- *Blood pressure:* systolic blood pressure between 12 and 24 kPa (90 and 180 mmHg); diastolic blood pressure between 6.67 and 13.3 kPa (50 and 100 mmHg).
- *Pulse:* between 50 and 110 beats per minute and regular. Lower values may be accepted in healthy athletes with endurance training.
- *Temperature:* oral temperature not exceeding 37.5 °C.
- *Weight:* donors weighing less than 50 kg may donate a volume of blood proportionally less than 450 ml in an appropriate volume of anticoagulant, provided that all other donor requirements are met.

Donors shall be free from any infectious skin disease at the venepuncture site and of skin punctures or scars indicative of abuse of intravenous drugs.

4.5 *Additional requirements applicable to donors for plasmapheresis*

All phases of apheresis, including explaining to donors what is involved in the process and obtaining their informed consent, should be performed under the direct supervision of a licensed physician or by trained personnel reporting to such a physician.

4.5.1 *First-time plasma donors*

When prospective plasma donors present themselves to a centre for the first time, initial screening shall begin only after the procedure of plasmapheresis has been explained and the donor has given consent.

The following information shall be permanently recorded:

- Personal information and identification. If the donor is to participate in an ongoing programme, an effective means of identification is especially important. The use of identity numbers, photographs or other equally effective measures should be considered.
- A preliminary medical history as required for blood donors, covering infectious diseases and the donor's general state of health.

If there are no contraindications to plasmapheresis, preliminary laboratory tests shall be carried out, namely reading of the erythrocyte volume fraction or haemoglobin concentration, determination of total serum protein and screening for protein and sugar in the urine. The haemoglobin concentration or erythrocyte volume fraction of the donor's blood shall be within normal limits, as defined by the national control authority or the national blood transfusion authority.

> Many countries specify minimum haemoglobin concentrations of 125 g/l for women and 135 g/l for men, or, for microhaematocrit determinations, minimum erythrocyte volume fractions of 0.38 for women and 0.41 for men.

If normal values are also obtained in the other laboratory tests, evaluation of the potential donor by the physician begins.

> In some countries, specially trained non-physicians are permitted to conduct these routine examinations under the supervision of a physician.

Donors participating in a programme in which plasmapheresis is more frequent than is blood donation for those eligible for whole-blood collection shall be examined by a licensed physician on the day of the first donation, or not more than one week before that donation. This examination shall include measurement of temperature and blood pressure, auscultation of the heart and lungs, palpation of the abdomen, assessment of neurological signs, urine analysis and blood sampling for tests required by the national control authority. Liver function tests (e.g. for alanine aminotransferase), tests for HBsAg, anti-HIV and anti-HCV, and quantification of plasma proteins by electrophoresis or another suitable method shall also be included. The physician shall obtain informed consent after explaining the procedure of plasmapheresis and describing the hazards and adverse reactions that may occur. At this stage, donors shall be given an opportunity to refuse participation. If they consent, it must be on the condition that their legal rights to recover damages are not waived.

In some countries, the first plasmapheresis procedure may be performed before the results are available for the liver function tests, the serological tests for syphilis (if required by the national control authority) and the tests for HBsAg, anti-HCV and anti-HIV. The results of the tests for quantifying plasma proteins should be reviewed by the physician before subsequent plasmapheresis procedures.

4.5.2 *Donors who have undergone plasmapheresis previously in the same programme*

For donors who have already taken part in a plasmapheresis programme:

- The receptionist shall note the date of the last donation (at least two days must have elapsed since that time). No more than two donations shall be permitted within a seven-day period.
- The medical history and weight of the donor shall be recorded; blood pressure, temperature, pulse rate and haemoglobin concentration shall be measured by trained personnel. On the day of each donation, in addition to meeting the general requirements for donors, plasma donors shall be shown to have a total serum protein concentration of not less than 60 g/l.

The medical evaluation of plasma donors shall be repeated at regular intervals, as specified by the national control authority, and tests carried out as specified in section 4.5.3.

Whenever the result of a laboratory test is found to be outside the established normal limits or a donor exhibits any important abnormalities of history or on physical examination, the donor shall be excluded from the programme. The donor shall not be readmitted to the programme until the results of relevant tests have returned to normal and the responsible physician has given approval in writing. It is the responsibility of national health authorities to define normal ranges and standard deviations of test results on the basis of data from a sufficiently large sample of healthy individuals not undergoing plasmapheresis.

In the case of hepatitis C, the results of liver function tests frequently return to normal before rising again. Test results obtained over a period of adequate length must therefore be evaluated by the physician before the donor can be readmitted to the programme.

4.5.3 *Tests for plasma donors*

The following tests shall be performed at each donation:

- Measurement of haemoglobin concentration or erythrocyte volume fraction.
- Determination of total serum protein concentration, which shall be at least 60 g/l.
- An approved test for HBsAg, which shall be negative.
- An approved test for anti-HIV, which shall be negative.
- An approved test for anti-HCV, which shall be negative.

The following tests shall be performed initially and then every four months or after every 10 donations, whichever time interval is longer:

- If required by the national control authority, a serological test for syphilis, which shall be negative.
- Urine analysis for glucose and protein, which shall be negative.
- Serum protein electrophoresis: this shall be normal (unusual changes in a donor's results may be more significant than absolute values). The albumin and globulin concentrations may be calculated from the known total protein value, and shall be: albumin, minimum 35 g/l; IgM, minimum 0.5 g/l; IgG, between 5 and 20 g/l.
- Liver function tests.

  When determination of serum alanine aminotransferase is required, the enzyme concentration measured photometrically using approved reagents shall be no more than two standard deviations above an established normal mean.

4.6 *Donors for platelet and leukocyte apheresis*

In general, platelet and leukocyte donors shall meet the general criteria for donors and the specific criteria for plasma donors (sections 4.1–4.5). In addition, platelet donors should not have taken aspirin or other platelet-active drugs for at least 72 h before donation.

The requirements to be satisfied in the performance of plateletpheresis and leukapheresis in order to ensure that there is no danger to donors and that the products obtained are of satisfactory quality are under active investigation in many countries. The following recommendations may be useful as guidance.

On the day of each donation, donors for plateletpheresis should have an absolute platelet number concentration ("count") of not less than 200×10^9/l and donors for leukapheresis should have an absolute granulocyte number

concentration of not less than 3×10^9/l. Both types of donor should have a normal differential leukocyte count and haemoglobin level.

Although levels of circulating platelets and leukocytes recover promptly in donors, data are not at present available from which the maximum numbers of platelets and leukocytes that can be safely collected from donors can be defined. The long-term effects of the repeated removal of cellular elements are not known.

Leukapheresis may entail the administration of drugs to donors and their exposure to colloidal agents to enhance the yield of granulocytes. Appropriate precautions should be taken to protect donors, such as investigation for latent diabetes by means of a glucose tolerance test if a donor is to be given corticosteroids.

Leukapheresis should be performed as part of the treatment of a patient with chronic myeloid leukaemia only if approved by the patient's attending physician. It is inadvisable to use the leukocytes from such patients.

4.7 *Donor immunization and plasma for special purposes*

4.7.1 *Plasmapheresis in donors with naturally acquired antibodies and other types of medically useful plasma*

Plasma may be collected by plasmapheresis from donors who have acquired immunity through natural infection or through active immunization with approved vaccines for their own protection, and from donors with plasma useful for diagnostic purposes as a result of acquired or congenital underlying conditions.

Donors with medically useful plasma may be identified by screening whole blood donations and by examining patients convalescing from specific diseases or vaccinated individuals, e.g. veterinary students who have received rabies vaccine or military recruits who have been immunized with tetanus toxoid. Unnecessary immunizations can be avoided by this approach.

The following are examples of medically useful plasma:

- Antibody-rich plasma for control reagents in diagnostic tests, such as those for anti-HIV, hepatitis A and B, cytomegalovirus, rubella, measles and uncommon infectious agents; plasma should be collected in appropriately isolated premises when products are being prepared that are known to be capable of transmitting infection.
- Plasma containing antibodies to human cellular and serum antigens of diagnostic use, for example in HLA (human leukocyte antigen) typing reagents, erythrocyte typing reagents and immunoglobulin allotyping reagents.
- Plasma containing reagents useful for diagnostic tests, such as reagin, rheumatoid factors, heterophile antibody and C-reactive protein.
- Factor-deficient plasma for specific assays, such as factor-VIII-deficient plasma. Donors who have received factor VIII are at increased risk of transmitting hepatitis B, hepatitis C and HIV; their plasma should therefore be collected in appropriately isolated premises.

4.7.2 *Precautions to be taken when handling blood or blood products containing infectious agents*

All blood and plasma may contain unknown infectious agents and must be handled accordingly. In addition, special precautions must be taken when handling infected donors and blood products known to contain infectious agents. The precautions to be taken might include:

- isolation by means of the appropriate timing or location of the procedures, special labelling and quarantine of the products collected, use of protective packaging with double wrapping in impervious plastic;
- disinfection of all work surfaces and equipment with a disinfectant of known efficacy, such as freshly prepared 0.25% sodium hypochlorite solution;
- protection of staff by means of adequate training, avoidance of aerosols and use of gloves, gowns, masks and eye protection; it is strongly recommended that such staff also be protected by immunization with hepatitis B vaccine;
- fulfilment of the labelling, shipping and waste-disposal requirements appropriate to the etiological agents in question.

4.7.3 *Immunization of donors*

There is a clinically valid need for specific immunoglobulins and plasma for therapeutic, prophylactic and diagnostic uses. Deliberate immunization of healthy volunteers may be necessary in addition to collection of plasma from convalescent patients and donors selected by screening for high levels of specific antibodies. The immunization of donors requires informed consent in writing and shall take into consideration all the requirements of the previous sections.

Donors shall be immunized with antigens only when sufficient supplies of material of suitable quality cannot be obtained from other appropriate donors, from donations selected by screening, or in the form of safe and efficacious licensed monoclonal antibodies. Donors must be fully informed of the risk of any proposed immunization procedure, and pressure shall not be brought to bear on a donor to agree to immunization. Women capable of child-bearing shall not be immunized with erythrocytes or other antigens that may produce antibodies harmful to the fetus. Donors of blood and those undergoing plasmapheresis shall, if necessary, undergo investigations that can reveal hypersensitivity to a proposed antigen (see also Part B, section 6).

An approved schedule of immunization shall be used. Every effort shall be made to use the minimum dose of antigen and number of injections. In any immunization programme, the following shall be taken into consideration as a minimum: (a) the antibody assay; (b) the minimum level of antibody required; (c) data showing that the dose, the intervals between injections and the total dosage proposed for each antigen are appropriate; and (d) the criteria for considering a prospective donor a non-responder for a given antigen. No donor shall be hyperimmunized with more than one

immunizing preparation unless the safety of the multiple procedure is demonstrated.

Potential donors should be:

- informed by a licensed physician of the procedures, risks and possible sequelae and how to report any adverse effects, and encouraged to take part in a free discussion (which, in some countries, is achieved in small groups of potential donors);
- encouraged to seek advice from their family doctor before agreeing to immunization;
- informed that any licensed physician of their choice will be sent all the information about the proposed immunization procedure;
- informed that they are free to withdraw consent at any time.

All vaccines used for immunizing donors shall be registered or recognized by the national health authority, but may be administered at doses and with schedules differing from those recommended for routine prophylactic immunization. Erythrocyte and other cellular antigens shall be obtained from an establishment approved by the national control authority.

Donors shall be observed for approximately 30 min following any immunization in order to determine whether an adverse reaction has taken place. Because reactions often occur 2–3 h after immunization, donors shall be advised of this possibility and instructed to contact the facility's physician if a reaction is suspected in the first 12 h after immunization. Reactions may be local or systemic. Local reactions, which may be immediate or delayed, take the form of redness, swelling or pain at the injection site. Systemic reactions may include fever, chills, malaise, arthralgia, anorexia, shortness of breath and wheezing.

4.7.4 *Immunization with human erythrocytes*

Erythrocyte donors. A donor of erythrocytes for the purposes of immunization shall meet all the general health criteria for donors (see sections 4.3 and 4.4). In addition, the donor shall not have had a blood transfusion at any time.

The volume of erythrocytes drawn from a donor should not exceed 450–500 ml of whole blood in any eight-week period.

At each donation the donor shall be found to be negative for syphilis, HBsAg, anti-HIV, antibody to hepatitis B core antigen (anti-HBc), anti-HCV and antibodies to human T-cell lymphotropic viruses (anti-HTLV). The serum level of aminotransferases should be within normal limits as established by the national control authority.

Erythrocyte phenotyping shall be done for ABO as well as for C, D, E, c, e, Kell and Fy^a. Phenotyping for other specificities is often desirable and is recommended especially for Jk^a, Jk^b, Fy^b, S and s.

Ideally erythrocytes obtained for immunization purposes should be frozen for at least 12 months before use and the donor should be recalled and retested for anti-HIV, anti-HCV, anti-HBc, HBsAg and anti-HTLV before the stored cells are used for immunization.

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_o) 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_o) antigen is present, each unit of blood shall be classified according to Rh blood type on the basis of