.22g	2.22g	2.22g
Dextrose	25.5g	31.8g	44.0g
Adenine	-	0.275g	0.55g
Water	1 litre	1 litre	1 litre

For both CPD and CPDA solutions, 14 ml (pH 5.6 to 5.8) of the solution is added for each 100 ml of blood.

2.3.2 Preservation of Red cells - Optimal Additive Solution (OAS)

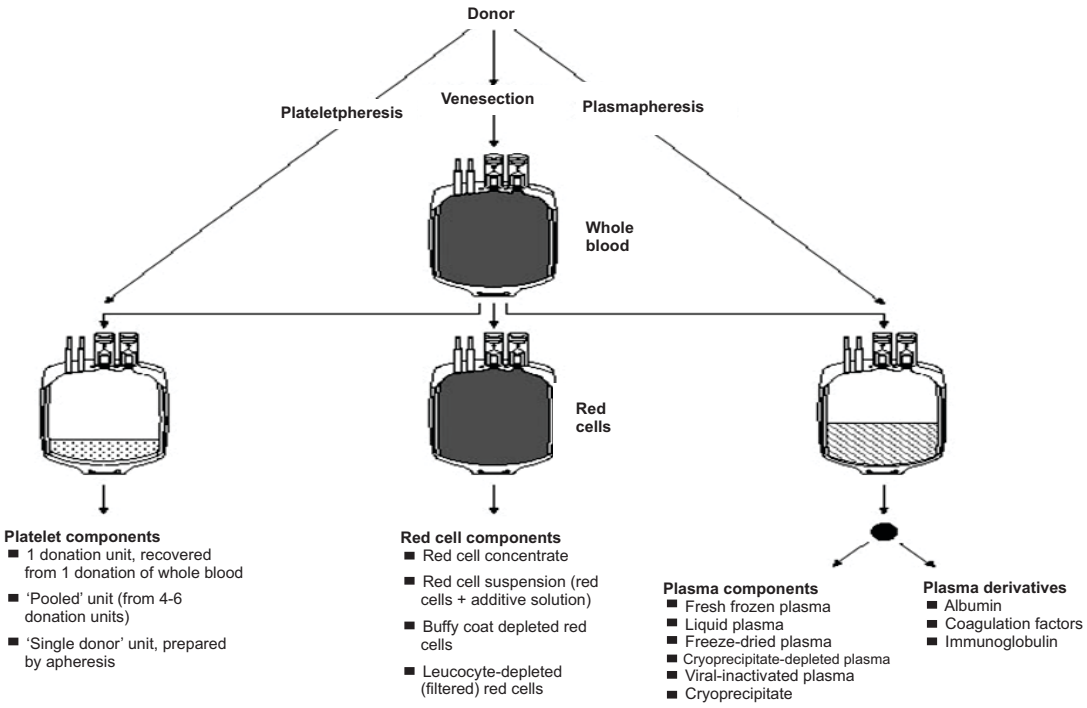
Different types of additive systems are now in use. These solutions contain saline, adenine and glucose and are added to the red cells after separating them from plasma. The advantages of using optimal additive solution (OAS) to red cells are as follows:

1. Volume (ml) = Better storage condition for red cell preparation and lowering of viscosity for ease of transfusion.
2. Increased yield of plasma for plasma fractionation.
3. Removal of unwanted buffy coat
4. Additional 7 day storage time for red cell preparation
5. Avoid unnecessary transfusion of plasma

Blood is collected into a multiple bag system including one plastic bag containing 100 ml of optimal additive solution.

After collection the whole blood is centrifuged and maximum amount of plasma along with the buffy coat is expressed into a transfer bag for further processing. Red cells are now suspended by running down the optimal additive solution (100 ml) to the main bag containing the red cells.

Typical blood container arrangement for use with additive solution



Currently available additive solutions are

1. **CPD-SAG** (Citrate phosphate dextrose - saline, adenine, glucose) - In this besides CPD as the primary anticoagulant, a satellite bag contains the additive solution - 100 ml physiological saline with adenine and glucose. CPD-SAG causes undesirable amount of hemolysis.
2. **CPD-SAGM**- In this, the additive solution also has Mannitol to prevent lysis of red cells. The mean 24 hr. post transfusion survival is better with the additive.
3. **CPD-ADSOL**- This additive solution is similar to CPD-SAGM but has greater quantities of glucose, adenine and Mannitol. It has better red cell preservation and 24 hr post-transfusion survival. The shelf life with this solution can be increased to 42-49days

Table 2.3: Composition of CPD-SAGM and CPD-ADSOL

Composition	CPD-SAGM	CPD-ADSOL
Primary bag	63 ml CPD	63 ml CPD
Additive bag	Constituents	Constituents
Sodium chloride	8.77g	9.0g
Dextrose	8.99g	22.0g
Adenine	0.16g	0.27g
Mannitol	5.25g	7.5g
Distilled Water to make	1 Litre	1 Litre

2.3.3 Preservation of Red Cells – Freezing (for Prolonged Storage)

Red cells can be stored for longer period in frozen state. Frozen red cells improve cell survival and increase shelf life to 5 years or more. It has been seen that freezing and subsequent thawing of red cells produces cellular dehydration and mechanical trauma due to intracellular ice formation which ultimately causes haemolysis.

A cryoprotective agent can prevent this freezing injury. The two agents commonly in clinical use are:

- Glycerol
- Dimethyl sulphoxide (DMSO)

Table 2.4: Advantages and Disadvantages of Freezing of red cells

S.No	Advantages	Disadvantages
1)	Long term storage of rare blood groups	High cost
2)	Transfusion requirement for patients with antibodies against high frequency antigens	Short post-thawing shelf-life (24 hrs)
3)	Storage of autologous donations in patients with rare blood groups.	Increased red cell loss in processing
4)	Fewer febrile and allergic transfusion reaction	Increased processing time in emergency
5)	Lower incidence of alloimmunization to HLA in transplant recipients	
6)	Ability to store rejuvenated outdated red cells	
7)	High level of 2,3- DPG in transfused blood.	
8)	Restoring blood stock and inventory.	

For freezing, red cells are used within 6 days of collection, however if a rejuvenating solution is used, the cells can be frozen even 2-3 days after expiry.

Two commonly used methods for freezing by glycerol employ:

1. Low glycerol solution (20%)
2. High glycerol solution (40%)

The rate of freezing and storage temperature determine the optimal glycerol concentration.

2.4 Storage

2.4.1 Effect of mixing red cell during storage

Red cells stored in SAGM or ADSOL show less spontaneous lysis and show fewer micro vesicles if the suspension is mixed, probably because of the dissipation of acid metabolites on mixing, which collect in the bottom layer of stored red cells.

2.4.2 Physical and Biochemical Effects of Storage

The conditions of storage will invariably produce changes in the physical and chemical properties of blood and blood components and in turn effect red cell recovery. The medical officers must be aware of these in order to consider the clinical efficacy of transfusion.

Abnormalities resulting from storage of blood are collectively known as storage lesions. These can be discussed as:

2.4.3 Storage lesions

a) Effect of collection on red cell recovery

b) Effect on red cell function and survival

c) Effect of anticoagulant used

d) Effect on pH

e) Effect of temperature

f) Effect on electrolytes & coagulation factors

g) Effect on cellular elements

a) Effect of collection on red cell recovery

The cells drawn at the start of donor bleeding are subjected to an acidic and hypotonic anticoagulant solution which results in irreversible damage to some of the cells. These cells, in contrast to those drawn towards the end of phlebotomy, deteriorate more rapidly on storage.

b) Effect on red cell function and survival

- Storage effect on red cell metabolism: The red cell is dependent on anaerobic glycolytic pathway for the formation of ATP, which plays a central role in determining its viability and maintaining its shape. During preservation the metabolic cycle must continue in vitro for red cells to remain viable with adequate post transfusion survival and function.
- Effect on O_2 release and 2, 3 Diphosphoglycerate (DPG): 2,3-DPG is known to profoundly lower the affinity of Hb for O_2 at concentration found in red cells. Depletion of 2,3-DPG in stored blood temporarily adversely affects oxygen release by Hb.
- Effect on survival: In almost all cases, cells that survive 24 hrs will remain viable and circulate for the remainder of their expected life span.

Maximum allowable storage time, referred to as shelf life is defined by requirement of 70% recovery at 24 hrs i.e. at least 70% of the transfused red cells remain in the recipient's circulation 24 hrs after transfusion.

c) Effect of anticoagulant-preservative

One of the most important factors influencing red cell recovery after blood storage is the anticoagulant solution used.

Table 2.5: Anticoagulant – Preservative Storage & Viability

Anticoagulant – preservative	Storage/ Viability
Trisodium citrate	Rapid deterioration, only 50% cells viable after 1 week.
Heparin	Rapid deterioration. Added disadvantage of being progressively neutralized by plasma. Therefore, most unsuitable for storage.
ACD-A	Storage/viability for 21 days, 24 hrs survival 77%, DPG level better maintained maximum for 1 week
CPD	Storage/viability for 28 days. 24 hrs survival 80%, DPG level better maintained for 10-14 days because of the favourable effect of higher pH.
CPDA-1/2	Storage/viability 35 days - improved storage due to adenine which maintains high ATP level in the RBC.

d) Effect on pH

There is a gradual fall in pH during storage due to accumulation of lactic acid.

e) Effect of temperature

Optimum storage temperature for whole blood and red cells is between 2^0-6^0C with occasional elevation to 10^0C (e.g. during transportation) being acceptable.

Delaying refrigeration increases the loss of 2,3-DPG over this period. Platelets and granulocyte retain better function when stored at room temperature. Labile coagulation factors in plasma are best maintained at temperature of -20^0C or lower.

Refrigeration and freezing additionally minimize proliferation of bacteria that might have entered the unit during venepuncture.

Table 2.6: Biochemical changes of stored CPD and CPDA - 1 blood

Biochemical changes	CPD				CPDA-1	
	Days of storage				Days of storage	
	0	7	14	21	0	35
% of viable cells (24 hrs post transfusion)	100	98	85	80	100	79
Plasma pH (measured at 37^{0}C)	7.20	7.00	6.89	6.84	7.6	6.98
ATP (% initial value)	100	96	83	86	100.0	56.0
2,3 - DPG (%initial value)	100	99	80	44	100.0	<10.0
Plasma Na meq/l	168	166	163	156	169.0	155.0
Plasma K meq/l	4.2	11.9	17.2	21.0	4.2	27.3

f) Effect on electrolytes & coagulation factors

- Electrolytes: The only important electrolyte change in stored blood is that of K. During blood storage there is a slow but constant leakage of K+ from cells into the surrounding plasma. In severe kidney disease even small amount of K+ fluctuations can be dangerous and relatively fresh or washed red cells are indicated. Due to a higher K+ content of stored blood, blood < 5 days old is recommended for neonatal exchange and top-up transfusion.
- Coagulation factors: Labile coagulation factors, Factors V and VIII lose their activity by 50% within 48-72 hrs of storage.

g) Effect on cellular elements

White cells lose their phagocytic and bactericidal property within 4-6 hrs of collection and become non-functional after 24 hrs of storage. It is important to remember that they do not lose their antigenic property and are capable of sensitizing the recipient to produce non-haemolytic febrile transfusion reactions.

Few lymphocytes may remain viable even after 3 weeks of storage.

Platelets lose their haemostatic function within 48 hrs in whole blood stored at 4°C.

2.4.4 Rejuvenation of Stored Red Cells

Storage of RBCs causes reduction in the intracellular levels of 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP). Stored red cells even at the end of their allowable shelf-life can be rejuvenated with solutions containing pyruvate, inosine, phosphate and adenine. Incubation with the rejuvenating solution at 37°C for 3-4 hours greatly improves viability and restores 2,3 DPG levels.

Method:

1) Add 50 ml of rejuvenating solution to the red cell concentrate.
2) Incubate at 37°C for 1 hr prior to glycerolization and freezing.

Before transfusion, rejuvenated RBCs are to be washed to remove the rejuvenation solution. The rejuvenated RBCs can also be frozen for long term storage. At the time of transfusion, deglycerolization is performed, which accomplishes the removal of the rejuvenation solution and the glycerol.

RBCs preserved in CPD or CPDA-1 can be rejuvenated up to 3 days after the expiration date of the RBCs as long as the storage conditions are met. RBCs preserved in Adsol maybe rejuvenated until 42 days, but not after the expiration date. Rejuvenated CPD or CPDA-1 RBCs can be maintained in frozen storage for10 years; and Adsol preserved RBCs can be stored frozen for only 3 years.

2.5 Storage of Blood and Blood Components

After the processing of blood into blood components safe and adequate storage and transportation are of immense value to preserve the viability and therapeutic efficacy of the components during their shelf-life or till the time it is transfused to the recipient.

Safe storage and adequate transportation of blood and blood component are vital for two main reasons.

- To maintain therapeutic efficacy and life span of the constituent
- To prevent bacterial over growth.

Table 2.7: Comparative chart of storage and expiry of blood components

Component	Storage	Temperature	Expiry
Whole Blood Red Blood Cells	Blood Bank Refrigerator	2-6°C ± 1°C	35 days for CPDA Bags 42 days for CPD SAGM
Fresh Frozen Plasma Cryoprecipitate	Freezer	-30°C or below	12 months
Platelet concentrate	Platelet agitator	22 ± 2°C	5 days
Cryo poor plasma	Freezer	-30°C or below	5 year ; usually sent for plasma fractionation

2.5.1 Whole blood

Whole blood and red cell concentrate must always be stored between 2^0C and 6^0C. A fall in temperature less than 2^0C can cause freezing injury to the red cells leading to hemolysis. If hemolysed blood is transfused to a patient, it can lead to fatal consequences.

Temperature more than 6^0C can lead to overgrowth of bacteria, which may have entered the blood unit during collections or component preparation.

As red cells consume glucose for their continued metabolism, storing a blood unit at 2- 6^{0}C will also decrease the rate of glycolysis. The shelf life of whole blood and red cell concentrate in commonly used anticoagulant, CPDA-1 is 35 days.

2.5.2 Plasma

a) Fresh frozen plasma (FFP)

FFP is usually transfused to restore or to maintain the clotting mechanism or as a volume replacement when crystalloids and colloids are not used. Fresh frozen plasma is prepared by removing plasma from a unit of blood within 6 hours of collection and snaps freezing it to - 30^{0}C.

The speed of freezing is very important for maintaining the coagulation factors. FFP must always be stored at - 20^{0}C or lower, otherwise the amount of clotting factors such as F VIII and F V will be greatly reduced.

FFP should preferably be stored in cardboard boxes (containing full information about the ABO blood group, donation unit number and screening test results,) to prevent fixing up with the adjacent bags while freezing, as it may lead to breakage of the bag. Packing should be in manner to leave tubing indent on frozen plasma.

Bag containing FFP meant for preparation of cryoprecipitate should be placed in between aluminium metal plates, to achieve a uniform thickness of plasma, which helps in rapid core snap freezing essential to maintain high yield of FVIII in the cryoprecipitate.

2.5.3 Platelets

Platelets are usually stored as concentrates. They are harvested from a single unit of blood either from PRP (platelet rich plasma) or from buffy coat and resuspended in 50 ml of autologous plasma.

Platelets should be stored at 22^{0}C in plasma under conditions in which the pH is maintained at values above 6.8. Shelf life of platelet concentrate is no more than 5 days in platelet storage bag. The functional integrity of platelets depends on many factors.

Storage temperature: Ideal temperature for platelet storage is 20 to 24^{0}C. Temperatures below 200C during platelet preparation and storage cause a striking increase in the number of large aggregates in the platelet-rich plasma.

pH: A fall of pH of platelet concentrate due to lactate production from platelet glycolysis leads to loss of viability. The rate of fall of pH is affected by:

a. Number of platelets

b. Volume of plasma in which stored

c. Availability of O_2

Plastics: Type of plastics used for storage of platelets is important. Availability of plastics with an increased permeability to O_2 has made it possible to lengthen the period to which the platelets may successfully be stored. New second generation plastics such as polyolefin allow satisfactory storage upto 5 days. Other plastic bags in use are Teruflexa (Terumo) made of polyvinyl chloride (PVC) with reduced thickness and larger surface area, F-720 (Biotrans) made of PVC with a phthalate ester analogue as plasticizer, PL 2209 (Fenwel) made of PVC plasticized with BTHC. Platelet storage bag are marked with platelet or '5' mark.

Agitation: Platelet concentrates must be gently and constantly agitated either on a flatbed agitator for better viability. Without agitation, there is a rapid fall in pH, due to collection of lactic acid metabolites.

Contamination with leucocytes: leucocyte contamination is associated with:

1. Fall in pH.
2. Loss of glycoprotein-lb (GP-Ib) from platelet surface leading to loss of platelet responsiveness to ristocetin and thrombin.

Storage of platelets in frozen state

Best results are found using 5% DMSO (dimethyl sulfoxide) as the cryo-preservative agent. They can be stored satisfactorily in liquid nitrogen (-196^{0}C) or in -80^{0}C deep freeze.

2.5.4 Granulocytes

Granulocytes are stored generally in the same medium in which they are collected i.e. citrated donor plasma containing hydroxyl ethyl starch. Leucocytes tolerate storage poorly and lose their highly integrative function i.e. chemotaxis, within 24 hrs.

Temperature: Storage is better at 20 to 24^{0}C rather than 4^{0}C.

Granulocyte concentrate should be transfused as soon as possible and should not be stored for more than 1 day.

2.6 Quality Control of Blood Storage

All efforts must be made to store and transport blood, blood components and plasma in as safe a way as possible. Important elements of maintenance of temperature are:

1. Equipment to store and transport
2. Organization of staff responsible for maintenance of safe temperature controlled storage.

In some of the hospitals, operation theatre maintains a separate storage space for blood and blood components. The staffs of blood transfusion centre should maintain the storage conditions in distant storage places also.

2.6.1 Temperature monitoring

The blood storage equipment must have uniform temperature distribution, an alarm system to detect gross irregularities in temperature and a temperature chart recorder.

In all blood storage equipment, regular monitoring of temperature using good thermometers is essential. A daily temperature recording chart may be affixed on the front door of the refrigerator and an 8 - hourly temperature recording should be done by responsible laboratory staff member

8-hourly Temperature Record				
Date	**Time**	**Shelf**	**Temperature**	**Signature**

Maximum and minimum thermometers can also be used to detect how low or high the temperature had been. The temperature should be recorded in different shelves on different occasions to check for uniformity of temperature.

The out-dated blood bags may be sent for bacterial culture, which can also act as a quality control for sterility of blood collection and storage condition.

2.6.2 Transportation of Blood and Blood Components

Blood units or blood components may need to be transported either within the hospital complex or from one centre to the other. On receiving the blood units, these should be grossly checked for any sign of deterioration or haemolysis either due to wide temperature variation or due to bacterial infection.

Look for any

1. leakage or breakage
2. any change in colour of plasma
3. change in colour of red cells
4. any clots or abnormal mass
5. any foul smell
6. any change in interface between cell and plasma as a fuzzy interface (suggests haemolysis)

a) Transportation of frozen blood components

As FVIII and FV are heat labile coagulation factors, fresh frozen plasma meant to supplement FVIII and FV levels in a patient with bleeding manifestation has to be stored and transported at 30^0C or lower.

The temperature of plasma freezer must be checked twice a day and must be recorded. If temperature shows a wide fluctuation above - 20^0C, the storage freezer must be checked urgently. Fresh frozen plasma once thawed must be transfused within 4hours. Thawed plasma should not be refrozen. It should be discarded if not used.

Fresh frozen plasma or frozen plasma should be transported in light temperature proof containers with plenty of ice packs and should be placed in cardboard boxes to prevent the adjacent bags from getting frozen on the surface of the other bag.

b) Transportation of platelet concentrate

All efforts must be made to ensure that platelets (and granulocyte) concentrates are maintained at room temperature i.e. between 20^0-24^0C during transportation. A well-insulated container without added ice is often sufficient. If the ambient temperature is high and the distance is great, transportation should be done with special coolant pouches that will maintain a temperature around 20^0-24^0C. Agitation is not required.

2.6.3 Blood returned to blood bank

If a unit is being returned to the blood bank, note the time of arrival, look for any visible signs of haemolysis/contamination, leakage and check the temperature by hand. If the unit has any sign of opening or of hemolysis and had been out of refrigerator for more than two hours, do not use the blood unit for therapeutic purposes.

2.7 Thawing of plasma

Once a request for fresh frozen plasma is received in the blood bank, the frozen plasma should be thawed as follows:

1. Place the plasma unit wrapped in a plastic overwrap in a temperature controlled water bath strictly maintained at 37^{0}C. The ports of the bag should always be above the water level in the water bath. The water in the water bath must be clean and frequently changed to prevent bacterial or fungal growth.
2. If there is delay in issue of thawed plasma, the bag may be kept for few hours at 2-8^{0}C till transfused.

CHAPTER 3

COMPONENT SEPARATION BY APHERESIS

3.1 Introduction

APHERESIS, PHERESIS or hemapheresis, is used to refer to automated blood component collection process. It is derived from the Greek word “aphairos” which means "to take from." During the apheresis procedure the whole blood is separated into components and the desired component is removed/modified, whereas the remaining components are returned to the donor or patient. The techniques used could be based either on centrifugation or membrane-based filtration methods. In the United States, centrifugal technology has been developed for apheresis applications. Whereas, in the other parts of the world, primarily Europe and Japan, have seen the additional development of membrane filtration technology for some donor procedures.

The status of donor apheresis at present parallels the evolution in technology. Development of an automated, online, centrifugal technologies relinked with the development of the first devices to allow donor apheresis in large-scale applications. Early device developments allowed the donation of platelets, plasma, and granulocytes. As the technology continued to develop, equipment, disposables, and software programs became increasingly sophisticated for the separation of components by apheresis is now possible. This chapter discusses the special consideration given to the regulatory aspects involved with donors, products, testing, and other requirements specifically related to apheresis.

Any one of the components of blood can be removed and the procedures are specified for the component selected. The process of removing the plasma from red cells is termed plasmapheresis. Similarly terms are given to the removal of other components, including platelets (plateletpheresis), red cells (erythrocytapheresis) or leukocytes (leukapheresis). Apheresis can be non-therapeutic or therapeutic. It can be done manually or using automated equipment. Automated cell-separator machines are used for both component preparation and therapeutic applications. In manual apheresis whole blood is collected in multiple bags system and centrifuged off-line. It involves great care that the bags are labelled correctly and its contents are returned to the donor. With the current available automatic technology now the manual process is seldom used.

3.2 COMPONENT COLLECTION

Collection of components by apheresis follows many of the same rules and guidelines that apply to whole blood donation. For instance, like whole blood donors, donors who are to undergo a procedure involving apheresis must be given information so that their consent to donate is informed. Although apheresis collection and preparation processes differ from those used for whole-blood-derived components, the storage conditions, transportation requirements, and some quality, control steps are the same for both. Yet another similarity is that the facility must maintain written procedures and protocols for all types of collections used and must keep records for each procedure as required by regulatory authority. The circumstances unique to apheresis collection are addressed in the sections that follow.

The advantages of single-donor components are:

1. Reduced multiple donors exposure
   a. Reduced risk of alloimmunisation
   b. Reduced incidence of transfusion-transmitted diseases
2. Full and effective transfusion dose
3. Higher quality product

4. Purer product leukocytes-reduced products
5. Ability to match donor to patient Platelet matched or HLA matched
6. Fewer donor reaction due to - Crystalloids and anticoagulants
7. Return of fluid - Longer period in apheresis help refilling of intravascular compartment, with fluid from interstitial spaces

Smaller needle technology-Venous access injuries are less

General Requirements for Apheresis

1. A qualified, licensed physician is responsible for all aspects of the apheresis program.
2. Equipment should be good, reliable and in proper working condition.
3. Well-trained and motivated staff is essential to an effective apheresis program.
4. Operator (nursing or technical personnel) of apheresis machine must know all aspects of its operation and trouble shootings.
5. An apheresis operator must be friendly and must be able to relieve the anxiety of the Donor/patient.
6. There must be manual readily available to nursing and technical personnel, giving detail description of each type of procedure, and trouble shootings specific for the machine.
7. Records for laboratory findings and data for each apheresis procedure should be kept.

General Criteria for Selecting Apheresis-donor

1. Donor undergoing an occasional apheresis procedure (performed not frequently than once every 4 week) must meet the same criteria as a whole blood donation.
2. Donor should be preferably repeat donor - might have given blood 1-2 times earlier.
3. Written consent of the donor is taken after explaining the procedure in detail, time taken, and about possible hazards and benefits.
4. Venous access is an important consideration in apheresis-donor and veins should be examined at the time of the selection of a donor as:
   I). long needle-in and needle-out times
   ii). prolonged flow rate
   iii). frequent need for two venepunctures with continuous-flow equipment
5. Donor should be screened prior to apheresis for markers of infectious diseases transmitted by the transfusion of blood and its components in the same manner as for the whole blood.
6. Tests for hemoglobin, ABO group, Rh type, and screening for unexpected antibody are done.
7. More stringent regulations govern the donor who participate in serial apheresis program (procedure performed more frequently than every 4 weeks).
   I). Interval between two procedures should be at least 48 hours and the loss of red cells should not exceed 25 ml per week,
   ii). If donor's red cells could not be reinfused during a procedure, or if the participant donates a unit of whole blood, 12 weeks should elapse before subsequent apheresis procedure,
   iii). Careful monitoring of weight, blood cells count, serum protein levels and quantitation of immunoglobulins is required.

Age should be between 18-50 years.

Weight be 60 Kg or more.

Hemoglobin - 12.5 g/dl or more

PLATELETPHERESIS

3.2.1 Platelets

Apheresis platelet collections are used to obtain platelets from volunteer donors, from patients' family members, or from donors with HLA or platelet antigen compatible phenotypes. By design, apheresis procedures are intended to collect large numbers of platelets from an individual, thereby providing a more consistent product with fewer donor exposures for the patient. Standards requires that an apheresis platelet component contain at least 3×10^{11} platelets in 75% of sampled units. With newer technology and more efficient processes, higher yields of platelets may be obtained from one donor, and the original apheresis unit may be split into multiple units, each of which must meet minimum standards. Some instruments are programmed to calculate the yield from the donor's haematocrit, platelet count, height, and weight.

For alloimmunized patients, refractory to random allogeneic platelets, platelets derived from an apheresis donor selected on the basis of a compatible platelet cross match or matched for HLA antigens may be the only way to achieve a satisfactory post transfusion platelet increment.

In a plateletpheresis procedure, a portion of the donor's platelet and some plasma is removed with the return of the donor's RBCs, WBCs, and remaining plasma. A routine plateletpheresis procedure usually takes 1 to 1.5 hours. The product is prepared in closed system and can be stored for 5 days. Platelets can be prepared without or with extra plasma in a separate bag which can be transfused or stored as fresh-frozen plasma (FFP). If extra plasma is collected during plateletpheresis, all precautions are taken as in the plasmapheresis. Routinely, the number of platelets in an apheresis product is equivalent to 6 to 10 random platelet concentrates.

Specific Criteria for the selection of donor for plateletpheresis:

1. Donors who have taken antiplatelet medications that irreversibly inhibit platelet function are deferred for specific intervals before donation (aspirin/aspirin-containing medications, 48 hours; piroxicam, 48 hours; clopidogrel, 14 days; /ticlopidine, 14 days) because apheresis platelets are often the sole source of platelets given to a patient.
2. The interval between procedures should be at least 48 hours. A donor shall not undergo the procedure more than 2 times in a week or 24 times in a year.
3. A platelet count is not required prior the first procedure or if the interval between plateletpheresis procedures is at least 4 weeks.
4. If plateletpheresis is performed more frequently than every 4 weeks, a platelet count should be done and must be more than 150,000/µl prior to performing subsequent plateletpheresis. and value to be entered in apheresis equipment to calculate processing volumes as per final product yield.
5. Platelet may be collected from donors who do not meet the requirement if the component is of particular value to the patient - HLA matched donors.
6. If extra plasma is collected and if the procedure is performed more than once every 4week, the procedure should not be done if the total serum protein is less than 6.0 g/dl or if there has been an unexplained weight loss.

a) Laboratory Testing

Tests for ABO group, for Rh type, for unexpected alloantibodies, and for markers for transfusion-transmitted diseases must be performed by the collecting facility in the same manner as for other blood components. Each unit must be tested unless the donor is undergoing repeated procedures to support a specific patient, in which case testing for infectious disease markers needs to be repeated only at 30-day intervals.

If red cells are visible in a product, the haematocrit should be determined. If the component contains more than 2 ml of red cells, the red cells must be ABO compatible with recipient plasma and be cross matched. In such cases, a sample of donor blood for compatibility testing is attached to the container. In some in-stances it may be desirable for the donor plasma to be ABO compatible with the recipient's red cells (e.g., if the recipient is a child or an ABO-mismatched allogeneic progenitor cell transplant recipient). In the United States, to be considered leukocyte reduced, apheresis platelets must contain fewer than 5 x 10^6 leukocytes per unit, and platelets must meet the specifications of the apheresis device manufacturer.' In Europe, the guideline for leukocyte reduced components is less than 1 x 106white cells (WBCs) per unit.

Record-Keeping complete records must be kept for each procedure. All adverse reactions occurring during collection procedures (or transfusion) should be documented along with results of thorough investigations. Records of all laboratory findings and collection data must be periodically reviewed by a knowledgeable physician and must be found to be within acceptable limits. FDA guidelines require a periodic review of donor records to monitor platelet counts. Facilities must have policies and procedures in place to ensure that donor red cell loss during each procedure does not exceed acceptable limits.

3.2.2 Plasma

Apheresis devices can be used to collect plasma as transfusable FFP or as Source Plasma for subsequent manufacturing. The FDA has provided guidance with regard to the volume of plasma that is allowed to be collected using automated devices. The distinction is made between infrequent plasmapheresis, in which the donor undergoes plasmapheresis no more frequently than once every 4 weeks, and serial plasmapheresis (or Source Plasma collection, the process to collect plasma for fractionation into plasma components), in which the donation is more frequent than once every 4 weeks. For donors in infrequent plasmapheresis programs, donor selection and monitoring requirements are the same as those for whole blood donation, and plasma obtained by these processes is intended for direct transfusion.

For serial plasma collection (Source Plasma collection), using either automated instruments or manual techniques, the following principles apply:

Donors must give consent for the procedure, and they must be observed closely during the procedure. Emergency medical care must always be available.

Red cell losses related to the procedure, including samples collected for testing, must not exceed 25 mL/week so that no more than 200 mL of red cells are removed in 12 weeks. If the donor's red cells cannot be returned during an apheresis procedure, haemapheresis or whole blood donation should be deferred for 12 weeks.

At least 48 hours should elapse between successive procedures. Donors should not undergo more than two procedures within a 7-day period. However, exceptions are permissible when plasma is expected to have special therapeutic value for a specific recipient.

At the time of initial plasmapheresis and at 4-month intervals for donors undergoing serial (large-volume) Plasmapheresis (donors undergoing plasmapheresis more often than once every 4 weeks), serum or plasma must be tested for total protein and for serum protein electrophresis or for quantitative immunoglobulins. Results must be within normal limits. This requirement applies to donors undergoing, large-volume plasma collections, where the total annual volume of plasma collected exceeds 12 L (14.4 L for donors weighing more than 80kg or if the donor is a frequent (more often than once every 4 weeks) plasma donor.

Qualified licensed physician, knowledge blood donation. Donors giving a single unit of able about all aspects of haemapheresis, must be responsible for the program.

3.2.3 Granulocytes

The use of granulocyte transfusions has been controversial for a number of years. Analysis of randomized controlled trials of granulocyte transfusions in adults has indicated that an acceptable minimum dose (>1 x 10^{10} granulocytes/day) and cross match compatibility (no recipient antibodies to granulocyte antigens) have a major bearing on effectiveness. Recently, there has been renewed interest in granulocyte transfusion therapy because much greater cell doses may be obtained from donors who have received recombinant colony stimulating factors.

a) Agents Administered to Increase Yields

A daily dose of at least 1 x 10^{10} granulocytes is necessary to achieve a therapeutic effect in adult patients. For infants and children, a dose of 10 to 15 ml/kg may provide adequate granulocytes per dose. To collect this number of cells in a unit of granulocytes, one must administer drugs or sedimenting materials to the donor. The donor's consent must include specific permission for any of these drugs or sedimenting agents to be used.

a.1) Hydroxyethyl Starch: A common sedimenting agent, hydroxyethyl starch (HES), causes red cells to aggregate, thereby sedimenting them more completely. Sedimenting agents enhance granulocyte harvest by causing increased sedimentation of the red cells, thereby enhancing the interface in the collection device and resulting in minimal red cell content in the final product. Because HES can be detected in donors as long as a year after infusion, facilities should have a process to control the maximum cumulative dose of any sedimenting agent administered to the donor in a given interval during granulocyte collection HES is a colloid, which acts as a volume expander. Donors who receive HES may experience headaches or peripheral oedema because of expanded circulatory volume.

a.2) Corticosteroids: Corticosteroids can double the number of circulating granulocytes by mobilizing granulocytes from the marginal pool. The common protocol is to use 60 mg of oral prednisone as a single or divided dose before donation in order to collect significant numbers of granulocytes with minimal systemic steroid activity. Another protocol uses 8 mg of oral dexamethasone. Donors should be questioned about relevant medical history (hypertension, diabetes, cataracts, or peptic ulcer can be relative to absolute contraindications) before they use systemic corticosteroids.

a.3) Growth Factors: Recombinant hematopoietic growth factors, specifically granulocyte colony-stimulating factor (G-CSF), can effectively increase granulocyte yields. Hematopoietic growth factors given alone can result in collection of up to 4 to 8 x 10^{10} granulocytes per apheresis procedure. Typical doses of G-CSF are 5 to 10 µg/kg given 8 to 12 hours before collection. Preliminary evidence suggests that in-vivo recovery and survival of these granulocytes are excellent and that growth factors are well tolerated by donors.

b) Laboratory Testing

Testing for ABO and Rh, for red cell antibodies, and for infectious disease markers is required on a sample drawn at the time of phlebotomy. Red cell content in granulocyte products is inevitable; the red cells should be ABO compatible with the recipient's plasma. If >2 mL of red cells are present, the component should be cross matched. Both Rh compatibility and HLA compatibility are recommended.

c) Storage and Infusion

Granulocyte function deteriorates rapidly during storage, and concentrates should be transfused as soon as possible after preparation or stored maximum for 24 hours at 20°C - 24°C without agitation. Irradiation is required for Immuno deficient recipients and is indicated for nearly all recipients because their primary diseases are likely to involve deficiencies in their immune systems. Use of a Micro aggregate or leukocyte reduction filter is contraindicated because it will remove the collected granulocytes.

3.3 KEY POINTS

1. Apheresis components must meet many of the same basic regulatory requirements (eg. donor consent, storage conditions, transportation requirements, etc) as whole-blood-derived components, although more specific requirements apply for each type of apheresis component collection.

2. Plasma can be collected by apheresis as Transfusable Fresh Frozen Plasma or as Source Plasma for subsequent manufacturing. The Food and Drug Administration has provided guidance with regard to the volume of plasma that is allowed to be collected using automated devices.

3. In multicomponent donations, a variety of components or combinations of components may be collected with apheresis technology. Regulations specific to this practice apply to donor selection and monitoring, quality control, and records.

4. Red cells can be removed concurrently with other components, or a double unit of red cells may be collected.

5. Several instruments and systems have been developed and/or adapted for apheresis collection of blood components, using different technologies. Some are appropriate for collection of only one type of component, and others can collect multiple components.

6. Granulocyte collection differs from that of other components. Specific techniques and conditions should be considered for the optimal collection of granulocytes by apheresis.

CHAPTER 4

QUALITY CONTROL OF BLOOD / BLOOD COMPONENTS

At least 1% of total components prepared are subjected to quality control at random. The individual parameters to be assessed are as follows and should be fulfilled by at least 75% of the components tested (Drugs and Cosmetics Act, 1940).

Indian standards for quality control of blood components:

- Drugs and Cosmetics Act 1940, Rules 1945 (Schedule F, Part XII-B), Government of India Blood Bank Standards of NACO, Ministry of Health and Family Welfare, Government of India
- NABH Accreditation Standards for Blood Banks

When to Perform Quality Control?

For platelet products it should be done on expiry date (end of storage period) of the component.
On installation and after repair of equipments (refrigerator, centrifuges, deep freezers etc.)
Modification in procedure for components preparation.

Recruitment of new personnel.

4.1 Whole blood and red cell concentrate - QC

Table 4.1: Quality control of whole blood
(Ref: (DGHS Technical manual)

Parameter	Quality requirement	Frequency of control
Volume	350/450ml ±10%	1% of all units
Anticoagulants	49/63 ml	1% of all units
PCV (Hct)	30-40%	4 units / month
Serology (HIV1+2, HBsAg, HCV, MP, Syphilis)	Negative	All units
Sterility	By culture	4 units/month whichever is higher

Table 4.2: Quality control of Red cell concentrate
(prepared from 450 ml blood) (Ref: DGHS Technical manual)

Parameter	Quality requirement	Frequency of control
Volume	280 ml ± 40 ml	1% of all units
PCV (Hct)	70% + 5%	Periodically
Sterility	By culture	Periodically (1% of all units)

Rest investigations as whole blood.

Table 4.3: Quality control of Red cell concentrate in preservative solution (Adsol/ SAGM) (Ref: DGHS Technical manual)

Parameter	Quality requirement	Frequency of control
Volume	350 ml ± 20 ml	1% of all units
PCV (Hct)	60% + 5%	Periodically
Sterility	By culture	Periodically (1% of all units)

Hematocrit of product is an important QC criterion because too high hematocrit will decrease RBC survival in the unit and this will also decrease rate of transfusion in the patient.

Procedure:

- Randomly select WB or PRBC units which are ABO and Rh grouped and screened, and stored at 2 to 6°C.
- Note down the bag details: Manufacturer, date of manufacture and expiry date of the bag, lot no., unit no., type of component, date of collection, date of expiry and blood group of the component selected.

Perform the quality control tests in the following order and record the results.

Visual examination of the bag

Look for any leakage from the bag or satellite tube, precipitate or gel formation, gas formation, colour change or any evidence of haemolysis.

Volume measurement

Weigh the whole unit on a calibrated weighing balance. The volume is calculated as

$$\text{Volume (V) (ml)} = \frac{\text{Total wt. of the bag (gm)} - \text{Wt. of empty bag (gm)}}{\text{Specific gravity of whole blood / RBC}}$$

Collection of sample and sending for culture

- Strip the tube of the bag with stripper thoroughly so that the contents of the bag fill the tube sufficiently after the stripper is released. Nearly outdating samples are preferred and randomly selected for the purpose
- Clean the tube with spirit swab and wait for 2 minutes.
- Immediately insert a sterile syringe into the selected site on the tube, release the clamp, and withdraw 10 ml of sample from the bag. The tube of the bag is then sealed by a dielectric sealer.
- After fitting the needle into the syringe inject about 4-5 ml in each of the two appropriately labelled culture bottles minimal for aerobic and anaerobic culture. (These should be sent to the Dept. of Microbiology along with properly filled forms). Collect the remaining sample (in the syringe) in a labelled test tube. The blood culture is to be done for 15 days as per norms of Indian Pharmacopoeia.

4.2 Fresh frozen plasma & Cryoprecipitate - QC

Table 4.4: Quality Control of Fresh Frozen Plasma (FFP)
(Ref: DGHS Technical manual)

Parameter	Quality requirement	Frequency of control
Volume	200 to 220 ml	4 units /month/
Stable coagulation factors	200 units of each factor	4 units /month/
Fibrinogen	200 - 400 mg	4 units /month/
Factor VIII	0.7 units/ml	4 units /month/

Table 4.5: Quality control of Cryoprecipitate
(Ref: DGHS Technical manual)

Parameter	Quality requirement
Volume	10-20 ml
Factor VIII	80-120 units
*von-Willebrand factor	40-70% of the original
*Factor XIII	20-30% of the original
Fibrinogen	150-250 mg
*Fibronectin	55 mg

- 75% units sampled and tested should have the values indicated above
- Parameters marked with an asterisk (*) are not mandatory

Factor VIII concentration is measured by factor VIII assays using commercial available Factor VIII deficient plasma.

Factor VIII level should be 0.7 I.U. per ml. in case of Fresh Frozen Plasma and more than 80 I.U. per bag in case of cryoprecipitate

Fibrinogen is measured by dry clot weight method. Fibrinogen level should be 250-300 *mgl* bag in FFP and 150*mgl*bag in case of cryoprecipitate.

I. One stage assay for factor VIII

Principle: It is based on comparing the ability of dilutions of patient's plasma and standard plasma to correct the APTT of substrate plasma lacking F VIII

Reagents

1. Platelet poor plasma of patient (test plasma)
2. Standard plasma (normal pooled plasma)
3. Substrate plasma deficient in F VIII
4. Kaolin 5mg/ml in barbitone buffered saline
5. Cephalin

6. $CaCI_2$
7. Plot clotting times of test and standard against concentration of F VIII on semi log paper.

Procedure

1. Place kaolin, cephalin and $CaCI_2$, and 37^0C.
2. Prepare 1 in 10, 1 in 20, 1 in 40, and 1 in 100 dilutions of standard plasma and 1 in 10 of plasma in buffered saline (keep at ice bath)
3. Place 0.1 ml of diluted plasma (test and standard) in glass tubes separately.
4. Add to each dilution 0.1 ml of freshly reconstituted substrate plasma.
5. Warm up to 37^0C.
6. Perform APTT and record the time.
7. Plot clotting times of test and standard against concentration of F VIII on semi log paper.

II. Fibrinogen Assay

Principle

It is based on precipitation of fibrinogen by ammonium sulphate and measuring the height of column of precipitate.

Reagents

1. Plasma for testing.
2. Saturated ammonium sulphate solution.

Procedure

1. Saturate 200µl of patient's plasma with 600µl of saturated ammonium sulphate (80% saturation).
2. Put in a Wintrobe's tube and spin at 3000 rpm for 30 minutes.
3. Read the height of column and multiply by 120.
4. Result is expressed in mg/dL.

Fibrinogen assay can also be performed using a *commercial kit*

Fibrinogen Assay (Clauss Technique)

Principle

Diluted plasma is clotted with a strong thrombin solution; the plasma must be diluted to give a low level of any inhibitors (e.g. FDPs and heparin). A strong thrombin solution must be used so that the clotting time over a wide range is independen to the thrombin concentration.

Reagents

- Calibration plasma. With a known level of fibrinogen calibrated against an International Reference Standard
- Plasma and a control.
- Thrombin solution. Freshly reconstituted to 100 NIH units/ml in 0.9% NaCl Owren's veronal buffer. pH 7.4.

Procedure

1. A calibration curve is prepared each time the batch of thrombin reagent is changed or there is a drift in control results; this is used to calculate the results of unknown plasma samples.
2. Prepare dilutions of standard plasma in Imidazole buffer to give a range of fibrinogen concentrations (eg. 1/5, 1/10, 1/15, 1/20 dilutions)
3. Pipette duplicate 0.2ml volume of each dilution in glass tube and warm to 37 degrees for 2 minutes.
4. Add 0.2ml of thrombin and clotting time is measured using a stop watch.
5. All tests are run in duplicate
6. Plot the mean clotting time versus fibrinogen concentration (g/l) on log/log graph paper. Take the 1/10 dilution to represent standard value.
7. Prepare a 1/10 dilution of test plasma and determine the clotting time and read the corresponding value of the graph.
8. Fibrinogen level can be read directly from the graph if clotting time is between 5 and 50 seconds. Outside this range, a different dilution and mathematical correction is needed. eg. If fibrinogen level is low and 1/5 dilution is needed then the answer is divided by 2. In case a 1/20 dilution is needed then the answer is multiplied by 2.

The high concentration of thrombin used raises the risk of carryover into subsequent tests.

4.3. Platelet Concentrates – QC

Table 4.6: Quality control of Platelet concentrates, prepared from whole blood (Ref: DGHS Technical Manual)

Parameter	Quality requirement	Frequency of control
Volume	50-70 ml	4 units / month
Platelet count	>5.5 x 10^{10}	4 units / month
pH	> 6.0	4 units / month
RBC contamination	< 0.5 ml (5.5 x 10^{9} RBCs)	4 units / month
WBC contamination	< 5.5 x 10^{7} - 5 x 10^{8}	4 units / month

On visual inspection, unit which does not have a pink or red discolouration may be assumed to contain insufficient red cells to cause immunization.

Table 4.7: Quality control of Platelet concentrate prepared from buffy coat

Parameter	Quality requirement	Frequency of control
Volume	70-90 ml	4 units / month
Platelet count	> 6-9 x 10^{10}	4 units / month
pH	> 6.0	4 units / month
RBC contamination	Traces to 0.5 ml	4 units / month
WBC contamination	> 5.5 to 10^{6}	4units / month

Table 4.8: Quality control of Platelet Concentrate prepared by apheresis

Parameter	Quality requirement	Frequency of control
Volume	200-300 ml	4 units / month
Platelet count	> 3.0-7.0 x 10^{11}	4 units / month
pH	> 6.0	4 units / month
RBC contamination	Traces to 0.5 ml	4 units / month
Residual leucocytes	< 5.0 x 10^{6}	4 units / month

Visual examination of the bag:

Look for any leakage from the bag or tube, precipitate or gel formation, gas formation, turbidity and RBC contamination. Any reddish or pinkish discolouration / tinge are recorded as red cell contamination.

Swirling test:

Swirling is a reliable test indicating the viability of platelets during storage. Viable platelets exhibit a swirling pattern of movement indicating their maintenance of discoid shape during storage. Hold the bag against a background of bright source of light and with opposing fingers squeeze one corner of the bag gently. Platelets are seen moving cross the bag in a random I swirling pattern.

Record results: if present (+) or absent (-).

Volume measurement:

Weigh the platelet unit on a calibrated weighing balance. The volume is calculated as:

$$\text{Volume (V) (ml)} = \frac{\text{Total wt. of the bag (gm)} - \text{Wt. of empty bag (gm)}}{\text{Specific gravity of platelets}}$$

Collection of sample and sending for culture:

(a) PC

(b) BC-PC

- Sample collection-As in collection of sample and sending for culture of whole blood and red cell concentrate

Alternate method for Collection of sample and sending for culture

- Strip the tube of the bag with stripper thoroughly so that the contents of the bag fill the tube sufficiently after the stripper is released.
- Clean
- Clean the tube with spirit swab and wait for 2 minutes.
- Insert sterile needle with 10 ml syringe in the tube, release the clamp, and withdraw 10 ml of sample from the bag. The tube of the bag is then sealed by a dielectric sealer. Inject about 5 ml in appropriately labeled culture bottles. Collect the remaining sample (in the syringe) in a labeled test tube.

4.4. Apheresis platelet concentrate (AP-PC) - QC

The sample for quality control is taken from the pouch attached to the transfer bag.

Rests of the steps are the same as described above.

4.5. pH measurement

It is done using the pH meter. Readings should be taken with buffers and from components at storage temperature. The pH indicator solution is mixed with the sample in a separate test tube. Match the colour of the resultants solution with that displayed on the label of indicator solution bottle, and note the pH reading.

4.6. Platelet count

Run the sample in cell counter and note the reading of platelet count. At least 1% of total components prepared are subjected to quality control at random. The individual parameters should be assessed and should be fulfilled by at least 75% of the components tested.

Further Readings

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3. Food and Drug Administration. Guidance for industry and FDA review staff: Collection of platelets by automated methods. (December 17, 2007) Rockville, MD: CBER Office of Communication, Outreach, and Development, 2007. [Available at http://www.fda.gov/cber/guidelines.htm (accessed November 22, 2010).]

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6. Technical manual, DGHS, MOHFW Govt. of India 2003.

7. Drugs and Cosmetic Act 1945 and rules thereof.

8. National Blood Policy, NBTC, Ministry of Health and Family Welfare.

9. National Policy for Access to Plasma Derived Medicinal Products from Human Plasma for Clinical/Therapeutic Use: Addendum to National Blood Policy, NBTC, Ministry of Health and Family Welfare.

10. Action Plan to Blood Safety NBTC, Ministry of Health and Family Welfare.

11. Training Module for Blood Bank Medical Officers And Lab Technicians.

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