DRAFT MONOGRAPHS FOR COMMENTS

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Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Comments received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in **before the last date for comments.**

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General Requirements: Blood Components Derived from Human Source

Blood/blood components transfusion is an integral part of the modern clinical practice. Technologic advances in blood collection, separation, anticoagulation and preservation have resulted in increased component preparation from whole blood units. Transfusion can be used in two ways:

1. the entire donor unit is administered as whole blood,
2. a specific part of the blood unit is selected as component

If whole blood is used then the patients may receive components of blood not required by them, which may increase the risks of adverse events related to blood transfusion. Transfusion of only the required component of blood will optimize utilization with more patients benefiting from a single unit of blood as well as reducing the risk of adverse events related to Blood Transfusion.

Components are constituents of blood that can be prepared by centrifugation, filtration and freezing using conventional blood center methodology.

Definitions

Whole blood. It is blood collected from the donor into an approved container containing an anticoagulant preservative solution.

Blood product. Any therapeutic substance prepared from human blood

Blood component. A constituent of blood, separated from whole blood by physical methods of separation, such as packed red cells, plasma, platelets, cryoprecipitate.

Plasma derivative. Human plasma proteins prepared under pharmaceutical manufacturing conditions, such as Albumin, Immunoglobulins, coagulation factor concentrates.

A) Blood bag system

Introduction of multiple plastic bag systems has greatly facilitated the preparation of high quality blood components. Polyvinyl chloride (PVC) has been found satisfactory for red blood cell storage. The biocompatibility of any plasticizers used must be assured. Storage of platelets at + 20° to + 24° requires a plastic with increased oxygen permeability. Leaching of plasticizers into blood or a component should not pose any undue risk to the recipient. Care should be taken to minimize levels of residual toxic substances after sterilization, for example ethylene oxide. Whenever new plastics are to be introduced an adequate study of component preparation and/or storage should be conducted. These studies would normally be carried out by the manufacturer before introduction of the new plastics and the results be made available to the transfusion services. In order to maintain a closed system throughout the separation procedure, a multiple bag configuration, either readymade or sterile docked, should be used. The design and arrangement of the pack system should be such as to permit the required sterile preparation of the desired component.

Although use of closed systems is recommended for all steps in component processing, open systems may sometimes be necessary for modification of blood components, example washing of red cells. When open systems are employed, careful attention should be given to use of aseptic procedures and the shelf-life of the component decreased depending on component and its storage temperature to prevent contamination and proliferation of microbial agents within 24 hours.

B) Anticoagulant-preservatives and Additive solutions

Red Blood cell (RBC) viability and functional activity require that RBCs be preserved in solutions that support their metabolic demands. All anticoagulant solutions contain citrate, phosphate and dextrose (CPD). These constituents function as an anticoagulant, a

buffer and a source of metabolic energy for the RBCs, respectively. Depending upon the collection system used, a single whole blood donation typically contains either 350 ml (±10 per cent) or 450 ml (±10 per cent) of blood collected from blood donors, withdrawn in

a sterile container that includes anticoagulant-preservative solutions licensed for this component. Whole Blood units are collected in an aseptic manner in a ratio of 14 ml of anticoagulant-preservative solution per 100 ml of whole blood. Apheresis components are collected into anticoagulant-preservative solution as recommended by the manufacturer.

The development of newer anticoagulant preservatives and additive solutions has led to an increase in the shelf life of RBC containing blood component from 21 days for CPD to 35 days for citrate-phosphate-dextrose-adenine (CPDA)-1 and to 42 days for the newer extended storage solutions (additive solutions). Additive solutions may be mixed with the red cells remaining after removal of most of the plasma.

Platelet additive solutions (PASs) are electrolyte solutions intended for storage of platelets, and they are used to modulate the quality of the platelets by adding specific ingredients. Addition of PASs allows for removal of more plasma as well as decreasing the titer of ABO isoagglutinins thus facilitating transfusion of platelets across the ABO barrier.

Various anti-coagulant preservative solutions are available in which whole blood can be collected for in-vitro storage.

They are:

1. Acid-citrate-dextrose (ACD)
2. Citrate-Phosphate-Dextrose (CPD)
3. Citrate-Phosphate-Dextrose-Adenine 1 (CPDA-1)

Table 1: Basic compositions of Anticoagulant-Preservative solution

Composition	**ACD**	**CPD**	**CPDA-1**
Sodium citrate (Dihydrate) (g)	22.0	26.30	26.30
Citric acid (Monohydrate) (g)	8.0	3.27	3.27
Citric acid (anhydrous) (g)	7.3	2.99	2.95
Dextrose (Anhydrous) (g)	22.3	23.2	29.0
Dextrose (monohydrate) (g)	24.5	25.5	31.9
Sodium dihydrogen phosphate (Dihydrate) (g)	-	2.51	2.22
Adenine (g)	-	-	0.275
Water for injection I.P. (ml)	1000	1000	1000
Preservative (ml) / 100 ml blood	15	14	14
Preservative (ml) / 450 ml blood	67.5	63	63
Preservative (ml) / 350 ml blood	52.5	49	49
Initial pH of preservative	5.0 ± 0.5	5.6 ± 0.3	5.6 ± 0.4
On first day pH of blood in bag	7.0	7.2	7.0 - 7.3
Storage time (days) at 2 - 6°	21	21	35

*49 ml for 350 ml collection and 63 ml for 450 ml collection

Table 2: Basic composition of Additive Solutions (AS)

Composition	AS-1 (ADSOL)	AS-3 (NUTRICEL)	AS-5 (OPTISOL)	SAG-M
Sodium citrate (Dihydrate) (g)	-	0.588	-	
Monobasic sodium phosphate (Monohydrate) (g)	-	0.276	-	
Dextrose anhydrous (g)	2.20	l.1	0.9	0.9
Adenine (g)	0.027	0.03	0.03	0.169
Mannitol (g)	0.750	-	0.525	0.525
Sodium chloride (g)	0.9	0.41	0.877	0.877
Water for injection I.P (ml)	100	100	100	100
Primary bag anticoagulant	CPD	CPD	CPD	CPD

Table 3: Basic composition of various Platelet Additive Solutions

Element	PAS- B	PAS- C	PAS - D	PAS- E	PAS - F	PAS - G
Sodium chloride (g)	111.5	77.3	90	69.3	90	110
Potassium chloride (g)	-	-	5	5	5	5
Magnesium chloride (g)	-	-	1.5	1.5	3	37.5
Sodium citrate (g)	10	10.8	11		-	7.5
Sodium acetate (g)	30	32.5	27	32.5	27	15
Sodium bicarbonate (g)	-	-	-	-	-	26
Sodium gluconate (g)	-	-	23	-	23	-
Sodium phosphate (g)	-	28.2	-	28.2	-	4
Glucose (g)	-	-	-	-	-	30
Water for injection I.P (ml)	1000	1000	1000	1000	1000	1000

Preparation of Blood Components

Preparation of blood and blood components must follow the principles of Good Manufacturing Practice (GMP) and shall comply with suitable regulations such as Drugs & Cosmetics Act, 1940 and rules 1945 there under and national and international guidelines. The premises used for the processing of blood components must be kept in a clean and hygienic condition and the microbial contamination load on critical equipment, surfaces and in the environment of the processing areas must be monitored. Protocol shall be developed and validated for all processing activities. These must include time limits for the processing of blood components. Validated manual, semi-automated or fully automated procedures may be adopted for any of these processes.

Collection of Blood for component preparation

Blood components can be prepared from whole blood or apheresis donations.

1. **Single whole blood donation**. A person fulfilling all the donor selection criteria is selected for whole blood donation. After proper selection of a blood bag, requisite volume of blood is collected through strict aseptic measures with a single sharp venipuncture and constant agitation for proper mixing of blood with anti-coagulant-preservative solution. A unit of whole blood consists of 450 ml ± 10 per cent of blood plus 63 ml of anticoagulant-preservative solution, or, 350 ml ±10 per cent of blood plus 49 ml of anticoagulant-preservative solution.

Preparation of blood components is done in double/triple/quadruple blood bags having closed integral tubing. These blood bags have a primary bag containing the anticoagulant-preservative solutions which is connected to satellite bags through sterile tubing. After collection of a unit of whole blood in the primary bag, blood components can be separated from one another by differential

centrifugation in a refrigerated centrifuge due to differences in their specific gravities. After their separation, various components can be transferred from one bag to another in a closed circuit thus avoiding exposure to the external environment and maintaining the sterility. Blood should be processed for component separation within 6 hours of collection. If Whole blood is used for transfusion, no post-donation processing into components is required.

2. **Apheresis.** This is a procedure in which a suitable donor is connected to an automated cell separator machine (that is essentially designed as a centrifuge) through which whole blood is withdrawn, the desired blood component is retained and the remaining of the blood is returned back to the donor. Depending on the component that is separated and removed, the procedure is called plasmapheresis and cytapheresis (plateletpheresis, leukapheresis, erythrocytapheresis etc.)

C**omponents prepared by Whole blood method**

There are two ways by which components can be prepared from a whole blood unit, either by platelet rich plasma (PRP) method or by buffy coat removal method.

1. **Platelet rich plasma method**. This is done most commonly initially by performing a soft spin, which separates the heavier RBCs from platelet rich plasma. For separation of platelets from plasma, a hard spin then is performed which yields platelet concentrate (PC) at the bottom of the bag and supernatant plasma. The resulting PC can be stored as multiples of single units or pooled with other donor PCs. The typical volume of a unit of plasma collected from whole blood is approximately 180 - 220 ml. In order for the plasma to be labeled as fresh frozen plasma (FFP), the unit must be separated from the other blood components and stored at -30^{o} within 6 hours of collection.

2. **Buffy Coat removal method**. Buffy coat method for blood component preparation utilizes principles of differential centrifugation and separation. Firstly, a hard spin (or high g-force centrifugation) is given which separates whole blood into three layers, based on their specific gravities. The topmost layer comprises of plasma, middle layer is the buffy coat layer comprising of white blood cells, platelets and red blood cells. The bottom layer comprises exclusively of red blood cells. The supernatant plasma is transferred to the satellite bag dedicated for plasma storage. The red blood cells at the bottom layer are transferred to the respective bag with additive solution. The remaining buffy coat along with some plasma and red cells is allowed to rest for 2 hours. Now the soft spin (or low g-force centrifugation) is given to the buffy coat bag. The resulting supernatant contains platelet concentrate which is transferred in platelet bag. This method of blood component preparation reduces the leucocyte content of the packed RBCs and platelets to less than 5×10^{8} i.e. 1 log reduction. Such components have lower rate of Febrile Non Haemolytic Transfusion Reactions (FNTRs) post transfusion. The yield of plasma and platelet components is also better with this method.

Components prepared by Apheresis method

Apheresis is a procedure to harvest a particular component, while returning the rest of blood back to donor. This entails a process where an automated apheresis instrument draws blood into an external circuit mixed with anticoagulant-preservative solution, separates the components by centrifugation or filtration, collects the desired component, and returns the remaining blood components to the donor. These methods provide larger quantities of the desired component than whole blood collection methods. Since the red cells are returned to the donor, the process can be repeated at frequent intervals. Depending on the component collected, procedure is called as plasmapheresis if plasma is collected or platelet apheresis if platelets are collected.

Blood Component Modifications

I. **Leucodepleted components**

A unit of whole blood generally contains ≥1 to 10×10^9 leucocytes. Leucodepletion may be achieved by in-process collection (apheresis) or filtration. Filtration may be done: 1) soon after collection (pre storage), 2) after varying periods of storage in the laboratory, or 3) at the bedside (post-storage). The method used in the laboratory for leucodepletion is subject to quality control testing; leucodepleted components prepared at the bedside are not routinely subjected to quality control testing. Leucodepletion will

decrease the cellular content and volume of blood according to characteristics of the filter system used. Leucodepleted blood components shall have a residual content of $<5.0 \times 10^6$ leukocytes. Leucodepletion filters variably remove other cellular elements in addition to white cells. Washing, buffy coat removal or irradiations are not a substitute for leucodepletion.

II. **Irradiation**

Blood components that contain viable lymphocytes may be irradiated to prevent proliferation of T lymphocytes, which is the immediate cause of transfusion-associated graft-versus-host disease (TA-GVHD). Irradiated blood is prepared by exposing the component to a radiation source. The standard dose of gamma irradiation is 25 Gy targeted to the central portion of the container with a minimum dose of 15 Gy delivered to any part of the component. Details of irradiated blood components are provided in "Guidelines for Irradiated Blood & Components".

III. **Washing**

Washing of cellular components can be done using isotonic saline (0.9 per cent Sodium Chloride) by validated procedures. Washing removes unwanted plasma proteins, including antibodies from the units. There will also be some loss of red cells and platelets, as well as a loss of platelet function through platelet activation. The shelf life of washed components is no more than 24 hours at 10° to 60° or 4 hours at 22 ± 2°. Washing is not a substitute for leucodepletion.

IV. **Frozen red cells, aliquoting and pooling of components**

Frozen/ cryopreserved RBCs

Red cells can be frozen for prolonged periods to preserve rare phenotypes or to build inventory for emergencies/use in disasters. Red cells are frozen with cryoprotective agents like glycerol. Glycerol should be added slowly with constant mixing by an approved and validated procedure. It can be preserved at -80°, with high-concentration glycerol (40 per cent) or at -196° in a low-concentration glycerol (20 per cent). Red cells must be frozen within 6 days of the collection after removing plasma and/or additive solution. The shelf life of frozen RBCs is 10 years.

For the recovery of frozen red cells, units should be thawed slowly with gentle agitation at 37°. Glycerol has to be removed by washing with sterile saline solution.

The cells are suspended in 0.9 per cent sodium chloride and 0.2 per cent dextrose solution.

Shelf-life of deglycerolized red cells:

Using open system: 24 hours at 4 ± 2°.

Using closed system: 14 days (when suspended in additive solution) at 4°.

Aliquoting of RBCs

Aliquoting of components may be done for specific volume requirements (per Kg body weight) for transfusion. It is done generally for paediatric transfusions, hemoglobinopathies, intra uterine transfusions etc.

Aliquoting may be done in an open or closed system (Details given later). Aliquoted component shall have a new identification number and shall be traceable to Primary/ mother blood bag. Label shall carry the identification of Primary blood bag, volume of component, date and time of aliquoting, date of expiry, along with other general labelling requirements.

Open/Closed System

Components may be required to be transferred to another bag for aliquoting, pooling or processing (e.g. washing, addition of additive solution etc.). This can be done either by Open or Closed system.

Closed system

When components are transferred without compromising the integrity of bags, then it is called as a closed system. Blood bags that allow transfer of component without breakage of the seal shall be recommended. If the seal is not broken, stability of the component is assured. The seal shall not be considered broken if a sterile connection device or sterile tube welder is used. When a device for making sterile connection is used, the system can be regarded as closed providing that the process of joining and sealing has been validated and shown not to lead to an increased risk of microbial contamination of the component. The procedure for use should ensure that the operator carefully checks the suitability of every weld and also pays particular attention to effective cleaning of the working parts of the equipment. Cleaning of the equipment should be done by validated procedure with regular checks to ensure conformance to procedures. Expiry of components prepared in the closed system will have the original expiry of the mother (primary) bag.

Open system

If the seal is broken during processing or transfer of components and integrity is compromised, it is called as an open system. Every effort shall be made to prevent microbial contamination by operating in a clean environment (using laminar airflow), using sterilized materials and aseptic handling techniques. The sterility of components prepared in an open system should be monitored using validated methods. Blood components prepared by an open system should be used as soon as possible. If storage is unavoidable, components with a recommended storage temperature of 22 ± 2° should be used within six hours and at 4 ± 2° should be used within 24 hours. Once the frozen components are thawed, these shall be transfused at the earliest and positively within 6 hours.

Expiry of the components prepared in an open system is as follows:

1. Red cells (2°- 4°): 24 hours
2. Pooled platelet concentrate: 6 hours
3. Pooled cryoprecipitate: 6 hours

Testing Requirements and Quality Control

Mandatory serological and infectious disease markers screening and other tests are required for all whole blood donations. 1 per cent of all blood components or 4 units, whichever is higher will be subjected to quality control testing including sterility check. A minimum of 75 per cent of those components tested for the parameters mentioned in individual monographs shall meet the specified values.

Storage and transport temperature of blood components

Blood Component	Storage period	Storage temperature	Transport temperature
Transport of pre-processed whole blood from collection site to	NA	NA	• Between +20° to +24° if platelet conc. is to be

processing lab			prepared, otherwise 4 ± 2°.
Whole blood	• 35 days if CPDA-1 is used	• Between 4 ± 2°. Must not go below 1°.	• Between +2° and +10° with a maximum transit time of 24 hours.
Red cell components • Packed red cells • Leuco-depleted red cells	• 21 days if stored in CPD • 35 days if stored in CPDA-1. • 42 days if stored in red cell additive solutions	• Between 4 ± 2°. Must not go below 1°.	• Between +2° and 10° with a maximum transit time of 24 hours.
Platelet components • Random donor platelets • Single donor platelets • Pooled platelets	• 5 days with continuous gentle agitation	• Between ± 22°	• Between ± 22° maximum transit time of 24 hours • Agitation can be interrupted up to 30 hours.
Plasma components • Fresh Frozen Plasma • Cryoprecipitate • Cryoprecipitate depleted plasma	• 1 year for FFP and Cryoprecipitate • 5 years for CPP	• Below -30o	• Below -30°
• Granulocyte concentrate	• Should be used as soon as possible after their preparation not more than 24 hours	• 22°± 2° without agitation	• 22°± 2° without agitation

Labelling

Every blood component unit should be properly labeled during the time of collection, processing, storage and compatibility testing. The following shall be included on the label of all Blood Components (* = mandatory, eye-readable and where applicable, approved barcode format)

- Name of the blood component
- Details of Blood Centre
- Blood Centre License number
- Unique identity numbers
- ABO and Rh(D) groups (color coded)
- Date of collection
- Date of expiry
- Volume and/or Weight of the blood component
- Storage temperature
- TTI Non-reactive label
- Additional component information: leucodepleted, irradiated, washed red cells, directed donation, paediatric bags etc. (as appropriate)
- Additional instructions to the clinician as per the components