Blood processing and components

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Introduction

A unit of whole blood collected from a donor is a precious asset. A donation of blood should be seen as a bag containing all the different constituents of whole blood – red cells, white cells, platelets, and plasma with plasma proteins such as clotting factors and protective antibodies. In modern transfusion medicine the aim is to transfuse the patient with only the component required as far as is practical. Component therapy also maximises the use of one donation. The products from a single donation can benefit multiple patients. See Section 18: Indications for transfusion, for more information about the use of whole blood and different blood components.

Whole blood may be processed into various components and each component can then be stored under ideal storage conditions (i.e. temperature and movement) to ensure that the product is most effective when it is used. Special preservative solutions and blood bags are used to lengthen the expiry time and improve product quality.

This section will cover very broadly, the processing of blood into components.

Learning objectives

By the end of this section, reader will be able to understand the processing of blood into components and to discuss the requirements of a component programme under the following headings:

- Processing blood into components
  - Sterile systems
  - Bag systems for blood collection
  - Single bag
  - Double bag
  - Triple bag
  - Quadruple (quad) bag
  - Top and bottom bag

- Principles of centrifugation
  - Blood processing equipment
  - Blood bag centrifuge
  - Scale
  - Balances
  - Plasma extractor
  - Automated blood processing machines
  - Pilot tube sealer
  - Sterile connecting device
  - Platelet agitator

- Preparation of specific blood components
  - Whole blood
  - Red cell concentrate (RCC)
    - RCC in plasma
    - RCC, buffy coat removed, in additive solution (RCC, leucocyte-reduced)
    - RCC, filtered (RCC, leucocyte-depleted Prestorage)
    - On demand
    - In line
    - Add on
    - RCC, paediatric
    - RCC, cryopreserved (frozen)
    - RCC, washed
  - Plasma
    - general information
    - fresh frozen plasma
    - freeze-dried plasma
    - cryoprecipitate
  - Platelet concentrate (PC)
    - general information
    - preparation of PC by platelet-rich plasma method
    - preparation of PC by buffy coat method
      - conventional quad bag method
      - top and bottom bag method
      - single unit PC from buffy coat
    - pooled PC from buffy coat
      - pooling kits
      - chain method
      - apheresis platelets
      - quality control for PC

- Irradiated products
- Measures to prevent transmission of pathogens
- Labelling and records
- Overview of plasma fractionation

Processing blood into components

Figure 1 shows the three main component types (red cells, plasma, platelets) that can be processed from whole blood and lists some of the blood products that may be prepared by processing further.
To process whole blood into components requires a basic understanding of:
- Sterile systems.
- Blood bag systems.
- Principles of centrifugation.
- Blood processing equipment.

Sterile systems
Blood bag manufacturers must ensure that all blood bags plus anticoagulants and additive solutions are sterile (free of contamination by bacteria or viruses) and pyrogen free (do not contain endotoxins or micro-organism debris).

Collection of whole blood is described in Section 9: Blood collection.

The routine processing of blood into components relies on the availability of closed blood bag systems. Individual bags in multiple bag systems are connected with one another via tubing and thus constitute a closed system. The advantage is that the components can be separated into the attached bags (after centrifugation) without affecting sterility, as the primary bag is never opened, and the entire process occurs in a closed system.

Bag systems for blood collection
A wide variety of polyvinyl chloride (PVC) plastic blood bag systems are available from many suppliers. The choice of bag type depends on the requirements of the particular blood service, based on the following criteria:
- Affordability.
- Clinical demand for components (such as RCC, plasma, PC).
- Whether units will be processed manually or automatically.
- Level of product storage to be achieved, such as the use of additive solutions and special plastic bags for platelets.
- Whether filtration to remove white cells (leucodepletion) is required.

Single bag
This is the simplest of the bags available. The donation is taken into the bag and the pilot tubing is then sealed. No further processing into components is performed and the unit is transfused as whole blood. The bag contains an anticoagulant solution (usually CPDA). CPDA contains sodium citrate that prevents clotting, and citric acid (C), monobasic sodium phosphate (P), dextrose (D), and adenine (A) that provide buffers and nutrients for enhanced red cell survival.

Double bag (two bag system)
In a multiple bag system, the bag with anticoagulant into which the donation is taken is referred to as the primary bag; in a double bag system, an additional empty bag is attached to the primary bag (called a transfer or satellite bag). Contents of the primary bag are prevented from entering the transfer bag by the presence of a breakable seal at the point where the transfer tubing joins the primary bag. After centrifugation of the whole blood, the seal between the two bags can be broken (without compromising the hermetic seal and the sterility of the system) and the plasma transferred through the tubing to the attached transfer bag creating two components, a red cell concentrate (RCC) suspended in a small amount of plasma in the primary bag and plasma in the transfer bag.

Triple bag and quadruple (quad) bag systems
A triple bag differs from a double bag only by having an additional transfer bag. Once plasma is separated into the first transfer bag, there is still another empty transfer bag attached to it. This configuration is used to manufacture PC from platelet-rich plasma, or to harvest cryoprecipitate from fresh frozen plasma. A quad bag system is similar to the triple bag system but has an additional bag containing red cell additive solution and is usually used in automated systems to prepare RCC, plasma, and platelets produced by the buffy coat production method. Quad bag systems may be designed to have transfer bags attached to both the top and the bottom of the primary bag (so called bottom and top method) or just to the top of the bag (so called top and top method). Details of how triple and quadruple bags are used to make components are described later in this section, under the heading Preparation of specific blood components.

Principles of centrifugation
Blood constituents can be separated by centrifugation because they differ in size and density and will sediment at different rates when centrifugal force is applied.
When whole blood is centrifuged, the red cells settle at the bottom of the blood bag because they have the highest density (have a greater mass/weight than the other components).

Being less dense, the white cells and platelets do not settle as quickly and remain in suspension for longer. As centrifugation continues, the white cells sediment above the red cells, and finally the platelets form a layer above the white cells and leave the original suspending fluid (now clear plasma plus anticoagulant) at the top. Figure 2 illustrates the separation of components as a result of moderate or hard centrifugation of a unit of whole blood.

It is important to select the correct speed and time of centrifugation to be used in order to separate the desired component. For example, if platelet-rich plasma is required then centrifugation should be such that the platelets are not sedimented. A lower centrifugation speed for a longer period is required. If, on the other hand, completely cell-free plasma is required then a faster centrifuge speed for an adequate time period would yield clear plasma and densely packed red cells with the white cells and platelets layered above and cell-free plasma on top.

It is important that the optimal conditions for a good separation be carefully evaluated for each centrifuge to obtain the desired components. To establish optimal centrifugation:

- Collect parameters that indicate the desired outcome of the procedure (e.g. whole blood centrifugation should yield RCCs with a haematocrit (Hct, also called the packed cell volume) between 0.65 and 0.75 and a certain volume of plasma (minimum 200 ml) as well as a buffy coat (BC) with a particular platelet yield. Hct is a measurement of the proportion of the blood that is made up of the red cells, expressed as a fraction. For example, an Hct of 0.65 means that there are 65 ml of red cells in 100 ml of blood.
- From the centrifuge manual or other procedure manuals establish a base line centrifuge setting with regard to speed (measured in revolutions per minute, i.e. rpm, or gravitational force equivalents, i.e. g) and time.
- Prepare a number of products (at least 10) using this setting and measure all parameters.
- Repeat the procedure with reduced or increased speed and time combinations and compare parameters until the optimum setting for the centrifuge is established.

After centrifugation, the bag system is gently removed from the centrifuge, taking care to prevent mixing or swirling, and the primary bag is placed in a plasma extractor, or an automated component extractor, for...
separation. Pressure is applied to the bag and the component layers are then transferred, in order, into one or more of the transfer bags connected in the closed system.

**Blood processing equipment**

**Blood bag centrifuge**

Blood bag centrifuges are essential pieces of processing equipment. Several manufacturers market centrifuges capable of spinning between four and 12 blood bags at a centrifugal force of up to 5000 \( g \). These are large machines that are usually floor-standing and require dedicated floor space and electrical supply. Professional installation and a good maintenance programme are essential to ensure staff safety and consistently reproducible centrifugation of product.

A typical centrifuge has an electric motor that turns a rotor housed in a very thick metal chamber. As the rotor is spinning the door on this chamber locks automatically to prevent access by the operator during operation as this would be extremely dangerous. The rotor is designed to hold a certain number of metal buckets. These vary in size and shape depending on the type of blood bag system being centrifuged (e.g. a quad bag system with additive solution will require a bigger bucket than a simple double bag). Each bucket has a plastic insert that can easily be loaded into, or unloaded from, the metal bucket. The inserts make the centrifugation process easier to perform and are easy to clean. Figure 3 shows a centrifuge bucket insert, metal bucket and rotor that fits into a centrifuge housing.

When loading a rotor, the bucket/insert/blood pack combinations that are placed opposite one another must be of equal weight. The metal buckets are rarely removed and should be placed in the rotor according to matching weights.

Two plastic insert and blood bag combinations are placed on a balance (or scale). Weight (mass) in the form of plastic or rubber strips, is added to the lighter combination until the two bag/insert combinations are of equal mass. The loaded and balanced inserts are then placed into the metal buckets opposite one another in the rotor. Failure to balance the buckets before operation, can cause the centrifuge to vibrate when spinning starts, and could cause serious damage to the centrifuge or injury to personnel. In addition, the product will not be adequately centrifuged. The use of water or other liquids is not recommended for balancing the opposing buckets as the liquid may become bacterially contaminated, and moisture may smudge or dislodge the blood bag label. The plastic or rubber strips used for mass correction should be disinfected regularly.

Blood pack centrifuges must also have a refrigeration capacity that enables the temperature in the chamber to be controlled during processing.

In order to make quality blood components, a centrifuge must be able to perform within tight parameters. The amount of centrifugation (i.e. spin) that a product requires can be measured in terms of speed and time (e.g. 2000 rpm for 10 minutes). This, however, is not the most accurate way, as the amount of gravitational force exerted is much greater in centrifuge heads with a longer radius. For example, 2000 rpm in a centrifuge with a radius of 30 cm produces less force than 2000 rpm in a centrifuge with a 50 cm radius. It is therefore better to calculate the \( g \)-force for a particular spin and specify the requirements in terms of gravitational force and time (e.g. 1900 \( g \) for 10 min). This figure takes the centrifuge radius into account and can be used to set
similar centrifuge settings on different makes and models. The handbook related to the blood pack centrifuge provides the formula to convert rpm to gravitational force for each specific centrifuge rotor. Some centrifuges have built-in software to do the conversion automatically. Centrifugation under a low $g$-force is referred to as a soft or light spin.

Some centrifuges also take into account the time taken to reach the desired speed (acceleration) and the time taken to stop (deceleration or braking), as these will vary according to centrifuge load. It is also possible to link computer software to a blood pack centrifuge and record all operational data for total process control. This means that the software is able to capture information like operator’s name, date and time processed, and details of the centrifuge time and speed parameters against the donation identification number. In the event of quality problems, full traceability of the centrifugation data is thus available.

**Scale**

A laboratory scale capable of weighing components to at least the nearest gram is essential when making components. The mass forms a critical part of quality control, for example:

- Whole blood must meet mass requirements in order to be suitable for component production.
- Each component produced must fall within a specified mass range.
- The mass may be used to calculate the volume of a component (gross weight of component minus empty bag weight, multiplied by specific gravity of component).

Systems need to be in place to ensure the accuracy of the scale on an ongoing basis and a calibration/service should be performed at least once per year. Figure 4 shows a laboratory scale for weighing blood components.

**Balance**

A balance has two weighing platforms and is used to prepare combinations of blood bags/centrifuge inserts of equal mass to be placed opposite one another in a centrifuge. The balance should ensure that combinations do not vary by more than 1 g. Clean plastic or rubber pieces are added to the side with the lighter combination until it is equal in mass to the heavier side. The display will indicate the mass difference in grams and give the operator a guideline as to how much balance material to add. A calibration/service should be performed at least once per year. Figure 5 shows an example of a balance with two weighing platforms for preparing blood bags of equal mass for centrifugation.

**Plasma extractor**

A plasma extractor (or blood press) is a commercially available device that is used to apply pressure to a centrifuged unit of blood in order to transfer part of it (e.g. plasma or BC) to an attached transfer bag. The design of the device is such that a controlled amount of pressure is applied to the bag that should allow reasonable flow of liquid from one bag to the next without danger of bursting the bag or causing excessive frothing of the component being transferred.

Regular cleaning and checking of the device are essential and if it is not performing correctly it should be repaired before use. Additional pressure should not be applied by squeezing the plates together by hand to ‘speed things up’ or compensate for a lack of pressure as a result of a defect. Figure 6 shows a typical plasma extractor.

To operate the plasma extractor, the following steps are taken:

1. Use the handle to open the front pressure plate.
(2) Hold it in the open position using the hook provided.
(3) Carefully remove the centrifuged blood bag from the centrifuge bucket.
(4) Hang the primary bag on the hooks located on the backing plate. Great care must be taken not to disturb the interface between red cells and plasma.
(5) Carefully reposition the transfer bags still attached to the primary bag and place them on the workbench next to the plasma extractor.
(6) Release the handle and slowly allow the front pressure plate to apply pressure to the primary bag in the press (without disturbing the interface).
(7) Once the pressure is applied, break the seal on top of the blood bag to allow plasma, and later BC if desired, to flow via the connecting tubing into the transfer bag. (Do not release pressure in mid-flow as this will cause mixing in the bag.)
(8) Stop the flow at the desired point by using forceps or plastic tubing clamps to create a temporary seal in the tubing.
(9) Now remove the separated bags of components from the extractor.
(10) Permanently seal the blood pack tubing for the separation of the bags into individual components.

Automated blood component extractor

Automated blood component extractors are commercially available devices that can be configured to process a centrifuged unit of blood into the required components with little or no manipulation by the operator other than to load and unload the machine.

There are several different types available that vary from machines that perform basic separations to more sophisticated machines that perform advanced separations and record the details of each separation for total quality management purposes.

The machines use light sensors to detect blood cells in the primary bag and tubing to activate the selected programming that controls the opening and closing of tubing clamps and regulates flow between bags. Some machines also have built-in scales that weigh the product transferred to the bags (e.g. BC) and use this information to activate the clamps. Fully automated blood component extractors will also perform the sealing of tubing between bags as part of their process.

Automation is justified when:
- There is an adequate number of units requiring processing each day.
- The blood centre has made a decision to produce leucocyte-reduced RCC (i.e. BC removed) that are suspended in red cell additive solution.
- The blood centre wants to produce PC from BCs.
- Personnel are motivated by the idea of automation and the quality improvement that can be realised by moving to it.
- Adequate technical support is available in the area for the repair, maintenance and calibration of the machines.

The manual processing of units of whole blood into RCC suspended in additive solution, involving the removal of BC and plasma into separate transfer bags, is an extremely labour-intensive operation to perform routinely without the aid of automation.

Pilot tube sealer

During manual or semi-automated processing, the tubing between separated components is temporarily sealed using forceps or plastic clamps. These temporary seals are replaced with permanent seals in the tubing as soon as possible. Seals are also used in the tubing of prepared red...
cell products to make segments of approximately five cm in length, that contain red cells from the product for use in testing without compromising the sterility of the bag contents.

A tubing sealer is a fairly simple device that, by means of heat, creates permanent seals in the PVC tubing of blood packs. The ideal seal is made quickly without generation of excess heat and will be about two mm wide with a ‘split line’ down the middle to enable easy parting of the tubing when firmly pulled apart. Figure 7 shows an example of a pilot tube sealer.

There are several types available:
- Hand-held models that are either electric or battery powered and are used at the bedside and in operations where the sealer is brought to the bag.
- Bench models that are positioned in specific areas where the bags are brought for sealing and are used when a large number of seals need to be made. They are generally quicker and less prone to overheating than the hand-held machines.

Great care needs to be taken to ensure that the machines are functioning correctly. Without a proper cleaning, maintenance and quality checking system, blood products with faulty or leaking seals might get into the blood supply.

Sterile connecting device

A sterile connecting device (SCD) is used to attach an additional transfer bag (or bags) to a primary blood bag without breaking the sterile integrity of the system. The shelf life of components thus prepared is the same as if the product had been prepared in a closed system.

The pilot tubing of the primary bag is placed into a slot on the SCD. The tubing of the transfer bag to be joined is placed in an adjacent slot running parallel to the first. On starting the operation, a disposable wafer is superheated by the SCD and then drawn through the tubing in the slots. Simultaneously, the tubing is moved to align the ends to be sealed, and the wafer is withdrawn. The ends to be sealed are welded together instantly and the closed system is extended by another bag (or set of bags).

There is another technology available which uses a permanent heating element for the docking, avoiding the need for wafer. However, the manufacturer has introduced a number counter that obliges to equally pay for every seal made.

Figure 8 shows a sterile connecting device and illustrates how the tubing of two separate bags is joined to extend the closed system.

The ability to extend a closed system has many advantages as it allows the technologist to weld in bags with filters, smaller bags to create paediatric units, and to create platelet pools. All SCD welds must be inspected for quality, integrity, leaks, air bubbles and alignment. The consequences of passing a faulty weld can be very serious indeed, so procedures must be in place to ensure that the correct course of action is taken when faults are detected. Record keeping must include full documentation of products welded and weld quality control results.

Regular (once per year minimum) servicing and calibration of weld strength is vital to safe use of the SCD.

Platelet agitator

A platelet agitator is a device designed to fulfil the need for PC to be agitated during storage and is an essential part of the equipment in a modern component’s laboratory. The best mixing action is provided by a machine that moves a flat tray/shelf in a gentle horizontally oscillating motion (side to side) at approximately 60 cycles per minute.

Other types, which rotate end over end, or in an elliptical action, are considered to have too robust an action and are not ideal. Figure 9 is a sketch of a small platelet agitator.
The oscillating tray/shelf is made of mesh or stainless steel sheet with multiple holes punched through it so that when a PC bag is placed on it, air can circulate all around the bag. This helps to fulfill the need for platelets to exchange gas through the walls of the special bags used for platelet storage.

Another requirement for PC storage is that they be held at a controlled temperature of 22°C ± 2°C. Platelet agitators that are built into a temperature-controlled cabinet are ideal, but if the agitator is operated in a room where a controlled temperature is maintained (and recorded) they are not essential. See Platelet Concentrate, General Information later in this section for more on platelet storage requirements.

A strict cleaning routine according to the manufacturer’s specifications should be in place to ensure that the platelet agitator (in particular the oscillating shelves) is clean and free of bacterial growth. Records indicating the frequency of cleaning and personnel responsible should be kept.

Checks need to be in place to ensure that temperature and oscillation requirements are being continually met, and these should be recorded and reviewed regularly by a senior technologist.

Plasma snap freezer

The main aim of freezing fresh plasma is to preserve the clotting factors (most notably factor VIII). During freezing, pure ice is formed and the solutes (salts) in the plasma are concentrated in the remaining water. If the plasma freezing is carried out in a conventional deep freeze, it could take up to 24 h (or longer) before the plasma is solidly frozen. Under these conditions, the factor VIII molecules are exposed to a high concentration of solutes for a prolonged period and become inactivated. This damage to clotting factors is avoided when the plasma is frozen rapidly to a core temperature below -30°C in less than one hour from the time the freezing process commences (e.g. -32°C core temperature in 45 min would exceed the requirement).

A variety of machines that can achieve these specifications are commercially available:

- Blast freezers blow super-cooled air over the product that is placed in special cassettes to give the plasma units a uniform flat shape.
- Plate freezers hold plasma units between two super-cooled plates to achieve freezing and uniform flat shape.
- Some devices use liquid that is super-cooled mechanically or by the addition of dry ice (solid carbon dioxide). Care must be taken to ensure that the liquid used cannot penetrate the container or that the container is not in direct contact with the coolant liquid.

In all instances, the snap-freezing system must be validated to ensure that it can achieve the desired freezing rate. Every run should be recorded, and temperature and time data collected to ensure that the machine is operating within specifications.

Low-temperature freezer

A freezer capable of cooling to temperatures as low as -80°C is required for cryopreserved (frozen) blood storage. See Red cell concentrate, cryopreserved later in this section. These freezers are usually set at approximately -70°C in order to ensure that the frozen blood units are constantly maintained at a temperature lower than -65°C, and that there is reserve cooling capacity. Section 12: Blood storage and transportation, provides more information about storage freezers.

Laminar flow cabinet

On rare occasions it may be necessary to perform a blood processing operation that cannot be made in a closed system or by the use of a SCD (e.g. addition of wash solutions from a bottle or preparing bottles of plasma for freeze-drying). In this event, every precaution should be taken to ensure that bacteria present in the air do not enter the bag.

A laminar flow cabinet provides an enclosed clean area that is kept clean when not being used by ultraviolet (UV) radiation. When the cabinet is being used, the UV light is switched off and air, sterilised by being passed through a series of filters capable of removing bacteria, is pumped into the cabinet creating a steady flow towards the operator. This positive pressure prevents micro-organisms from the environment surrounding the laminar flow cabinet from entering. The operator wears protective clothing and
sterile gloves and works through the restricted space in front of the working surface of the cabinet. This reduces the likelihood of contamination of the product being processed, by the operator. Figure 10 is a diagram to show a laminar flow cabinet.

At the end of an operation the area under the hood and the working surface is cleaned thoroughly with a bactericidal agent and closed. The UV light is then switched on and remains on to inhibit bacterial growth until the hood is required again. The use of laminar flow may be needed in some processing laboratories but generally it is not an essential piece of equipment because multiple blood bags and sterile connecting devices, to maintain the closed system, are readily available.

Preparation of specific blood components

Blood components can be made in many different ways, which depend largely on the requirements of the blood service and availability of resources (donors, personnel, disposables, funding and space). The following explanations are not designed as standard operating procedures, but to provide students with an overview of each component and outline the processing methods (Fig. 11).

Whole blood

Whole blood is the source material for blood component preparation and if not processed any further maintains various properties for different periods of storage time at 4°C ± 2°C. For example, red blood cells retain their oxygen-carrying capacity for the entire duration of the storage period, labile plasma factors (particularly factor VIII) will decrease in concentration after the first 24 h of storage, and platelet counts will decrease but may retain haemostatic function for up to 2 weeks.

Red cell concentrate (RCC) in plasma

Red cell concentrate in plasma is prepared by removing part of the plasma from centrifuged whole blood. Enough plasma is removed to increase the Hct to between 0.65 and 0.75, and white cells and platelets remain with the red cells. This is a simple separation of whole blood that is usually collected into a double bag.

Red cells in plasma are used for replacement of blood or red cell loss. As white cells are not reduced and the storage medium for the red cells is not improved using additives, they do not offer much advantage over whole blood other than reduced volume in the transfusion. However, the separated plasma may be used as another component or forwarded to a fractionation facility.

Red cell concentrate, buffy coat (BC) removed, in additive solution (RCC, leucocyte-reduced)

For more processing options in the provision of components, blood collected into triple or quad bags is preferred.

These systems typically include an attached bag containing approximately 100 ml additive solution. Additive solutions from different suppliers may include sodium chloride, adenine, glucose, mannitol, citrate, phosphate or guanosine dissolved in water in differing combinations and amounts.

Some additive solutions have brand names, and, in some areas, personnel may refer to these solutions using these names, or they can simply be named according to their composition (e.g. SAGM contains saline, adenine, glucose and mannitol).

The component is derived from whole blood by centrifugation and removal of plasma and BC and the subsequent resuspension of the red cells in nutrient additive solution. The actual volume of additive in the concentrated red cells varies depending on the volume of whole blood collected from the donor (e.g. 500 ml collection = 111 ml SAGM; 450 ml collection = 100 ml). The
amount of the solution added is designed to give a final product haematocrit of 0.5–0.7 to facilitate good infusion flow rates and easy administration.

Removal of the BC creates a product with less than $1.2 \times 10^9$ leucocytes per bag, and this is considered to be leucocyte-reduced (not to be confused with leucocyte-depleted, which is a filtered product and has even fewer leucocytes, typically less than $1 \times 10^6$).

The main advantages of BC removal include:

- Microaggregate formation during storage is greatly reduced (compared to whole blood or RCCs stored with BC).
- The incidence of recipient febrile reactions is reduced.

Storage in additive solution can support red cell viability and function if stored at 4°C ± 2°C for up to 42 days from the date of donation.

**Red cell concentrate, filtered (RCC, leucocyte-depleted)**

Red cell concentrate with extremely low white cell counts of less than $1 \times 10^6$ per unit are essential in the treatment of patients known to have leucocyte antibodies and to prevent alloimmunisation to leucocyte antigens in patients where transfusions are likely to be ongoing. The use of leucocyte-depleted red cells is considered an acceptable method to prevent the transmission of CMV.

In recent years many blood services (and indeed countries) have adopted a policy of universal leucodepletion of all cellular products in order to reduce febrile transfusion reactions, HLA alloimmunisation, CMV transmission, and possibly the risk of transmission of variant Creutzfeldt-Jakob disease (vCJD).

Leucocyte-depleted red cells can be prepared in a number of ways depending on the local requirements, policy adopted and available finance. Table 1 shows several ways in which blood services meet demand for filtered (leucocyte-depleted) RCCs.

**Prestorage filtration** means that the unit of blood is filtered as soon as possible, preferably within 48 hours of donation. Blood services applying this policy have usually decided to carry all, or part, of their stock as filtered RCCs and are able to supply the product routinely when ordered.

**On-demand filtration:** A blood service may apply an on-demand policy, filtering units only when requested. This has obvious cost-saving advantages as filters are expensive and there is little wastage because of expiry.

**In-line filter systems** are supplied with a filter built into the closed system to ensure sterility and ease of operation. The system may be designed to filter the whole blood donation prior to further processing or to filter the RCC after removal of the plasma and BC.

**Add on filter systems** involve a separate bag with integral filter that is attached to the unit to be filtered by pushing the spikes (cannulas) into its port or by connecting it using an SCD.

If the connection is made using an SCD then the shelf life of the component is unaffected. Other methods require that the product is used within 24 h of the ‘add on’ when stored at 4°C ± 2°C.

**Prestorage filtration has several major advantages:**

- Filtration is carried out when the unit is still fresh and white cells still intact.
- White cells are removed before they fragment and release micro-organisms into the plasma.
- White cells are removed before they release cytokines that can cause immune modulation in a recipient.
- Personnel, who are trained and certified competent, perform the procedure.
- Good laboratory practice (GLP) can be applied.
- Quality control of product is better. It is easier to collect quality testing samples from a representative number of filtered units when the process is performed in a processing laboratory. Samples are also taken into the correct test tubes after thorough mixing of the product.
- There is little storage lesion and red cell expiry time is unaffected.

**Prestorage in-line products are used in two ways to filter blood:**

1. A filter is located in the tubing between the whole blood donation bag and a second transfer bag. On receipt in the component’s laboratory, the whole blood is filtered into the transfer bag, which then becomes the new primary container of filtered whole blood. Red cell and plasma components made from this filtered whole blood are leucocyte-depleted. Most

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Table 1 Options for filtration of red cells prior to transfusion

| Prestorage filtration | On-demand filtration |
|-----------------------|----------------------|
| **In line** | **In line** | **Add on** | **Add on** | **Add on** | **Add on** |
| Whole blood prior to processing | RCC during processing | Sterile weld | Processing laboratory | Blood issue laboratory | Bedside filtration |
whole blood filters also remove platelets, so the method is unsuitable for making BC platelets.

(2) A filter is located in the tubing between the primary bag and an additional attached transfer bag. On receipt at the components laboratory the whole blood is processed as usual into components (RCC, plasma, BC). The BC reduced RCC is then filtered through to the additional transfer bag that is then appropriately labelled leucocyte-depleted RCC.

Prestorage add on systems require a sterile transfer bag with a leucocyte filter in line. This is sterile welded to a BC reduced RCC prepared from top and top or top and bottom bags in a routine separation procedure. The red cells, generally after adding an additive solution, are then allowed to flow through the filter and into the transfer bag, which must be correctly numbered and labelled as leucocyte-depleted RCC.

A blood service may benefit from the flexibility of this system. With proper management, selected numbers and blood groups can be processed with little wastage resulting from expiry.

On-demand systems generally meet the need for filtration in situations where prestorage products are not available. On-demand filtration has several disadvantages:

- The unit can be at any stage of its shelf life at the time of filtration.
- A larger number of personnel in various laboratories and hospital wards need to be trained to perform the procedure.
- It is likely that personnel performing the task do so only infrequently, and competency may be reduced as a result of lack of practise.
- GLP is difficult to apply to a procedure performed in multiple centres.
- Taking the required number of quality testing samples in the correct way to obtain accurate counts is difficult if not impossible in some areas.
- White cell fragmentation occurs as a result of extended storage prior to filtration.

On-demand processing of filtered red cells in the Components Laboratory is the most practical way to supply the component when usage is low and only occasionally requested. In this instance the technologists who are most familiar with component production perform the filtration in a laboratory setting where GLP may be applied. Quality testing is possible because personnel are familiar with quality testing requirements and how to take the samples for testing. Connection of the filter/transfer bag can be achieved using an SCD (expiry date of component therefore unaffected) or by using the cannula (open system – expiry reduced to 24 h when stored at 4°C ± 2°C).

On-demand processing by blood bank technologists in a crossmatching laboratory may be practical in some situations. Connection of the filter/transfer bag is likely to be made using the cannula just before issue (open system – expiry reduced to 24 h when stored at 4°C ± 2°C). As the number of individuals performing the procedure is limited to blood bank trained personnel, there is some quality control and measure of GLP. It is likely that personnel performing the task do so only infrequently and competency may be reduced because of lack of practise.

On-demand bedside filtration is achieved by using a transfusion set with an in-line white cell filter, and the red cell product is filtered as it is being transfused. The blood service is not involved in the filtration process, training or quality control as this responsibility has shifted to the hospital personnel responsible for setting up the transfusion.

Red cell concentrate, paediatric

Neonates and very young children have a smaller circulating blood volume, generally higher haematocrit values, reduced metabolic capacity and immature immune systems. These factors need to be taken into consideration when preparing components for their use.

A paediatric dose of RCC is prepared either from RCC with BC removed, or filtered RCC by dividing the adult unit into quantities suited to the smaller patient’s needs (usually from 25 to 100 ml).

Additional bags may be built into the blood bag system used for the collection to ensure that sterility is maintained during transfer of red cells (closed system). To use this method the need for the product must be identified at the outset and the special bag must be committed to the process at the time of blood collection. For this reason, it is more common to make paediatric units from stock blood by welding on additional bags using an SCD.

Premature infants are particularly susceptible to CMV infection. The use of filtered RCC, when preparing paediatric units minimises the risk of CMV transmission.

Care must be taken to ensure that separated paediatric doses of RCC are identified with unique numbers that can be clearly traced to the original donation.

When a paediatric patient requires blood over several days, all the split units should be reserved for that patient to limit the number of donor exposures received. The greater the number of donations used to provide components, the greater the risk of exposure to TTIs and also to donor-derived antigens.

Red cell concentrate, cryopreserved (frozen)

When frozen, the shelf life of red cells can be extended up to 30 years if the units are correctly protected during freezing, and ultra-low freezing temperatures (colder than −65°C) are maintained throughout the entire storage period.
Damage to red cells if frozen without protective solutions:

- Red cell dehydration: This occurs when extracellular water freezes before intracellular water, creating a difference in osmotic pressure between red cells and their surroundings, and resulting in intracellular water diffusing out of the red cells, collapsing them.
- Intracellular ice: The red cells rupture from mechanical trauma caused by ice crystals that form inside the cells.

The most commonly used cryoprotective solution is a high concentration of glycerol (40%). Other protective solutions such as low concentrate glycerol and hydroxyethyl starch are less frequently used and are not described. Glycerol consists of a small molecule that can cross the red cell membrane, and this is known as a penetrating cryoprotective agent. After entering the red cell, it provides an osmotic force that will stop the migration of water from the cell as ice is formed outside it. The high concentration of glycerol also prevents the formation of intracellular ice crystals and thus prevents cell membrane damage.

Frozen storage of red cells is used mainly to preserve units of blood with rare blood types. It can be used to stockpile blood for emergency use in disasters, but the high cost and short shelf life after recovery from the frozen state make it impractical as a routine tool to manage inventory.

Procedures for glycerolisation of cells vary. Usually, all plasma and/or additive solution is removed from whole blood or RCC within six days of collection. The glycerol solution must be added slowly with adequate, constant mixing to allow equilibration of solution and cells. Failure to equilibrate during addition results in a high degree of cell damage that is seen as excessive haemolysis and poor red cell recovery when the unit is thawed.

The original blood collection bag can be used for frozen storage, but the volume capacity is rather small to facilitate mixing of cells and glycerol so it may be preferable to use a larger volume bag (800 ml). Some polyvinyl chloride (PVC) bags can cause cellular damage during the freeze/thaw process so these larger bags are usually made of polyolefin plastic that minimises cell damage. Polyolefin bags are also less brittle when frozen to the very low temperatures required and are less likely to break during storage and transportation.

Donations destined for freezing can be collected into special primary packs with these specifications, but it is also possible to connect a suitable freezing bag to any stock donation.

Automated equipment and disposables that perform the entire freezing and thawing operation in a closed system are available.

Notes on freezing and thawing technique

- Red cells prepared with a final glycerol concentration of 40% (weight to volume) are placed in metal or cardboard protective canisters to minimise breakage during the long period of frozen storage during which they might be handled or transported several times.
- The canisters and units are placed in a mechanical freezer capable of operating at −80°C. Although the freezer can run at this temperature, it is more likely to run at about −70°C to maintain the temperature of the cells below the required −65°C.
- If temperatures are maintained continuously below −65°C the units have an expiry date set at up to 30 years from date of donation.
- Most donations that are stored frozen are rare blood types, so when this type of donation reaches expiry it is not discarded. Scientific evidence indicates that cell recovery and viability from units stored for up to 21 years is acceptable, so if a frozen rare donation is required after its official expiry, it may be used as long as the reasons are clearly documented (e.g. no frozen units less than 10 years in storage and no other compatible donations or donors available).
- When frozen cells are to be recovered (deglycerolised) the unit is thawed with gentle agitation at 37°C for about 20 min.
- The thawed cells contain a high concentration of glycerol that should be removed gradually by washing with sterile saline solutions of decreasing osmolality to avoid red cell haemolysis.
- Using a typical three wash procedure, the cells are diluted with 12% saline for the first wash, with 1.6% saline for the second wash and finally with 0.9% (normal) saline for the third wash. After the addition of each wash solution and gentle mixing, the bag is centrifuged, and then the supernatant removed from the red cells and discarded. The solutions are always added slowly with mixing and plenty of time for osmotic equilibration.
- Whatever technique is used it should be properly validated for local conditions, and the final product should be free of cryoprotective agent, show minimal signs of haemolysis and yield at least 80% of the cells originally frozen.
- The freeze/thaw process involves adding solutions and extra bags and as this is usually performed using an open system it is carried out using a laminar flow cabinet. The red cells have a post thaw shelf life of 24 h at 4°C ± 2°C.
- Automated equipment, disposables and solutions that perform the thaw operation in a closed system are
commerically available and provide a post thaw shelf life of up to 14 days at 4°C ± 2°C.

Red cell concentrate, washed
Washed red cells are a specialised component prepared for patients with antibodies to plasma protein (e.g. anti-IgA) and those who have severe allergic reactions when transfused with blood products. This condition is uncommon, and components laboratories are not often asked to provide the product.

Washed red cells are usually prepared by further processing of RCC, BC removed, in additive solution. Some blood services use filtered RCC as the starting material.

- Approximately 250 ml of cold (4°C ± 2°C), sterile, isotonic saline is added to the RCC and the contents gently and thoroughly mixed. The saline bag can be attached to the RCC bag using an SCD and in this way it is possible to maintain a closed system throughout the process. If saline is added by making connections using the spikes (cannulas) on transfer bags and transfusion sets, then the connections should be made under laminar flow conditions, and the expiry time of the product reduced to 24 h provided that temperature is kept between 4°C ± 2°C.

After centrifugation in a refrigerated centrifuge set at approximately 4°C, the supernatant saline is removed and discarded. This is referred to as the first wash. The wash process (of adding saline, mixing and centrifuging) is usually repeated three or four times, with each wash resulting in the removal of more plasma protein from the product and finally producing washed red cells suspended in saline with less than 0.5 g protein per unit.

The technology is available to perform this wash procedure automatically but would require quite a large demand for the product to justify the cost of machine and disposables.

Plasma

General information
The first step in component processing is to remove the plasma from a centrifuged unit of whole blood. Fresh plasma contains proteins such as albumin, coagulation factors (most notably FVIII), and immunoglobulins. It is not practical to store plasma in liquid form as some fractions deteriorate rapidly, even if stored at refrigerator temperature (4°C ± 2°C). Liquid plasma stored for more than a few days at this temperature is suitable only as a blood volume expander. To preserve labile fractions, plasma must be stored frozen and then becomes known as fresh frozen plasma (FFP). If blood being donated is to be processed into FFP or cryoprecipitate, the time taken to make the donation should not be longer than 15 minutes as poor flow during donation leads to consumption of clotting factors.

Fresh frozen plasma is used therapeutically or is a starting material for the preparation of plasma derivatives in a fractionation facility. See Overview of plasma fractionation later in this section for more detail.

- Plasma must be frozen within a maximum of 24 h from time of collection.
- From the time of donation to the time of freezing, the donation must be kept at 22°C ± 2°C. For details on storage, refer to Section 12: Blood storage and transportation.
- The time taken to freeze the plasma to a core temperature colder than −30°C must not exceed 1 h from the time freezing is commenced. Core temperature refers to the temperature in the centre of the unit – the warmest part of the plasma pack during the freezing process. See Plasma snap freezers earlier in this section for more detail.

Fresh frozen plasma
Fresh frozen plasma for therapeutic use is prepared by snap-freezing the plasma as soon as practical after collection. Specifications require that this time should not be longer than 24 h from time of collection.

Once frozen, units are best stored at temperatures consistently colder than −18°C, and under these conditions have an expiry date of 12 months after date collected in the US and Canada. In Council of Europe regulations, plasma stored at −18°C has an expiry date of 3 months, while plasma stored at −25°C has an expiry date of 36 months.

Fresh frozen plasma is often stored in cardboard or polystyrene protective containers that minimise the risk of breakage of the brittle frozen product during storage, handling and transportation.

Labelling of frozen bags of plasma is difficult because stick-on labels will not adhere securely. Units are either labelled before freezing, and a rigorous checking system put into place to verify safe donations once TTI testing results become available, or donations are labelled using tie-on tags.

In all instances the use of computer records to identify safe donations is essential. Manual tracking of donations in a quarantine system is not recommended.

Plasma for fractionation
Plasma not used therapeutically may be supplied to a fractionation facility for the extraction of clotting factors, albumin and immunoglobulins. See Overview of plasma fractionation.
fractionation later in this section for more detail. Source plasma refers to plasma collected for further fractionation.

Fractionation facilities require bulk lots consisting of several thousand units of plasma frozen within 24 h of collection. Fresh plasma is preferred, as albumin, immunoglobulin and clotting factor concentrates can be made from the same pool.

Plasma that is separated and frozen after the deadline of 24 h from collection is considered to be ‘outdated’ plasma. Separation of this type of plasma may continue for up to 1 week after the expiry date of the whole blood provided that it was stored within the prescribed temperature range (4°C ± 2°C). The outdated plasma is not used for fractionation.

Any supplier of plasma as a raw material to a fractionation facility must comply with legislation and the fractionator’s guidelines. See Overview of plasma fractionation later in this section for more detail. The records of plasma forwarded to a fractionation facility must be supported by a comprehensive data capture system to ensure quality and traceably of every unit in the batch.

Freeze-dried plasma

A suitable product for use in outlying rural hospitals and emergency rooms can be manufactured using a process of freeze-drying (lyophilisation). By this method the clotting factors present in fresh plasma can be preserved in powder form and stored at ambient temperature (usually at 25°C or less). A measured amount of distilled water (the same amount as was lost during drying) is added to reconstitute the powder just before use.

In the process of lyophilisation fresh plasma is transferred to a glass bottle under laminar flow conditions and then frozen by rolling the bottles in a bath of alcohol and dry ice, which when mixed, results in a temperature of colder than −60°C. This process is called ‘shell freezing’ as the plasma freezes in a thin layer over the inner surfaces of the bottle so that a large surface area is made available for evaporation when the product is in the process of being dried.

After freezing, each shell frozen plasma bottle has its rubber bung replaced with a sterile vapour-permeable membrane, also under laminar flow conditions. The bottles are then loaded onto the shelves of a refrigerated freeze-drying machine, the door is sealed, and a vacuum is created in the chamber. In the presence of a vacuum, the plasma releases its water in the form of vapour, without passing through a liquid phase — the product does not melt. This process of sublimation (changing from a solid directly to a vapour) is called lyophilisation. The drying process is accelerated by mildly heating the shelves on which the bottles are placed. The vapour migrates from the plasma shell, through the permeable membrane positioned over the neck of the bottle, to the coldest area in the freeze-drier (the condenser) where it forms ice again.

Freeze-dried plasma in single donation units is not as commonly used as in the past. It has been superseded by pooled fresh plasma that is solvent/detergent treated and dried in a fractionation facility.

Cryoprecipitate

When the plasma of freshly donated blood is frozen shortly after collection, and later slowly thawed at 4°C ± 2°C, a white precipitate (called cryoprecipitate) may be seen in the plasma. This cryoprecipitate contains most of the factor VIII, von Willebrand factor and fibrinogen that was present in the original fresh plasma. After hard centrifugation at a temperature of 4°C ± 2°C (the precipitate will go back into solution if the plasma is warmed) the precipitate is concentrated in the bottom of the plasma bag. The cryoprecipitate-poor (‘cryo-poor’) supernatant plasma is then removed into an attached bag leaving only about 40 ml for resuspension of the cryoprecipitate. This product is also called wet cryoprecipitate or ‘wet cryo’.

Cryoprecipitate isolated from fresh plasma may be stored frozen for up to three years at temperatures colder than −25°C or for three months at temperatures of between −18°C and −25°C. Alternatively, it may be freeze-dried and stored for at least 1 year.

Platelet concentrates

General information

Platelets may be made by two main methods: random donor (or recovered) platelets refer to individual or pooled platelet products made from whole blood donations, while single donor platelets are made from one donor using apheresis technology.

If whole blood is being donated for the preparation of PC, the time taken to give the donation should not take longer than 12 min as poor flow during donation leads to consumption of platelets. Blood should be collected only from individuals who have not taken aspirin in the previous 72 h, since platelet function is adversely affected.

Whole blood used for the preparation of platelets should not be refrigerated, which would initiate clumping of platelets and reduce their functionality. Suitable donations should be stored in conditions validated to cool donated blood to 22°C ± 2°C as rapidly as possible after donation and then maintain this temperature range for up
to 24 h. This is usually achieved using an insulated transport box and coolant packs (or plates) filled with a specially selected coolant that has an appropriate melting point (e.g. butane diol with a melting point of 20.1°C).

A standard adult therapeutic platelet dose is derived from four to six whole blood donations. The yield of platelets from a single donation of whole blood (approximately 50–70 × 10^9) can be recovered and concentrated in a small volume (50–60 ml) by centrifugation using various techniques, and then administered to the patient in a total volume of 250–300 ml.

Platelet yield is the total number of platelets present in the final storage bag and is calculated using the platelet count per litre and adjusting it according to the total volume in which the platelets are suspended.

Platelet concentrate may be issued for therapeutic use as individual units but are more likely to be pooled into a single bag to provide the therapeutic dose in a convenient package for easier administration. When pooled in the components laboratory the connections are performed using an SCD and product sterility and expiry time are not compromised. It is also possible to use a leucocyte filter during pooling and provide filtered (leucocyte-depleted) PC pools.

Once the PC has been prepared, the storage conditions required to maintain viability and haemostatic activity are very different from other components:

- The PC must be continually stored at a controlled temperature of 22°C ± 2°C. This temperature is best maintained in a temperature-controlled room or cabinet. Though this is the best temperature for platelet storage, it is also a temperature that will encourage rapid growth of bacterial organisms that may contaminate the donation from the phlebotomy site or the donor's circulation. For this reason, the maximum storage period for PCs is 5–7 days, depending on the licensure of the specific bag and storage solution, and the use of bacterial detection methods.

- The PVC bags used for platelet storage differ from collection and transfer bags in that they are made from special plastics that are permeable to gases and guarantee availability of oxygen to the platelets, thus preventing a drop in pH. The bags are large; a bag of approximately 1000 ml is used for the storage of 300 ml of pooled platelets, to enhance oxygen transfer over a bigger surface area. The amount of oxygen required depends on the number of platelets in the bag – more platelets need more oxygen. Bag manufacturers usually give guidelines on the capability of their products.

- During storage, gentle and continuous agitation on a flatbed agitator rotating at approximately 60 cycles per minute is essential to prevent clumping of the platelets and to enhance oxygen transfer.

- the trays/shelves of the agitator should allow airflow to the underside of the bag (i.e. perforated or meshed).

- PCs must be placed individually on the rotator and not on top of one another as this inhibits airflow.

- the size and number of labels stuck on the platelet bag should also be kept to a minimum so as not to restrict airflow.

Methods of preparation: platelets

Figure 10 illustrates the production of whole blood-derived platelets by the platelet-rich plasma (PRP) method used in the USA, and the buffy coat method used in most other countries

Whole blood-derived platelets, Platelet-rich plasma method

A unit of whole blood is centrifuged at a speed and time validated to maximise the number of platelets and minimise the number of red cells and leucocytes in the plasma. This is the critical step in the production of PC by this method.

The centrifuged bag is then placed in a plasma extractor, or automated blood component extractor, and the platelet-rich plasma (PRP) is slowly expressed to an attached transfer bag. To minimise red and white cell contamination of the final product, great care must be taken not to disturb the interface between the red cells and plasma when loading, and the separation should be slow and stopped when about eight millimetres of plasma remains above the red cells.

The platelets in the PRP are sedimented to the bottom of the transfer bag by quite hard centrifugation (higher rpm or g for longer time). However, if the second centrifugation is too hard, then the platelet button formed in the bottom of the bag will not disaggregate. Care needs to be taken in selection of the correct centrifuge setting (see Principles of centrifugation earlier in this section). The supernatant platelet poor plasma is removed into an attached transfer bag leaving 50–70 ml of plasma with the platelet button.

Resuspension of the aggregated platelets can be difficult if the proper procedure is not followed. The bag must be allowed to rest undisturbed for between 1 and 2 h before gentle mixing by agitation on a flatbed agitator is commenced. Attempting to speed up the resuspension by manual manipulation of the platelet button is not recommended as this may cause irreversible aggregation.
After resuspension, four to six units of ABO-identical PCs may be pooled to make an adult therapeutic dose. In this process the PC bags are joined to a large volume platelet storage bag using SCD connections or by spiking the ports using laminar flow. SCD connections are preferable because the pool has the same expiry time as the individual units (5 days), whereas spiked pools are an open system and should therefore be used within 6 h of pooling.

As the first centrifugation is quite moderate, this method does not maximise the volume of plasma harvested from a donation. Removal of BC from an RCC and addition of red cell additive solutions is also more difficult when platelets are derived by this method, because the interface is not sharply defined.

**Whole blood-derived platelets, Buffy coat method**

Whole blood units stored for less than 24 h at 22°C ± 2°C, are centrifuged at a speed and time validated to sediment the platelets into the BC layer with the white cells. This centrifuged whole blood is usually separated using automated equipment but may be performed manually.

*Buffy coats can be prepared by one of two methods:*

1. **Top and top quad bag method**
   During separation of the centrifuged whole blood, all but a small amount of plasma is transferred to an attached bag and the BC, together with a small amount of plasma, is transferred to another attached bag, leaving the red cells in the original primary bag.

2. **Top and bottom bag method**, illustrated in Figure 12
   During separation of the centrifuged whole blood, all but a small amount of plasma is transferred to a bag attached to the top and the red cells transferred to another bag attached to the bottom leaving the BC (with the small amount of plasma) in the original primary bag.

Prepared BCs are stored at 22°C ± 2°C without agitation until they are further processed into PCs, preferably within 48 h of donation. The BCs can be processed as single or pooled units:

**Single unit PC from buffy coat**

The volume of plasma left in the BC should be 50–60 ml to ensure that the final product has sufficient volume to suspend the platelets. The BC bag should have an additional bag attached that is suitable for platelet storage. This bag may be an integral part of the bag system or added using a sterile connection.

After thorough but gentle mixing the BC is centrifuged at a speed and time validated to sediment the residual red cells and white cells whereas the platelets remain suspended in clear plasma supernatant. This centrifuge setting is critical to the successful production of PCs containing a large number of platelets with a low white and red cell count.

Because the volume of BC is very small, the bag should be supported vertically in a centrifuge bucket insert to ensure that there are no folds to trap pockets of cells. There should be a distinct interface between residual red cells and platelet-rich plasma after centrifugation. A special plate, with hooks to hang the bag, or packing the bucket insert with dummy bags, are the usual means of supporting the PC at the time of centrifugation.

After centrifugation the clear supernatant plasma containing the platelets is transferred to the PC storage bag. The BC bag containing the sedimented red and white cells is discarded. The PC is stored on a flatbed agitator. After suitable labelling the unit is ready for transfusion.
Pooled PC from buffy coat

Four to six BCs of the same ABO blood group may be pooled together before centrifugation. BCs used to prepare these pools are approximately 50 ml each, with a haematocrit of 0.4–0.5.

The pool is diluted either with plasma (from one of the BC donations), or with platelet additive solution (PAS). Although many different formulations of PAS are available, they are all designed to maintain pH and platelet viability better than plasma, and more plasma is therefore available for therapeutic use.

BCs may be pooled using a pooling kit or the chain method.

1) Pooling kits

- Pooling kits may be achieved using commercially available pooling kits, as illustrated in Fig. 13. Using an SCD, BC bags are welded individually to one of the multiple tubing ends of the pooling kit. The plasma or PAS is attached to a further tubing end.

- The BCs are drained into a 600 ml transfer bag. Ultimately the contents of each BC bag plus approximately 300 ml PAS or plasma are pooled into an attached transfer bag with a total liquid volume of approximately 600 ml.

- The pooling bag has an integrally attached platelet storage bag, and, if required, an in-line platelet filter may be included to provide a filtered pool.

The bag of pooled BCs (prepared using either the pooling kit or the chain method), with platelet storage bag attached, is centrifuged at a speed and time validated to sediment the red cells and white cells, leaving the platelets suspended in clear supernatant (plasma or PAS). The centrifuge setting is critical to the successful production of PCs with high numbers of platelets and low white cell and red cell counts. The centrifuged pool is carefully moved so as not to disturb the interface and is placed in a plasma extractor or automated blood processing device. Pressure is applied and the platelet-rich supernatant is transferred to the platelet storage bag. The transfer can be through a platelet filter or not depending on requirements. The BC bag containing the sedimented red and white cells is discarded.

During separation and pooling procedures critical labelling errors can occur. It is vital that strict checking procedures, that are preferably computer assisted, are in place to ensure that the identification link to the original donation is maintained for each unit in the pool.

Apheresis platelets (single donor platelets)

PC may be obtained by platelet apheresis of a single donor using automated cell separation equipment. In this procedure whole blood is removed from a donor and the apheresis machine harvests the platelets. All other components are returned to the donor, so he/she can return to donate again more frequently than a whole blood donor. The platelets collected may be leucocyte-depleted by additional centrifugation or filtration as part of the collection procedure.

Apheresis platelets can be stored in plasma or in a mixture of plasma and PAS. The bags used for storage and the temperature and movement conditions during storage are the same as for pooled platelets. Generally, the apheresis procedure is a closed system, and the product has a 5-day shelf life at 22°C ± 2°C.

The yield of platelets obtained from a single donor can vary depending on the donor, type of machine, and procedure used but is equivalent to between three and 13 random donor PCs.

Dividing apheresis platelet donations

The yield of platelets collected from a single apheresis procedure may be too large for storage in one storage bag, and can be divided to provide two (doubles) or three...
(triples) adult platelet doses. Most platelet apheresis collection kits have two storage bags that allow for the product to be split and stored with adequate oxygen exchange. Often the yield of platelets in each bag is sufficient to provide a standard adult therapeutic dose (approximately \(240 \times 10^9\) per pack, not less than \(200 \times 10^9\)) and each bag may be used for a different patient.

In other instances, the second bag may not be sufficient for an adult therapeutic dose and may be further split into smaller units suitable for paediatric use (more than \(60 \times 10^9\) platelets in 40–60 ml plasma or PAS per bag).

Quality control for platelet concentrates

- Quality control (QC) testing should conform to local or international standards that set guidelines for the number of units that need to be tested, and the specification range for volume, platelet content, white cell content and pH.
- Samples for QC can be taken from fresh product an hour after completion of processing using an attached sampling pouch (welded or integral). This testing does not waste product and provides early assurance that production is within specification.
- Some QC testing of expired units is essential to monitor pH and platelet yield per pack after storage. Testing of expired units should not be carried out after day six, and the correct storage temperature range and agitation must be maintained until samples are taken.
- It may not be possible to test every PC produced, so it is essential to keep daily records of QC testing and compare results over time to identify trends.
- A very simple non-invasive visual test can be performed on every PC at issue. By holding the PC up to a light source behind it and moving the contents in a circular motion with the thumb, a swirling phenomenon can be observed. This is based on light being scattered by platelets with normal morphology (discoid), and would be poorly, or not at all visible in units with only a few platelets, or with damaged platelets (spheres).
- Various strategies are used to test for bacterial contamination, including culturing a sample of all platelet concentrates, and/or performing a rapid test closer to the time of transfusion.

Irradiated products

Blood components that may contain viable lymphocytes could initiate graft vs. host disease (GvHD) in a recipient given the following circumstances:

- Recipient on immunosuppressive drugs.
- Neonate (poorly developed immune system).
- Recipient with immunodeficiency.
- Intrauterine transfusion.
- Component for transfusion is from close family member of recipient.

GvHD is an immune condition that occurs after transfusion when immune cells in the transfused blood (i.e. the graft) attack the tissues of the patient (the host). In these conditions listed above the patients are immunocompromised and may not recognise foreign viable lymphocytes in the transfused blood that then proliferate in the host (patient) usually with fatal results.

Lymphocytes exposed to 25–50 Gy of ionising radiation (Gy, i.e. gray, is a unit of measurement for ionising radiation) will be rendered non-viable, whereas other components (red cells, platelets) are not significantly affected.

Irradiation is accomplished in blood irradiators designed specifically for this purpose or in a hospital radiotherapy unit. Protocols describe the procedure and standardise exposure time. Periodic validation of the exposure source is essential.

Radiation sensitive labels are commercially available and if attached to a unit being irradiated will indicate, by change of colour, that the process has been successfully completed.

Red cell products to be irradiated should be less than 14 days old and have a maximum of 28 days shelf life.
Measures to prevent transmission of pathogens

Pathogens are defined in this publication as micro-organisms with the potential to infect recipients of blood products or plasma derivatives, and include bacteria, viruses and protozoa. Section 8: Blood donors, and Section 10: Donation testing, explain steps in donor screening and testing to prevent pathogen transmission. There are also several steps in blood processing that contribute to reduced pathogen transmission:

- Avoiding the immediate refrigeration of donated blood so that white cells in the blood bag ingest pathogens, such as bacteria, present in the donation.
- Prestorage leucocyte filtration of blood donations prior to processing, to remove white cell-associated micro-organisms, such as CMV.
- Storage of donated blood in a temperature range of 4°C ± 2°C for four days will prevent the transmission of Treponema pallidum (syphilis) because of its susceptibility to time and temperature.
- Storing and transporting blood components within the correct temperature range to minimise deterioration and the proliferation of bacterial contaminants.

Pathogen inactivation

Despite the measures listed above, some risks remain, such as pathogens not detected by current screening tests or pathogens that are not yet identified. Pathogens may also have been accidentally introduced into the donation at the time of phlebotomy. Bacteria present in a unit of blood may replicate during storage and this may not be detectable even with bacterial culture performed at the time of preparation.

Other technologies have relatively recently been developed for inactivating pathogens in labile blood components. Pathogen inactivation is aimed at preventing infectivity by damaging the nucleic acid of the pathogen. Unfortunately, it has been shown that some pathogens, such as small nonenveloped viruses including hepatitis A and hepatitis E, are not inactivated by currently available technologies.

Inactivation technologies include the following:

- Photochemicals, such as psoralen (amotosalen) with a high affinity for nucleic acid strands that under illumination with ultraviolet A (UVA) light, induce lesions to RNA or DNA, making the pathogen unable to replicate in vitro or after transfusion. Amotosalen and UVA light-based technology are able to inactivate bacteria, viruses and protozoa in plasma and platelet products. In this way both known and unknown micro-organisms may be rendered harmless.
- Methods using photosensitisers such as methylene blue and riboflavin are also available for treating plasma (methylene blue) or platelets and plasma (riboflavin). In the presence of these molecules, the illumination with visible or UV light provokes the generation of reactive oxygen species, i.e. chemically reactive chemicals containing oxygen, that will induce lesions to the nucleic acid of the pathogen, thus preventing its replication.
- Methods, based on the addition of amotosalen and riboflavin, damage DNA, preventing replication of lymphocytes in treated blood components. Treated components should not be able to cause TA-GvHD, and there is a growing body of evidence that these products will not need to be irradiated.

Procedures for pathogen inactivation are relatively complicated and expensive and have started to be implemented in developed countries for plasma and PC. More recently, methods are being developed for pathogen inactivation of whole blood and RCC. Studies are being performed in Africa to assess the ability of these methods to reduce the risk of transfusion transmissible malaria.

For information on pathogen inactivation and removal in plasma derivatives, see Overview of plasma fractionation later in this section.

Labelling and records

The labelling of blood components should comply with the relevant legislation (local and national), as well as conforming to international agreements (e.g. for barcode generation).

A unique donation identification number that links the donation to records and test results should identify every blood bag. This kind of information can best be accurately kept and updated if stored in a computer database. All functions of the computer and the software should be validated to check that they do exactly what they are intended to do without corrupting or deleting stored data. Accuracy of computer records is dependent on the quality of the software and the quality of data input. Input errors can best be minimised (or eliminated) by the use of barcodes, which can be electronically scanned directly into the database.

The label on a blood component ready for use should contain information (preferably in eye and machine-readable codes) necessary for safe transfusion, including the following:

- Name of producer (collection organisation/blood service).
• The donation identification number should incorporate a code for the responsible collection organisation, the year of donation as well as a serial number. The number should be unique and never used again to label another blood product.
• There should be full traceability by number, from the recipient back to the donor and the collection, testing, processing, storage, release, distribution and transfusion of every single donation that was processed into components or transferred to a fractionation facility.
• The name of the component (e.g. red cells, leucocyte-depleted).
• The ABO and Rh(D) blood group.
• Titre (if applicable).
• Name and volume of anticoagulant and/or additive solution.
• Additional information such as that indicating irradiation, leucodepletion, washing.
• Volume (or weight) of component.
• Date of donation and date of expiry.
• Temperature for storage and temperature for transportation.

The blood service (collection organisation) should supply product information in a booklet and/or a product information leaflet format. This should include information about the various components that are made available to clinicians with regard to composition, indications, and storage and transfusion practices.

All blood components and plasma derivatives issued for transfusion should carry a warning that the product must not be transfused if there is any abnormality noted, such as haemolysis in red cell components, or some other evidence of deterioration, such as cloudiness in plasma or fractionated products. The warning should also state that all blood components should be administered through a 170–200 micron filter.

Quality control

Making safe and efficacious blood components requires the continual application of GLP and good manufacturing practice (GMP). For more details, refer to Section 16: Quality.

Overview of plasma fractionation

Blood services as they develop from the collection and distribution of whole blood towards a comprehensive component therapy programme inevitably will generate plasma surplus to the needs for therapeutic FFP. The more successful the component programme (higher percentage of RCC) the more plasma is potentially available for fractionation.

This can provide a means of cost recovery for the blood service, as costs incurred through recruitment, collection, processing and testing of blood are recouped through the sale of excess plasma to the fractionation facility. Large blood services can generate significant quantities of plasma for fractionation, and the opportunity to embark on ‘contract fractionation’ becomes an option to be considered. This is the process whereby the plasma is supplied to a fractionation facility, a manufacturing fee is charged to produce the fractionated products, and the products are returned to the blood service.

Plasma for fractionation is obtained either as ‘recovered’ plasma from whole blood donations, or as ‘source’ plasma from apheresis donations. If such plasma is frozen to a core temperature below −30°C within 24 h of donation, labile clotting factors may also be harvested; if plasma is frozen after 24 h of donation, labile clotting factors can no longer be extracted.

The pool of plasma used as the starting material consists of plasma from several thousand blood donations, all of which must have tested non-reactive for TTIs. The safety of plasma can be improved by employing a process of quarantining the plasma in a ‘donor retest programme’ as previously described.

As the fractionation of plasma is not the function of a blood service and is generally governed by legislation applicable to medicinal products rather than blood components, this overview does not address the complexities of this pharmaceutical process.

Requirements for supply of plasma for fractionation

Maximising the safety of starting plasma from the blood service includes the points listed in ‘Measures to prevent transmission of pathogens’ in this section. Although plasma that is transferred to the fractionation facility is considered safe and has been found non-reactive for TTIs, micro-organisms are still presumed to be present (window phase donations or organisms not tested such as hepatitis A), and every effort is therefore made to either remove or inactivate known and unknown micro-organisms.

Although inactivation or removal processes may make plasma derivatives less likely to transmit pathogens, it is critical that the starting material is tested for HIV, HBV and HCV plus other TTIs in the region, so that only plasma that is non-reactive is forwarded to the fractionation facility and pooled for fractionating. Starting pools of plasma are retested by the fractionation facility for the presence of HIV, hepatitis B and C prior to proceeding, so that only non-reactive pools are fractionated.

The complete history from the demographics of the donor base, the recruitment, selection, testing, look-back
procedures, traceability, separation, freezing, storage and transportation of plasma supplied, is generally contained in a Plasma Master File (PMF). This is prepared by the supplying blood service and is subject to audit by the fractionator to verify that the information contained in the PMF is correct and that the processes and testing procedures are GMP/GLP compliant.

Fractionation process

The most widely used method of separation is cold ethyl alcohol fractionation. This was developed by Cohn-Oncley in the USA (1940s), and Kistler-Nitschmann in Switzerland (1964). Both methods involve the addition of ethanol at varying concentrations, to a large pool of plasma while simultaneously cooling it and controlling the temperature, pH and ionic strength. The methods rely on the different solubility of the protein fractions in plasma, and their behaviour when subjected to varying concentrations of alcohol, degree of acidity or alkalinity (pH), temperature and salt concentration (ionic strength). Fig. 15 provides a flowchart of the Kistler-Nitschmann fractionation process for large pool plasma.

Cold ethanol fractionation results in the desired protein fraction being isolated, either by precipitation as a paste, or by retention in solution, while other protein fractions are precipitated. This is achieved without destroying their biological function by processing at cold temperatures so that in the final form they remain efficacious, suitable for infusion (non-toxic, non-pyrogenic) and remain stable when stored.

The yield for the different clotting factors and protein fractions is not the same for each; some are more abundant in unprocessed plasma than others, and this affects the yield, as does the actual process. Albumin is the most abundant and FVIII gives the lowest yield. The process of fractionation is time-consuming; it takes days to isolate a fraction and weeks or months before the final product is bottled and available for therapeutic use. Each derivative has a unique storage temperature and shelf life (see Table 2 for further information).

The first stage of fractionation is concerned with the isolation of coagulation factors, notably factor VIII and fibrinogen in cryoprecipitate and factor IX or prothrombin complex from the cryo-poor plasma. FFP from the blood service is control-thawed to isolate cryoprecipitate in bulk, using a process of continuous flow centrifugation. This is normally carried out prior to commencing fractionation with ethanol, because of the labile nature of the coagulation factors.

After these clotting factors have been separated, the residual plasma pool is cold ethanol fractionated to extract the predominantly protein groups: gamma globulin (immunoglobulins) and albumin. This is a sequential process using conditions that will precipitate groups of proteins that are required and may then be isolated, or that are contaminants and must be removed, or that remain in solution for subsequent precipitation into a more concentrated form (paste). Once isolated into these groups other techniques are used to ultimately produce the final products.

Other processing techniques used in the fractionation of plasma

Although cold ethanol fractionation is the foundation of fractionation, refinements in protein separation techniques have added new benefits. These include new products from the plasma or plasma fractions, improved yields, increased safety, higher purity and enhanced stability. Of these, the developments in chromatography (such as size exclusion, anion exchange and immunoaffinity) and filtration (such as membrane filtration, ultrafiltration and nanofiltration) have had the biggest impact. This has enabled a better utilisation of the plasma and plasma fractions obtained from ethanol fractionation.

The development of these techniques also facilitated the introduction of viral inactivation/removal processes...
that significantly further enhance the safety of fractionated products.

**Infection control: inactivation or removal of pathogens**

Viral inactivation techniques are only considered effective if it can be demonstrated that they are able to destroy at least one element needed for viral replication (validated inactivation). Although not all viruses may be completely inactivated or removed, what is important is that the residual viral load within the pool is reduced to a level below which it could be infective when administered to a patient.

During the manufacturing process, chemical or heat treatment steps are included to inactivate both known and unknown micro-organisms that may have been in the starting pool (from window period donations or contamination at the time of phlebotomy). Unlike blood components derived from single donations, plasma derivatives are manufactured in large batches, and every bottle or vial in the batch is the same.

Some inactivation steps are in-process during cold ethanol fractionation; others are additional processes. Depending on their physical nature, both known and unknown microbes are similarly affected by these processes. There are numerous techniques that may be used to inactivate or remove microbial agents in fractionated products, and descriptions of these are not included in this publication. However, a few notes on microbial control are included for information.

| Blood component or plasma derivative | Shelf life | Storage temperature |
|--------------------------------------|-----------|---------------------|
| Whole blood in CPDA–1               |           |                     |
| Red cell concentrate                | 35 days   | 4°C ± 2°C            |
| Buffy coat removed (leucocyte-depleted) |          |                     |
| In additive solution, also leucocyte-depleted (filtered) |          |                     |
| Washed                              |           |                     |
| Irradiated: within 14 days of donation |          |                     |
| Irradiated: within 5 days of donation for intrauterine transfusion and neonatal exchange transfusion |          |                     |
| Cryopreserved in high glycerol      |           |                     |
| Cryopreserved/thawed                 |           |                     |
| Fresh frozen plasma and cryoprecipitate | 3–12 months depending on national standards | −18°C to −25°C |
| Frozen                               |           |         |
| Thawed                               | 1–3 years depending on national standards  | Below −25°C |
| Platelet concentrate (in constant agitation during storage) | If not used immediately, 24 h | 4°C ± 2°C |
| Isolated from platelet-rich plasma in a closed system | 5–7 days | 22°C ± 2°C |
| Irradiated                           |           |                     |
| Recovered from Buffy coat in a closed system |          |                     |
| Pooled from Buffy coats              |           |                     |
| Dried factor VIII (S/D treated)      | Open system: up to 6 h | Below 25°C |
| Dried factor XI (S/D treated)        | Closed system: 5–7 days |                     |
| Fibrinogen (dried)                   | Up to 3 years depending on manufacture |                     |
| Albumin (liquid)                     |           |                     |
| Immunoglobulin, intravenous (dried)  |           |                     |
| Immunoglobulin, intramuscular (liquid) |         |                     |
|                                    |           | 4°C ± 2°C            |
In-process
- The ethanol used in cold alcohol fractionation is in itself an effective virucidal and antimicrobial agent.
- Pooled plasma is likely to contain donor antibodies (such as anti-HBs) and this assists in the immune neutralisation of viruses of corresponding specificity (such as HBV) that may also be present in the pool.
- The pooling of large quantities of plasma, the use of large volumes of water and ethanol has a dilution effect.
- The process of partitioning viruses during the precipitation of fractions and the isolation of precipitates has been demonstrated to result in significant viral reduction.
- Filtration to remove precipitates and retain the fraction in solution, such as immunoglobulin, also removes microbes.

Additional processes for plasma derivatives

Heat.
- Pasteurisation (60°C for 10 h) in the final container inactivates viruses and is used in the final stage of albumin production.
- Dry heat in the final container.
- Steam treatment of dry product in the presence of steam under pressure.

Chemical.
- Solvent/detergent (S/D) treatment disrupts the viral membranes of lipid-enveloped viruses (such as hepatitis B and C and HIV) by means of an organic S/D solution. The solvent breaks the protective lipid envelopes of the viral particles and the detergent retains the lipids in solution. S/D is then removed and the product (such as FVIII) is filtered to remove any viral debris that may be present.
- Low pH treatment (pH 4.0) with or without pepsin, at a temperature between 30°C and 37°C, is used in the viral inactivation of immunoglobulin solutions.
- Beta propiolactone treatment followed by UV irradiation is performed using special equipment.

Filtration.
- Filtration using filters of appropriate pore size (nanofiltration) removes viruses with a protein membrane and not a lipid envelope.
- Aseptic membrane filtration (0.22 nanometres) is used to remove micro-organisms and sterilise bulk products prior to filling ampoules/final product containers.

Pharmacovigilance and product recall
Because large numbers of containers of product belong to a single batch of product and could potentially be infused into a large number of patients, it is essential to monitor the use and effect of the products on recipients. This is a formalised process called pharmacovigilance.

Pharmacovigilance is the structured approach to monitoring and assessing (measuring the effect) of medicinal drugs, including plasma derivatives, on patients. Reporting of untoward effects is encouraged by pharmacovigilance personnel and consideration is then given on how products may be improved, and reactions avoided. The process should be both proactive and reactive.

- Proactive measures include ongoing assessment of products before issue. Regular inspection of batches allows for the detection of deterioration, and batch recall.
- Reactive measures address reactions reported by clinical personnel. This may lead to a product recall to prevent further reactions of the same nature in other patients.

Abstract of blood storage conditions
Once blood has been processed to prepare components or has been fractionated to manufacture plasma derivatives, products are stored within the correct temperature range, and under the correct conditions. Information provided in this connection, within the preceding text, is consolidated and summarised in a table to review these unique conditions together.

Table 2 summarises the storage conditions required for blood components and plasma derivatives. Notes that relate to the summarised information in the table, are as follows:

- Local standards apply – these are general guidelines only.
- Shelf life of RCC in additive solution depends on the nature of the additive.
- When using an open system to prepare red cells, shelf life is reduced to a maximum of 24 h at 4°C ± 2°C.
- When using an open system to pool platelets, shelf life is reduced to a maximum of 6 h.
- Closed systems must be validated to ensure that they are safe before being put into use.
- RCC divided into paediatric packs using an approved closed system may be used for 4 weeks if stored at 4°C ± 2°C.
- Apheresis red cells and platelets have a similar shelf life and storage requirements as listed.
• Storage temperature of plasma derivatives depends on manufacturer’s recommendation.

Key Points
• Whole blood may be divided into three main component types: red cell concentrates, platelet concentrates and plasma.
• Blood bags are available in different configurations where different numbers and types of bags are integrally attached with tubing in a sterile closed system. Each bag configuration is designed for use in a specific way according to processing requirements.
• Blood components can be separated as they differ in size and density and will sediment differently when centrifugal force is applied. A blood bag centrifuge is an essential item of blood processing equipment.
• A scale capable of weighing components to at least the nearest gram weight is essential when making components.
• A plasma extractor (or blood press) is a device that is used to apply pressure to a centrifuged unit of blood in order to transfer part of it to an attached transfer bag.
• Automated blood processing machines are devices that can be configured to process a centrifuged unit of blood into components.
• A tube sealer is a device that will place permanent seals in the PVC tubing of blood packs.
• Sterile connection devices are used to weld additional transfer bags to a primary blood bag without breaking the sterile integrity of the system.
• A platelet agitator is a device designed to fulfil the need for platelet concentrates to be kept in motion during storage.
• Plasma snap freezers are designed to rapidly freeze fresh plasma in order to preserve the labile clotting factors.
• RCC, buffy coat removed, in additive solution, may be considered the red cell product of choice. The component is derived from whole blood by centrifugation and removal of plasma and BC and the subsequent resuspension of the red cells in nutrient additive solution.
• RCC filtered (RCC, leucocyte-depleted) are depleted of white cells by filtration. They are used for patients with leucocyte antibodies and to prevent alloimmunisation in patients expected to receive repeated transfusions.
• Some organisations (and countries) have adopted a policy of universal leucodepletion of all blood donations in an effort to minimise the risk of exposure to vCJD.
• RCC, paediatric, is usually made from either BC depleted RCCs or filtered RCCs by dividing the adult unit into smaller quantities in several bags (usually from 25–100 ml).
• RCCs cryopreserved (frozen) have a shelf life up to 10 years or longer if units are correctly protected during freezing and ultra-low freezing temperatures of colder than −65°C are maintained during the entire storage period.
• Frozen storage of red cells is used mainly to preserve units of blood with rare blood types. It can be used to stockpile blood for emergency use in disasters, but the high cost and short shelf life after recovery from the frozen state make it impractical as a routine tool to manage inventory.
• RCCs, washed, are a specialised component prepared for patients with plasma protein antibodies (e.g. anti-IgA).
• The first step in component processing is to remove the plasma from a centrifuged unit of whole blood.
• Fresh plasma contains coagulation factors (most notably FVIII), protein (e.g. albumin) and immunoglobulins (antibodies). To preserve labile components such as FVIII the plasma is stored frozen and then becomes known as fresh frozen plasma.
• FFP for therapeutic use is prepared by freezing the plasma component of whole blood within 18 h of collection.
• Because plasma has a long expiry time when frozen and stored below −18°C, it is possible to minimise the risks of TTIs by instituting a quarantine and release ‘donar retest’ programme.
• Freeze-dried plasma is a suitable product for use in outlying rural hospitals and emergency rooms. Freeze-drying preserves the clotting factors present in fresh plasma in a powder form and may be stored at ambient temperature (25°C or below) before use.
• Platelet concentrate from a single donation of fresh whole blood (approximately 50–70 × 10⁶) can be recovered and concentrated in a small volume (50–60 ml) by various techniques.
• PCs may be issued and transfused individually by hospital personnel or may be pooled in the components laboratory into a single bag to provide the therapeutic dose (4–6 units) in a convenient package.
• Whole blood used for the preparation of platelets should not be refrigerated, which would initiate clumping and
• reduce their functionality and should be stored in conditions validated to cool donated blood to 22°C ± 2°C rapidly and then maintain this temperature range for up to 24 h.

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• PCs should be continually stored at a controlled room temperature (22°C ± 2°C).
• BC platelets may be prepared as single or pooled units.
• BC platelets may be diluted in either plasma or platelet additive solution.
• Pooling of BCs may be achieved using commercial pooling kits or the ‘chain’ method.
• Platelet concentrates can also be obtained by platelet apheresis of a single donor using automated cell separation equipment.
• Quality control testing of PCs should conform to local or international standards that set guidelines for the number of units that need to be tested, and the specification range for volume, platelet content, white cell content and pH.
• The swirling test is a simple non-invasive visual check that may be performed on every platelet concentrate. The test is based on light scattering by platelets with normal morphology, and would be poorly, or not at all visible in units with damaged or few platelets.
• Irradiation of blood components is accomplished in irradiators designed specifically for this purpose or in a hospital radiotherapy unit.
• Systems that inactivate viral and bacterial pathogens in blood are available for some blood components (platelets) and are under development for others (red cells).
• The labelling of blood components should comply with the relevant legislation (local and national), as well as conforming to international agreements.
• Making safe and effective blood components requires the continual application of good laboratory practice (GLP) and good manufacturing practice (GMP).
• Ongoing product control should be carried out to ensure that the processing laboratory maintains a high and consistent quality of product.
• FFP (for fractionation) is supplied to a fractionation facility for the extraction of clotting factors, albumin and immunoglobulins. Fractionation requires bulk lots made up of several thousand units of ‘safe’ plasma.
• Component processing laboratories may be involved in the preparation of plasma for transfer in bulk to a fractionation facility, which has strict requirements related to quality and standards, and will audit potential suppliers to ensure that they comply.
• The most widely used method of separation of plasma fractions is cold ethanol fractionation, using ethanol at specific concentrations, together with pH and temperature control, to either precipitate a fraction or isolate it in the supernatant.
• Plasma derivatives manufactured in a fractionation facility are subjected to several steps, either in-process (such as filtration) or as additional processes, to remove or inactivate micro-organisms. Additional processes include pasteurisation and solvent/detergent treatment.