Where suitable facilities for freezing erythrocytes are not available, national control authorities may authorize the use of cells from a single donor to immunize no more than three persons (preferably who have not previously had a blood transfusion) in an initial 12-month period, during which monthly determinations of anti-HIV, anti-HCV, anti-HBc, HBsAg and serum alanine aminotransferase should be made in both the donor and the recipients. If, after 12 months, the initial three recipients show no clinical or laboratory evidence of hepatitis, HIV infection or other blood-transmissible diseases, the donor may be considered acceptable for providing erythrocytes for immunization. As small a number of donors of erythrocytes should be used as possible.

*Collection and storage of erythrocytes.* Erythrocytes shall be collected under aseptic conditions into sterile, pyrogen-free containers in an appropriate proportion of an approved anticoagulant. They may then be dispensed in aliquots under aseptic conditions into single-dose, sterile, pyrogen-free containers for storage. The microbiological safety of the dispensing environment shall be validated.

Erythrocytes should be stored frozen for at least 12 months to permit retesting of donors for disease markers. The method selected should have been validated such that there is 70% cell recovery *in vivo*. Erythrocytes should be washed after storage to remove the cryoprotective agent.

Adequate sterility data to support the requested shelf-life for stored erythrocytes should be submitted by the manufacturer to the national control authority. A test for bacterial and fungal contamination should be made on all blood dispensed in aliquots in an open system (*9*). The test should also be performed on at least one single-dose vial from each lot of whole blood that has been stored unfrozen for more than seven days. The test should be made on the eighth day after collection and again on the expiry date. Cultures for the sterility test should be maintained for at least 14 days, with subculturing on day 3, 4 or 5.

*Erythrocyte recipients.* The following additional testing of erythrocyte recipients is necessary:

- The recipient should be phenotyped for ABO, Rh, Kell and Duffy antigens before immunization. Kell-negative and/or Fy(a–) persons should not receive Kell-positive or Fy(a+) cells except for the specific purpose of producing anti-Kell or anti-$Fy^a$. Only ABO-compatible erythrocytes may be transfused. Matching of $Jk^a$, $Jk^b$, $Fy^b$, S and s phenotypes is also desirable.
- Screening for unexpected antibodies by methods that demonstrate coating and haemolytic antibodies should include the antiglobulin method or a procedure of equivalent sensitivity.

Prospective erythrocyte recipients in whom antibody screening tests demonstrate the presence of erythrocyte antibodies (other than those deliberately stimulated through immunization by the plasmapheresis centre) should be asked whether they have ever been pregnant or had a transfusion, a tissue graft or an injection of erythrocytes for any reason. This history should form part of the permanent record and should identify the cause of immunization as clearly as possible. Recipients should be notified in writing of any specific antibodies developed after injection of erythrocytes. The national control authority should be notified annually in writing of unexpected antibodies induced by immunization, and the immunized donor should carry a card specifying the antibodies.

*Immunization schedules.* Erythrocytes used for immunization purposes shall not be administered as part of any plasmapheresis procedure. Such immunization may be performed on the same day as plasmapheresis, but only after it and as a separate procedure.

To minimize the risk of infection to the donor, the immunization schedule should involve as few doses of erythrocytes as possible.

For primary immunization two injections of erythrocytes, each of about 1-2 ml and given three months apart, elicit antibody formation within three months of the second injection in approximately 50% of volunteers; the result is not improved by injecting larger amounts or giving more frequent injections.

It is advantageous to choose as donors of anti-D (anti-$Rh_0$) volunteers who are already immunized, since useful levels of anti-D are then usually attained within a few weeks of reimmunization. In some people, the level of antibody reaches its maximum within the first three weeks and will not increase after further immunization. In others, antibody levels may continue to rise for more than 12 months when injections of 0.5-1 ml of erythrocytes are given at intervals of five to eight weeks. About 70% of immunized volunteers eventually produce antibody levels well above 100 IU/ml. Once attained, such levels can be maintained by injections of 0.1-0.5 ml of erythrocytes at intervals of two to nine months, as required. If injections of erythrocytes are discontinued, antibody levels usually fall appreciably within 6-12 months.

The baseline antibody titre of every recipient of erythrocytes should be established, and the antibody response, including both type and titre, should be monitored monthly.

Erythrocytes to be used for immunization purposes should be selected, for each recipient, by a licensed physician.

*Risks to recipients.* Recipients of erythrocytes for immunization purposes may run the risk of:

- viral hepatitis (B and C) and HIV infection;
- other infectious diseases;
- HLA immunization;
- the production of unwanted erythrocyte antibodies that may complicate any future blood transfusion;
- a febrile reaction if the antigen dose is too great;

– the production of antibodies that may interfere with future organ transplantation if it is needed.

*Record-keeping.* Records of erythrocyte donors and of the recipients of their erythrocytes should be maintained and cross-referenced.

## 5. Collection of blood and plasma

A number of precautions must be taken in the collection of blood and plasma, as described in the following sections.

### 5.1 *Blood collection and apheresis procedures*

The skin of the donor at the site of venepuncture shall be prepared by a method that has been shown to give reasonable assurance that the blood collected will be sterile. Blood shall be collected into a container by means of an aseptic method. The equipment for collecting the sterile blood may be closed or vented provided that the vent is designed to protect the blood against microbial contamination.

With apheresis procedures, care shall be taken to ensure that the maximum volume of erythrocytes is returned to the donor by intravenous infusion. If the red cells cannot be returned to the donor, no further collection should be made until the donor has been re-evaluated. Several checks shall be made to ensure that donors receive their own erythrocytes, including identification of the containers of erythrocytes by donors before re-infusion. Haemolytic transfusion reactions are avoidable, since they are caused by the accidental infusion of incompatible erythrocytes. Personnel involved in reinfusion procedures should be adequately trained to prevent them. The signs and symptoms are hypotension, shortness of breath, stomach and/or flank pain, apprehension, cyanosis and haemoglobinuria.

If a haemolytic transfusion reaction occurs, the infusion of cells to all donors at the centre concerned should be discontinued until the identity of all containers of erythrocytes has been checked. Automated plasmapheresis is preferred to manual plasmapheresis in some institutions because of its greater safety.

#### 5.1.1 *Summary of minimum general requirements for apheresis*

*Equipment.* This must be electrically safe and non-destructive for blood elements; disposable tubing must be used wherever there is blood contact. In addition, equipment must be accessible to detailed inspection and servicing and its decommissioning should not significantly interrupt the programme. It should also be provided with suitable automatic alarms.

*Procedure.* This must be non-destructive for blood elements and aseptic; there must be adequate safeguards against air embolism.

*Disposables.* These must be pyrogen-free, sterile and biocompatible (e.g. there must be no activation of enzyme systems).

### 5.1.2 *Adverse reactions*

Provision must be made to prevent and treat any adverse reactions in donors. As with any medical procedure involving the treatment of individuals, adverse reactions may occur with blood collection and plasmapheresis. Almost all such reactions are mild and transient, but an occasional serious reaction may occur. The possibility of adverse reactions, though remote, should be anticipated and adequate provision should be made to ensure that care is available to donors. Initial and continuing training in emergency care is mandatory for personnel. If any serious adverse reaction occurs, a physician should be called.

### 5.1.3 *Types of adverse reaction*

*Vasovagal syncope.* This is most likely to occur with new donors. The signs and symptoms are hypotension, bradycardia, syncope, sweating and (rarely) convulsions.

*Local infection, inflammation and haematoma at the phlebotomy site.* Reactions of this type are best prevented by adequate preparation of the venepuncture site and by training phlebotomists in proper methods of initiating blood flow. The symptoms are localized pain and redness and swelling at the phlebotomy site.

*Allergic and anaphylactoid reactions.* These may occur during the introduction of saline into the donor while red cells are being processed, or during reinfusion of red cells. The signs and symptoms are urticaria, burning in the throat, tightness of the chest, wheezing, pain in the abdomen and hypotension.

*Systemic infection.* Care should be taken at all stages of plasmapheresis to avoid the transmission of infectious organisms to the donor.

## 5.2 ***Containers***

The original blood container or a satellite attached in an integral manner shall be the final container for whole blood and red cells, with the exception of modified red cells, for which the storage period after processing should be as short as possible and certainly not longer than 24 h. Containers shall be uncoloured and translucent and the labelling shall be placed in such a position as to allow visual inspection of the contents. They shall be sterilized and hermetically sealed by means of suitable closures so that contamination of the contents is prevented. The container material shall not interact adversely with the contents under the prescribed conditions of storage and use.

The specifications for containers should be approved by the national control authority (*10, 11*).

If sterile docking devices are not available, closed blood-collection and processing systems should be used to prepare blood components.

### 5.3 *Anticoagulants*

The anticoagulant solution shall be sterile, pyrogen-free and of a composition such as to ensure that the whole blood and separate blood components are of satisfactory safety and efficacy.

Commonly used anticoagulant solutions are acid–citrate–glucose, citrate–phosphate–glucose and citrate–phosphate–glucose–adenine; the amount of adenine used varies in different countries. Solutions of adenine, glucose and mannitol used for red cell preservation may be added after removal of the plasma.

For plasmapheresis, sodium citrate as a 40 g/l solution is widely used as an anticoagulant.

### 5.4 *Pilot samples*

Pilot samples are blood samples provided with each unit of whole blood or of red blood cells. They shall be collected at the time of donation by the person who collects the whole blood. The containers for pilot samples shall be marked at the collection site before the samples are collected, and the marking used must be such that the sample can be identified with the corresponding unit of whole blood. Pilot samples must be collected by a technique that does not compromise the sterility of the blood product.

Pilot samples should be attached to the final container in a manner such that it will later be clear whether they have been removed and reattached.

### 5.5 *Identification of samples*

Each container of blood, blood components and pilot and laboratory samples shall be identified by a unique number or symbol so that it can be traced back to the donor and from the donor to the recipient. The identity of each donor shall be established both when donor fitness is determined and at the time of blood collection.

When blood-derived materials are transferred to a fractionation plant, the following details shall be provided by the supplier:

- name and address of collecting centre,
- type of material,
- donor identification,
- date of collection,
- results of mandatory tests,
- conditions of storage,
- other details required by the fractionator,
- name and signature of responsible person,
- date.

# Part B. Requirements for single-donor and small-pool products

## 6. General considerations

These requirements for single-donor and small-pool products cover the methods used to prepare products directly from units of whole blood or of components collected by apheresis, starting with the testing of the units and proceeding to the separation of the various cell and plasma protein components. Among the products that may be prepared in small pools (12 donors or fewer) are cryoprecipitated factor VIII and platelets. In addition to tests on the units of whole blood that provide information on the safety, efficacy and labelling of the components, specific tests are included, where applicable, to ensure the quality of various components.

It is important to note that single-donor and small-pool products have certain specialized uses other than therapeutic application to correct deficits in patients. Although not dealt with further in these Requirements, these uses include the stimulation of plasma donors with red blood cells in order to raise antibody levels for the preparation of anti-D (anti-$Rh_o$) immunoglobulin (*12*) and special blood-grouping reagents. It is of the utmost importance that the donors of cells and plasma for such purposes be carefully studied both initially and on a continuing basis to minimize the likelihood of the transmission of infectious diseases to recipients. The use of red cells, stored frozen, that have been demonstrated to be free from infectious agents by retesting the donor 12 months after the initial collection reduces the risk of such transmission to volunteers for immunization.

Plasma donors may also be immunized with viral or bacterial antigens for the preparation of specific immunoglobulin products. All donor immunization procedures must be planned and carried out under the supervision of a physician who is familiar with the antigens being used and especially with the reactions or complications that may occur. Donors being immunized shall have been fully informed of all known hazards and shall have given their written informed consent to the procedures.

Donor immunization practices are considered in more detail in Part A, section 4.7.

Minimum general requirements for apheresis are summarized in Part A, section 5.1.1.

## 7. Production and control

### 7.1 *General requirements*

Single-donor and small-pool products shall comply with any specifications established by the national control authority. Cellular blood components and certain plasma components may deteriorate during separation or storage. Whatever the method of separation (sedimentation, centrifugation, washing or filtration) used for the preparation of cell components, therefore, it is important that a portion of plasma protein sufficient to ensure optimum cell preservation be left with the cells, except when a cryoprotective substance is added to enable them to be stored for long periods in the frozen state, or additive solutions (for example containing adenine, glucose and mannitol) are used for the same purpose for liquid storage.

The methods employed for component separation should be checked before they are introduced. The characteristics assessed might include yield of the component, purity, *in vivo* recovery, biological half-life, functional behaviour and sterility.

The nature and number of such checks should be determined by the national control authority.

Immediately before issue for transfusion or for other purposes, blood components shall be inspected visually. They shall not be issued for transfusion if abnormalities of colour are observed or if there is any other indication of microbial contamination or of defects in the container.

Blood components shall be stored and transported at the appropriate temperature. Refrigerator or freezer compartments in which components are stored shall contain only whole blood and blood components. Reagents required for use in testing may be stored in a separate section of the same refrigerator or freezer provided that they have been properly isolated and are in suitable containers.

## 7.2 *Testing of whole blood and plasma*

### 7.2.1 *Sterility*

Each donation of whole blood intended for transfusion and each preparation of component cells constitutes a single batch. Single batches shall not be tested for sterility by any method that entails breaching the final container before the blood is transfused.

The national control authority may require tests for sterility to be carried out at regular intervals on final containers chosen at random and at the end of the storage period. The purpose of such tests is to check on the aseptic technique used for taking and processing the blood and on the conditions of storage.

### 7.2.2 *Laboratory tests*

Laboratory tests shall be made on laboratory samples taken either at the time of collection or from the pilot samples accompanying the final container, labelled as required in Part A, section 5.

In some countries, test reagents, in particular those used for blood-grouping and for detecting anti-HIV, anti-HCV and HBsAg, must be approved by the national control authority.

The results of the tests shall be used for ensuring the safety and proper labelling of all components prepared from units of whole blood.

7.2.3 *Tests for infectious agents*

*Syphilis.* Each donation of whole blood shall, if required by the national control authority, be subjected to a serological test for syphilis. If so tested, only units giving negative results shall be used for transfusion or component preparation.

*Viral hepatitis.* Each unit of blood or plasma collected shall be tested for HBsAg and anti-HCV by a method approved by the national control authority and only those giving a negative result shall be used (*13*). Units giving a positive result shall be so marked, segregated and disposed of by a method approved by the national control authority, unless designated for the production of a reagent or experimental vaccine in an area designed and segregated for such production.

In some countries plasma pools are also tested.

The label on the container or the record accompanying the container should indicate the geographical source of the blood or plasma as well as whether and how the material has been tested for HBsAg and anti-HCV.

Liver function tests, such as serum transaminase determinations, are used in some countries to detect liver damage that may be associated with hepatitis.

*Anti-HIV-1 and anti-HIV-2.* Blood for transfusion and for use in the preparation of blood components must be tested by a method approved by the national control authority for antibodies to HIV-1 and HIV-2 and be found negative. However, when other important factors outweigh the benefits of such testing (e.g. in emergencies) formal arrangements, approved in advance by the national control authority, should be in place that enable the prescribing physician to have access to an untested product. In all such cases, retrospective testing of the pilot sample shall be performed.

*Other infectious agents.* It is important for the national control authority to reassess testing requirements from time to time in the light of current knowledge, the prevalence of infectious agents in different populations and the availability of tests for serological markers of infection. For example, human retroviruses other than HIV have been described (HTLV types 1 and 2) and more may be identified in the future.

## 7.3 Blood-grouping

Each unit of blood collected shall be classified according to its ABO blood group by testing the red blood cells with anti-A and anti-B sera and by testing the serum or plasma with pooled known group A (or single subtype $A_1$) cells and known group B cells. The unit shall not be labelled as to ABO group unless the results of the two tests (cell and serum grouping) are in agreement. Where discrepancies are found in the testing or the donor's records, they shall be resolved before the units are labelled.

In countries where polymorphism for the D ($Rh_0$) antigen is present, each unit of blood shall be classified according to Rh blood type on the basis of the results of testing for the D ($Rh_o$) red cell antigen. The D ($Rh_o$) type shall be determined with anti-D (anti-$Rh_o$) reagents.

With the high-strength antisera and sensitive techniques now available, it is usually considered unnecessary to use the $D^u$ test if the cells are found to be D-negative in routine testing.

## 7.4 *Red cells*

Whole blood for the preparation of all components shall be collected as described in Part A, section 5, and tested as described in Part B, section 7.2.

Red cells shall be processed under aseptic conditions and whenever possible in a closed system. The sterility of all components shall be maintained during processing by the use of aseptic techniques and sterile pyrogen-free equipment. The methods shall be approved by the national control authority, and a written description of the procedures shall be prepared for each product, covering each step in production and testing. Proposals for any procedural modifications shall be submitted to the national control authority for approval before they are implemented.

The following may be prepared for therapeutic purposes (see pages 40–41 for definitions):

- red cells;
- red cells suspended in additive solution;
- modified red cells:
  - red cells, leukocyte-depleted;
  - red cells, leukocyte-poor;
  - red cells, washed;
  - red cells, frozen;
  - red cells, deglycerolized.

### 7.4.1 *Methods and timing of separation*

Red cells shall be prepared from whole blood collected in plastic bags or in glass bottles.

Multiple-plastic-bag systems with sterile docking devices are preferable because they minimize the risk of microbial contamination by providing completely closed systems. They are easy to handle and are disposable. The use of glass bottles is cheaper but has the disadvantage that the system is then an open or vented one, so that separation must be carried out under strictly aseptic conditions in sterile rooms or laminar-flow cabinets and microbiological monitoring is necessary. The same conditions also apply to the separation procedure when plasma is transferred from disposable single plastic bags to separate containers.

All surfaces that come into contact with the blood cells shall be sterile, biocompatible and pyrogen-free. If an open plastic-bag system is used, i.e. the transfer container is not integrally attached to the blood container and the blood container is opened after blood collection, the plasma shall be separated from the cells under conditions such that the original container is kept under positive pressure until it has been sealed. If the separation procedure involves a vented system, i.e. if an airway is inserted into the container for withdrawal of the plasma, the airway and vent shall be sterile and constructed so as to exclude microorganisms.

In some countries, the sterility of products prepared in open systems is monitored by testing a sample of at least 2% of the units. The national control authority should approve the system used.

The final containers for red cells (but not necessarily modified red cells) shall be the containers in which the blood was originally collected or satellite containers attached in an integral manner. If pilot samples are detached from the blood container during removal of any component, such samples shall be reattached to the container of red cells. The removal and reattachment of the pilot samples shall be recorded conspicuously (with a signature) on the label of the unit. The final containers for all other components shall meet the requirements for blood containers given in Part A, section 5.2. If the final container differs from the container in which the blood was originally collected, it shall be given a number or other symbol to identify the donor(s) of the source blood. Whenever appropriate, the secondary final container shall be similarly labelled while attached to the primary final container.

The timing and the method of separation (centrifugation, undisturbed sedimentation or a combination of the two) will depend on the components to be prepared from the donation. When platelets and coagulation factors are being prepared from the same donation, the components shall be separated as soon as possible after withdrawal of the blood from the donor.

Separation should preferably be effected within 8 h of blood donation.

When platelets and coagulation factors are to be prepared, it is especially important that the venepuncture be performed in such a way as to cause minimal tissue damage so as to prevent the initiation of coagulation. The blood should flow freely without interruption and as rapidly as possible, and be mixed thoroughly with the anticoagulant.

If platelets are to be prepared from a unit of whole blood, the blood shall be kept at a temperature of 20-24 °C for up to 8 h until the platelet-rich plasma has been separated from the red blood cells.

Red cells may be prepared either by centrifugation or by undisturbed sedimentation before the expiry date of the original whole blood. Blood cells shall be separated by centrifugation in a manner that will not increase the temperature of the blood.

Sedimentation is the least expensive method for separation of red blood cells and does not require special equipment.

Repeated washing with saline and centrifugation and filtration are used to reduce the number of leukocytes and platelets and the volume of trapped plasma in red cells. Frozen red cells after thawing are also repeatedly washed with special solutions to remove cryoprotective agents while also preventing haemolysis.

7.4.2 *Expiry date*

The expiry date of whole blood and red cells prepared in a closed system from blood collected in acid-citrate-glucose or citrate-phosphate-glucose is generally 21 days after collection. The time of removal of plasma is not relevant to the expiry date of the red cells when the integrity of the container is not compromised.

The shelf-life of stored blood has been extended to 35 days by collecting the blood in acid–citrate–glucose supplemented with 0.5 mmol/l adenine or in a mixture of 0.5 mmol/l adenine and 0.25 mmol/l guanosine with extra glucose, and to 42 days by adding a solution containing adenine, glucose and mannitol. Recent studies indicate that it may also be possible to extend the shelf-life of stored blood to 35 days by collecting it in citrate–phosphate–glucose supplemented with 0.25 mmol/l adenine and extra glucose.

When red cells are prepared with very high erythrocyte volume fractions, an expiry date 14 days after collection is recommended in some countries because the cells may become glucose-deficient after this time. The erythrocyte volume fraction of red cells collected in citrate–phosphate–glucose–adenine should not exceed 0.9 if the expiry date is more than 21 days after collection.

The usefulness of acid–citrate–glucose is limited by the significant reduction in cell viability when the volume of cells collected is small, which is unavoidable for some donations.

Provided that sterility is maintained, the shelf-life of red cells is not influenced by the method of separation used. However, if an open system is used that does not maintain sterility, the expiry date shall be 24 h after separation and the cells should be used as soon as possible. Red cells and whole blood should be stored at $5 \pm 3$ °C and transported with wet ice in insulated boxes at $5 \pm 3$ °C. Care should be taken not to place containers directly on ice.

Refrigerated whole blood and red cells will warm up rapidly when placed at room temperature. Every effort should be made to limit the periods during which the products are handled at ambient temperatures in order to prevent the temperature from rising above 10 °C until they are used.

7.4.3 *Modified red cells*

*Red cells, leukocyte-depleted and red cells, leukocyte-poor.*

Because of the possibility of reactions, some countries require that red cells contain less than 2% of the leukocytes of the original whole blood.

Leukocyte depletion may be achieved by buffy-coat removal, freezing and washing, or by washing alone.

Leukocyte-poor red-cell concentrates are prepared by filtration.

*Red cells, washed.* Red cells can be washed by means of interrupted or continuous-flow centrifugation. If the first of these methods is used, the washing procedure shall be repeated three times.

Centrifugation should be carried out in refrigerated centrifuges. If such equipment is not available, the washing solution should have a temperature of 5 ± 3 °C.

Red cells can also be washed by means of reversible agglomeration and sedimentation using sugar solutions.

Washed red cells should be transfused as soon as possible and in any case not later than 24 h after processing if prepared in an open system that does not maintain sterility, unless the national control authority has specified a longer shelf-life. They should be stored at all times at 5 ± 3 °C.

Requirements for pilot samples, labels and storage and transport temperatures are the same as those for unmodified red cells.

*Red cells, frozen and red cells, deglycerolized.* Red cells less than six days old are usually selected for freezing in order to minimize loss of yield due to haemolysis during processing.

Frozen red cells are red cells that have been stored continuously at low temperatures (-65 °C or below) in the presence of a cryoprotective agent. The red cells must be washed to remove the cryoprotective agent before use for transfusion. The methods of preparation, storage, thawing and washing used should be such as to ensure that at least 70% of the transfused cells are viable 24 h after transfusion. Storage at temperatures below -65 °C is usually necessary to achieve 70% recovery.

The cryoprotective agent in most common use is glycerol. The temperature of storage should be between -65 °C and -160 °C, depending on the glycerol concentration used.

The shelf-life of frozen cells below -65 °C is at least three years and may be much longer under certain circumstances, but the reconstituted (thawed and washed) red cells should be used as soon as possible and not later than 24 h after thawing unless a closed system is used.

Frozen cells are usually shipped in solid carbon dioxide ("dry ice") or liquid nitrogen, depending upon the glycerol concentration used. Deglycerolized red cells should be stored at a temperature of 1–6 °C and shipped at 5 ± 3 °C.

Requirements for pilot samples and labels are the same as those for unmodified red cells.

## 7.5 *Plasma*

Single-donor plasma shall be obtained by plasmapheresis or from units of whole blood that comply with the requirements of Part A, section 5, and Part B, section 7.2.

Fresh-frozen plasma and frozen plasma should be stored in carefully monitored freezers equipped with recording thermometers and audio and visual alarms to give warning of mechanical or electrical failure. If refrigeration is interrupted for longer than 72 h and the temperature rises above -5 °C, the product may no longer be considered as fresh-frozen plasma, although testing may indicate that reasonable amounts of factor

VIII remain if the plasma has not become liquid. Repeated thawing and freezing may cause denaturation of plasma constituents and cause prekallikrein activation.

### 7.5.1 *Plasma, fresh-frozen*

Fresh-frozen plasma shall be prepared by separating plasma from whole blood and freezing it rapidly within 8 h of collection.

Ideally, fresh-frozen plasma should be prepared by rapid freezing using a combination of solid carbon dioxide and an organic solvent such as ethanol. If this procedure is used, it should have been shown that the container cannot be penetrated by the solvent or substances leached from the container into the contents. Fresh-frozen plasma should be stored at or below −20 °C, and below −30 °C if to be used for transfusion purposes.

Before use for infusion, fresh-frozen plasma should be thawed rapidly at 30–37 °C. Agitation of the container and/or circulation of water at a temperature of 37 °C during the thaw cycle will speed thawing. Once thawed, fresh-frozen plasma must not be refrozen. It can be stored at ambient temperature and should be used within 2 h of completion of thawing.

Fresh-frozen plasma shall have an expiry date one year from the date of collection.

Before its expiry date, fresh-frozen plasma may be used for preparing cryoprecipitated factor VIII. It may be used for the preparation of other pooled plasma fractions (e.g. factors I, II, VII, VIII, IX and X) at any time, even after its expiry date.

### 7.5.2 *Plasma, frozen*

Frozen plasma is, by definition, a plasma separated from whole blood more than 8 h after the latter has been collected, but the delay should be as short as possible. Frozen plasma may be used directly for transfusion or fractionation, or it may be freeze-dried as single-donor units. Plasma may be combined in small pools before freezing if it is to be used to prepare freeze-dried plasma.

The national control authority should determine the specific requirements for frozen plasma.

If frozen or freeze-dried plasma is intended to be used directly in patients without further processing, the blood shall be collected in such a manner and in containers of such a type as to allow aseptic handling, e.g. by means of closed systems.

In some countries, frozen plasma is given an expiry date five years from the date of collection.

Whenever the container of frozen plasma is opened in an open procedure, the method of handling shall avoid microbial contamination; as an additional precaution, sterile rooms or laminar-flow cabinets can be used. Delay in processing shall be avoided, and the ambient conditions shall be regulated so as to minimize the risk of contamination.

Plasma may be pooled at any time after collection.

### 7.5.3 *Plasma, freeze-dried*

Freeze-dried plasma shall be made from single units or small pools of fresh-frozen plasma or frozen plasma.

The storage conditions and expiry dates of different forms of freeze-dried plasma shall be approved by the national control authority. The product normally has a shelf-life of five years when stored at 5 ± 3 °C, but this will depend on the source material, storage conditions and residual moisture in the product. Pooled freeze-dried plasma has a significant potential for the transmission of infectious diseases. This is likely to be substantially diminished by the introduction of viral inactivation procedures applicable to plasma.

### 7.5.4 *Plasma, recovered*

Recovered plasma intended to be pooled for fractionation shall not be used directly for transfusion; a preservative shall not be added.

Plasma may be separated from whole blood at any time up to five days after the expiry date of the blood. The method used for separation shall avoid microbial contamination. As an additional precaution, sterile rooms or laminar-flow cabinets can be used.

If the plasma has been pooled, it shall be stored and transported frozen at or below -20 °C.

### 7.5.5 *Plasma, platelet-rich*

Platelet-rich plasma is a preparation containing at least 70% of the platelets of the original whole blood.

The preparation shall be separated by centrifugation, preferably within 8 h of collection of the whole blood. The temperature and time of processing and storage shall be consistent with platelet survival and maintenance of function.

To achieve the desired haemostatic effect, platelet-rich plasma shall be transfused as soon as possible after collection, and not later than 72 h afterwards, unless stored at 22 ± 2 °C in containers approved for a longer storage period.

## 7.6 *Platelets*

Platelets shall be obtained by cytapheresis or from whole blood, buffy coat or platelet-rich plasma that complies with the requirements of Part A, section 5, and Part B, section 7.2. Aspirin ingestion within the previous three days precludes a donor from serving as a source of platelets.

Whole blood or platelet-rich plasma from which platelets are derived shall be maintained at 22 ± 2 °C until the platelets have been separated.

The separation shall preferably be performed within 8 h of collection of the whole blood. Blood shall be obtained from the donor by means of a single venepuncture giving an uninterrupted flow of blood with minimum damage to the tissue. It must have been demonstrated that the time and speed of centrifugation used to separate the platelets will produce a suspension without visible aggregation or haemolysis.

The national control authority shall determine the minimum acceptable number of platelets that should be present in the products prepared (e.g. $5.5 \times 10^{10}$).

A pH of 6.5–7.4 shall be maintained throughout storage of platelets. The volume of plasma used to resuspend platelets will be governed by the required pH of the platelet suspension at the end of its shelf-life, but shall be no less than $50 \pm 10$ ml.

Licensed artificial suspension media may be used to replace plasma.

Platelets stored at 5 °C are inferior to the same product stored at $22 \pm 2$ °C. Cold storage should be avoided where possible.

When stored at $22 \pm 2$ °C, platelet products shall be gently agitated throughout the storage period.

Platelet products with high platelet counts that are stored at $22 \pm 2$ °C may need to contain as much as 70 ml of plasma or more if the pH is to be maintained above 6.5 throughout the storage period. This period may be as long as seven days for containers made of certain special plastics, but it is prudent to restrict platelet storage to five days because of the risk of bacterial contaminants.

The product should be ABO typed and, in countries where D ($Rh_0$) is polymorphic, D ($Rh_0$) typed; it may also be desirable to know the HLA type.

The material of which the final container used for platelets is made shall not interact with the contents under normal conditions of storage in such a manner as to have an adverse effect on the product.

The requirements for labelling the final container are given in section 7.9. In addition to the customary data, the label shall bear: (a) the recommended storage temperature; (b) the statement that, when stored at $22 \pm 2$ °C, the platelets should be gently agitated throughout storage to obtain maximum haemostatic effectiveness; and (c) a statement to the effect that the contents should be used as soon as possible, and preferably within 4 h once the containers have been opened for pooling.

### 7.6.1 *Monitoring the quality of platelets*

Units randomly selected at the end of their shelf-life shall be tested on a regular basis. They shall be shown to have: (a) plasma volumes appropriate to the storage temperature; and (b) a pH between 6.5 and 7.4.

The number of units and of platelets to be tested shall be specified by the national control authority.

Some countries require there to be no visible contamination by red cells.