WHO Expert Committee on Biological Standardization

Twenty-ninth Report

Technical Report Series
626

World Health Organization, Geneva, 197

Annex 1

REQUIREMENTS FOR THE COLLECTION, PROCESSING AND QUALITY CONTROL OF HUMAN BLOOD AND BLOOD PRODUCTS[1]

(Requirements for Biological Substances No. 27)

[1] Prepared by a team of WHO consultants and staff members whose names are given in Appendix 1.

INTRODUCTION

In the past a number of documents of the World Health Organization have been concerned with whole blood and its components, but each one has concentrated on guidelines mainly concerned with blood transfusion services and except for human immunoglobulin none has dealt with the requirements applicable to the quality control of whole blood and blood products.

A WHO Working Group on the Standardization of Human Blood Products and Related Substances [1] considered that there was an urgent need for international requirements for the processing and control of whole human blood and blood products. It emphasized that, as the quality of the source material played an important part in the quality of the final products, such requirements should cover all stages, from the collection of source materials to the quality control of the final product.

In the compilation of these international requirements for human blood products, advice and data from a number of experts have been taken into account. The names of these experts are given in Appendix 2.

GENERAL CONSIDERATIONS

The setting up of an organization for the collection and fractionation of human blood and blood components calls for a great deal of expertise

[1] WHO Technical Report Series, No. 610, 1977, p. 24 (Annex 1).

and considerable investment. Any country contemplating the establishment of such an organization should carry out a careful cost-benefit analysis to determine whether the investment is justified. The collection and distribution of whole blood, the separation of whole blood into components, and the fractionation of plasma batches is a logical developmental sequence for a comprehensive organization. It is not always possible to be specific about the details of the procedures employed, the in-process controls, or the tests applied at each stage of production. This is particularly the case with whole blood and component cells. Although the general principle of fractionation of plasma is well established, there are in practice numerous variations in the details of the various production steps. Therefore, any country wishing to begin the collection and fractionation of blood and blood components should send personnel for training to a plant that is operating successfully. WHO can help in arranging such training.

It would not be possible to rely on any product unless the relevant requirements for each step are complied with, and any attempt to reduce these requirements may have serious consequences for the safety of the final product. It is recommended, therefore, that these requirements be applied as a complete document.

One of the basic questions to be answered in considering whether the fractionation of plasma should be started is whether the country has a suitable donor population of sufficient size to guarantee an adequate supply of source material. It is not possible to set a lower limit for the quantity of source material that would be necessary to make such an operation economic because too many factors are involved. In order to maintain competence in production and to avoid certain contamination risks, it is important to have sufficient source material to maintain the fractionation facility in continual operation. In general it would be difficult to justify setting up a plant unless at least 250 litres or 1000 donor pools of plasma are available for fractionation at regular intervals. Even with this amount the fractionation plant would be working on a small scale, but it could serve as the basis for later expansion to a much larger project. Alternatively it could be used for specialized national services, or it could form part of an integrated service organized on a regional basis with neighbouring countries.

The greatest expense involved is in setting up the fractionation plant, but it would be possible to consider the collection of source material and the fractionation as quite separate operations. A country may wish to establish collection centres to separate the cell components and send the plasma to an established fractionation centre in another country. The

products would then be returned to the original country and the costs involved in this operation might be less than those needed to establish and operate a fractionation plant.

The general prevalence of viral diseases, such as various forms of hepatitis, and of parasitic diseases differs so markedly in different geographical regions that each national authority must decide for itself whether the application of the most sensitive test on each blood donation is cost-effective and whether it is feasible to collect suitable source material. A brief protocol of the collection of source material is in any case mandatory (see Appendix 3). In countries where the prevalence of hepatitis B virus (HBV) and parasitic diseases is so high that the supply of the most suitable source material is markedly restricted, greater emphasis should be placed on the production of fractions made by a process that experience has shown causes the least risk of contamination. For example, immunoglobulin prepared by the cold ethanol fractionation method of Cohn has a well established reputation of being free from contamination with HBV, as have albumin products prepared by the same method and heated for 10 hours at 60°C.[1] Nevertheless, the assurance of freedom of these products from infectious viruses requires extreme care in manufacture and cannot be assumed when new fractionation methods are introduced.

When source material with a high risk of contamination is handled, special care should be given to the protection of the health of the staff and appropriate protective measures approved by the national control authority insisted upon.

The transport of source materials from blood collecting centres and hospitals to the fractionation facilities requires special consideration. Thus refrigeration at the temperature range appropriate for the product must be efficient and reliable and proved to be so by monitoring. Thermal insulation must be adequate as a safeguard against a temporary failure of refrigeration. Containers of liquid source material should be filled so as to diminish frothing due to shaking. Because of the potentially infective nature of these biological materials, suitable safeguards should be taken in the event of breakage, spillage, or leakage of containers.

In this document the qualifying word " human " has been dropped from the names of the products derived from human blood. Blood products of animal origin are immunogenic, and their administration to man should be avoided wherever an equivalent product of human origin

[1] WHO Technical Report Series, No. 602, 1977, p. 45.

could be used in its place. Any such products of nonhuman origin should now carry the species of origin in its proper name. In order to avoid confusion while the recommendation is being implemented, it is advised that for an interim period national authorities introduce the animal species in the proper names of animal products before the qualification "human" is dropped.

These requirements have been formulated in the following five parts.

A. Requirements for the collection of source materials.
B. Requirements for single-donor and small-pool products.
C. Requirements for the manufacturing of human blood products.
D. Requirements for the control of plasma fractions.
E. National control requirements.

Each deals with a separate part of the whole process but all the parts taken together are intended to make a composite document.

The parts are divided into sections, each of which constitutes a recommendation. Text printed in type of normal size is written in the form of requirements so that, if a health administration so desires, these parts as they appear may be included in definitive national requirements. Paragraphs printed in small type are comments and recommendations for guidance.

Should individual countries wish to adopt these requirements as the basis for their national regulations concerning blood products and related substances, it is recommended that a clause be included that would permit manufacturing requirements to be modified on the condition that it be demonstrated, to the satisfaction of the national control authority, that such modified requirements ensure a degree of safety and efficacy of the products at least equal to that provided by the requirements formulated below. It is desirable that the World Health Organization should then be informed of the action taken.

The terms "national control authority" and "national control laboratory" as used in these requirements, always refer to the country in which the product is collected, manufactured or used, as appropriate.

Rapid technological developments in the measurement of biological activity of blood products and related substances require the establishment of international biological reference materials. The first two international reference materials (for anti-A and anti-B blood typing sera) were established in 1950, and a further six reference materials have been established in the last seven years. There are a number of materials currently under investigation for the preparation of new standards.

Furthermore, the increased demand for the use of blood products is resulting in the extensive movement of such products between countries. Internationally accepted requirements are therefore necessary in order that countries without any regulations concerning blood products and related substances may refer to these requirements when importing such products.

International standards and international reference preparations

The activity of blood and blood products shall be expressed in international units where an international standard or international reference preparation exists.

A list of international standards and international reference preparations appropriate for the control of blood products and related substances is given in Appendix 4.

These standards are in the custody of the laboratories in Copenhagen, London, Amsterdam and Bilthoven mentioned in Appendix 4.

Samples are distributed free of charge on request to national control laboratories. The international standards are intended for the calibration of national standards for use in the manufacture and laboratory control of human blood and blood products.

PART A:

REQUIREMENTS FOR THE COLLECTION OF SOURCE MATERIALS

A.1. DEFINITION OF CENTRES, ACTIVITIES AND SOURCES

A.1.1 Centres for the collection of source material

The following definitions are intended for use in this document and are not necessarily valid for other purposes.

Blood donor centre : an establishment in which blood and/or blood components are obtained from donors.

Placenta collecting centre : an establishment in which placentas and/or retroplacental blood or parts of either are received from hospitals, accumulated and stored.

A.1.2 Activities of collection centres

Blood collection : a procedure by which a single donation of blood is collected either in an anticoagulant and stabilizing solution or in a container of a kind that permits the separation of serum from coagulated blood.

Processing : any procedure used after collection and before compatibility testing with a prospective recipient.

Plasmapheresis and cytopheresis : procedures by which whole blood is separated by physical means into components and one or more of them returned to the donor.

A.1.3 Donors

Blood donor : a suitable person who gives blood.

A.1.4 Single-donor materials

Whole blood (sometimes referred to as " blood ") : the blood collected in an anticoagulant solution with or without the addition of nutrients such as glucose or adenine.

Whole blood, plasma-reduced (sometimes referred to as " plasma-reduced blood ") : the whole blood in which the erythrocyte volume fraction (" packed cell volume ") has been elevated to approximately 0.6 by the removal of plasma.

Whole blood, modified : the whole blood from which plasma has been separated for the purpose of obtaining cryoprecipitate, platelets, or leukocytes and the plasma returned to the blood cells.

Blood component : any part of blood separated from the rest by physical procedures.

Plasma : the liquid part of blood collected in a receptacle containing an anticoagulant.

Plasma, fresh frozen : a plasma frozen within 6 hours of donation and stored below −20°C (and preferably below −30°C).

Plasma, frozen : a plasma obtained from whole blood within a specified short time (but longer than 6 hours) of collection and maintained in the frozen state below −20°C (and preferably below −30°C).

Plasma, platelet-poor : a plasma from which most platelets have been removed.

Plasma, specific immune : a plasma that can be used either for passive immunization or for the manufacture of specific immunoglobulins.

Plasma, freeze-dried : any of the above forms of plasma that have been freeze-dried for preservation.

Plasma, recovered : a plasma that does not meet the requirements of " plasma, fresh frozen " or " plasma, frozen " and is intended for further processing.

Plasma, platelet-rich : a plasma containing at least 70% of the platelets of the original whole blood.

Cryoprecipitated Factor VIII : a preparation of Factor VIII that is obtained either from plasma from whole blood or by plasmapheresis, through a process involving chilling and precipitation.

Serum : the liquid part of coagulated blood or plasma.

Specific immune serum or plasma : a serum that can be used either for passive immunization or for the manufacture of specific immunoglobulins.

Red cell concentrate : whole blood from which most of the plasma has been removed and having an erythrocyte volume fraction (" packed cell volume ") greater than 0.7.

Red cell concentrate, washed : a red cell concentrate from which most of the plasma, leukocytes and platelets have been removed by one or more stages of washing with an isotonic solution.

Red cell concentrate, leukocyte-poor : a red cell concentrate containing at least 80% of the red cells and less than 25% of the leukocytes of the original whole blood.

Red cell concentrate, frozen : a frozen red cell concentrate to which a cryoprotective agent such as glycerol has been added prior to freezing.

Red cell concentrate, deglycerolized : a red cell concentrate, frozen, that has been thawed and has had the glycerol removed by washing.

Platelet-concentrate : platelets obtained either by separation of whole blood or by pheresis and suspended in a small volume of autologous plasma.

Leukocyte concentrate : a concentrate of leukocytes obtained either by the separation of whole blood or by pheresis and suspended in autologous plasma.

A.1.5 Postpartum source materials

Placenta : the placenta with or without the retroplacental blood from a single delivery.

Placental blood : the blood expressed from the placenta.

Retroplacental blood : uterine blood collected during and after delivery.

Retroplacental serum : the liquid part of the coagulated retroplacental blood.

A.2. PREMISES

The premises shall be of suitable size, construction, and location to facilitate their proper operation, cleaning, and maintenance in accordance with accepted rules of hygiene. They shall comply with the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] and in addition provide adequate space, lighting, and ventilation for the following activities where applicable :

(1) Medical examination of individuals to determine their fitness as donors of blood and/or blood components.

(2) Withdrawal of blood from donors and, where applicable, reinfusion of the components with minimum risk of contamination and errors.

(3) Care of donors, including the treatment of those who suffer reactions.

(4) Storage of whole blood and blood components in quarantine pending completion of processing and testing.

(5) Laboratory testing of blood and blood components.

(6) Processing and distribution of whole blood and blood components in a manner that prevents contamination, loss of potency, and errors.

(7) Performance of all steps in pheresis procedures.

(8) Labelling, packaging, and other finishing operations in a manner that prevents errors.

(9) Storage of equipment.

(10) Storage of finished products prior to distribution.

(11) Documentation and recording of data on the donor, the donated blood, and the ultimate recipient.

[1] WHO Technical Report Series, No. 323, 1966, p. 13.

The collection of blood can be achieved by mobile teams. Although the premises used by such teams may not comply with the more stringent requirements for centres built specially for the purpose, the facility must be adequate for the safety of both the donor and the collected blood or blood components.

A.3. EQUIPMENT

Equipment used in the collection, processing, storage, and distribution of blood and blood components shall be kept clean and shall be maintained and checked regularly. The revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] shall apply in every particular.

Equipment employed to sterilize materials used in blood or blood component collection or for the disposal of contaminated products shall ensure the destruction of contaminating microorganisms. The effectiveness of the sterilization procedure shall be not less than that achieved by a temperature of 121.5°C maintained for 20 minutes by saturated steam at a pressure of 103 kPa (1.05 kgf/cm^2 or 15 lbf/in^2) or by a temperature of 170°C maintained for two hours with dry heat.

Tests for sterility are indicated in the International Pharmacopoeia.[2]

The disposal of contaminated material should comply with the local by-laws controlling such procedures.

A.4. PERSONNEL

A blood or blood component collection organization shall be under the direction of a designated qualified person who shall be responsible for ensuring that all operations are carried out properly and competently. The director shall have an adequate knowledge and experience of the scientific and medical principles involved in the procurement of blood and, if applicable, the separation of blood components and the collection of plasma by plasmapheresis.

The director shall be responsible for ensuring that employees are adequately trained and acquire practical experience and that they are

[1] WHO Technical Report Series, No. 323, 1966, p. 13.

[2] *Specifications for the quality control of pharmaceutical preparations ; second edition of the International Pharmacopoeia.* Geneva, World Health Organization, 1967, p. 747.

aware of the application of accepted good practice to their respective functions.

The director should have the authority to enforce or to delegate the enforcement of discipline among employees.

The persons responsible for the collection of the blood and blood components shall be supervised by licensed physicians who shall be responsible for all medical decisions.

The personnel responsible for the processing, storage, distribution, and quality control of blood, blood components, and plasma shall be adequate in number, and each shall have a suitable educational background and training or experience to assure competent performance of assigned functions so that the final product has the required safety, purity, potency, and efficacy.

A.5. THE COLLECTION OF BLOOD AND BLOOD COMPONENTS

A.5.1 The selection of donors

Source materials for further processing are obtained from donations of blood or its components. The medical criteria for accepting donors—criteria relating to the safety, purity, potency, and efficacy of the final products—must be the same for donors of whole blood and of cellular components or blood components collected by pheresis.

Blood from donors with glucose-6-phosphate dehydrogenase deficiency, sickle-cell trait, or other inherited erythrocyte abnormalities may give rise to transfusion reactions under certain circumstances. Decisions regarding the suitability of such donors should be made by the national control authority.

A.5.2 Donors of whole blood

The physical fitness of a donor shall be determined by a licensed physician or a person under the direct supervision of a licensed physician. Donors shall be healthy persons of either sex between the ages of 18 and 65 years. The frequency of donations shall not exceed one every two months, with a maximum volume in any consecutive 12-month period of 2 litres.

The recruitment of volunteer (non-remunerated) donors should be the aim of any national blood programme. In some countries the upper and lower age limits of the donors may differ from 18 and 65 years.

The frequency of donation may have to be modified on an individual basis, and, in general, premenopausal female donors may be bled less frequently than males. Donors should be within normal weight limits (see section A.5.4).

A.5.3 Medical history

A.5.3.1 *General*

Before each donation questions shall be asked to determine that the donor is in normal health and has not suffered, or is not suffering, from any serious illness, e.g., malignant disease, diabetes, epilepsy, hypertension, renal disease.

Any donor who appears to be suffering from symptoms of acute or chronic disease or who is receiving oral or parenteral medication, with the exception of vitamins or oral contraceptives, may not be accepted for donation unless approved by a physician.

Any donor who appears to be under the influence of alcohol or any drug or who does not appear to be providing reliable answers to medical history questions shall not be accepted.

A.5.3.2 *Infectious diseases*

Donors shall have a negative history of viral hepatitis, of close contact with an individual with hepatitis within the past six months, of receipt within six months of human blood or any blood component or fraction that might be a source of transmission of viral hepatitis, or of tattooing within six months.

Acupuncture within six months may also present a risk.

In some countries donors with a history of viral hepatitis or of a positive test for hepatitis B surface antigen are permanently excluded. In other countries such donors are accepted providing that recovery occurred longer than one year previously and that the reaction for hepatitis B surface antigen is negative when tested by a sensitive technique.

Any donor shall be permanently excluded if a previous blood donation given by him was the only unit of whole blood or of a blood component administered to a patient who developed hepatitis within six months and who received no other blood fractions capable of hepatitis transmission during this period.

Donor populations showing a prevalence of acute or chronic hepatitis higher than that found in the general population should be avoided for collection both of single donor products (whole blood and its components) and of plasma for pooling for the manufacture of plasma fractions known to be capable of transmitting hepatitis, such as clotting factor concentrates.

Countries with a low incidence of hepatitis should not use whole blood or blood products obtained from source material collected from an area in which there is a high incidence of hepatitis.

The testing of blood or plasma for the presence of hepatitis B surface antigen shall be done by methods described in section B.1.

National health authorities shall develop policies designed to prevent the transmission of other infectious diseases based on the prevalence of these diseases in the donor population and the susceptibility of recipients to the same diseases.

In countries where malaria is not endemic, donors should have a negative history of malaria exposure during the past six months and a negative history of clinical malaria or malaria prophylaxis while residing in an endemic area within three years of donation. Such restrictions may be less important in countries where a high level of endemic malaria is present in both donors and recipients, except when blood products are required by visitors from non-endemic areas.

Other diseases that can be transmitted by blood include syphilis, brucellosis, trypanosomiasis (Chagas' disease), infectious mononucleosis, and cytomegalovirus infection. Precautions should be taken to avoid blood collection from persons known to have suffered acute or chronic brucellosis or trypanosomiasis in areas where these diseases are prevalent. Spread of herpes viruses (Epstein-Barr virus and cytomegaloviruses) by blood transfusion is a hazard not easily avoided owing to the high prevalence of asymptomatic chronic infection with these agents in the general population.

A.5.3.3 *Minor surgery*

Donors shall have a negative history of tooth extraction or other minor surgery during a period of 72 hours prior to donation.

A.5.3.4 *Pregnancy*

Pregnant women shall be excluded from blood donation. In general, mothers shall also be excluded for the period of lactation and for at least six months after full-term delivery.

The interval following pregnancy may be shorter in some cases—e.g., six weeks following an abortion during the first trimester.

In some countries donors are accepted when pregnant or during the period of lactation when the blood contains rare blood group antibodies. The volume to be taken should be determined by the physician responsible.

A.5.3.5 *Immunization*

Symptom-free donors who have recently been immunized may be accepted with the following exceptions.

- Those receiving smallpox vaccine shall be excluded until the scab has fallen off or until two weeks after an immune reaction.
- Those receiving attenuated vaccines for measles (rubeola), mumps, yellow fever, or poliomyelitis shall be excluded until two weeks after the last immunization or injection.
- Those receiving attenuated rubella (German measles) vaccine shall be excluded until eight weeks after the last injection.
- Those receiving rabies (therapeutic) vaccine or immunoglobulin shall be excluded until one year after the last injection.
- Those receiving passive immunization using animal serum products shall be excluded until four weeks after the last injection.

A.5.4 Physical examination

Donors shall have a weight, blood pressure, pulse rate, and temperature within normal limits. Donors with any measurements outside the established normal limits of weight, blood pressure, and pulse rate may be accepted only if approved by the responsible licensed physician.

The following recommendations may be useful for guidance:

(1) 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).

(2) Pulse. Between 50 and 100 beats per minute and regular.

(3) Temperature. Oral temperature not exceeding 37.5°C.

(4) Weight. Donors weighing less than 50 kg may be bled proportionately less than 450 ml in an appropriate volume of anticoagulant, provided all other donor requirements are met.

In some countries it is not required to take the body temperature but such decisions should be made by the national control authority.

Donors shall be free from any infectious skin disease at the venepuncture site and of skin punctures or scars indicative of addiction to narcotics.

A.5.5 Haemoglobin or haematocrit determination

The haemoglobin shall be not less than 125 g/l of blood for women and 135 g/l of blood for men or the haematocrit, if substituted, shall be not less than 38% or 41% respectively.

These limits are not universally accepted, and national control authorities should raise or lower them when appropriate.

A.5.6 Donors for plasmapheresis

All phases of plasmapheresis, including explaining to donors what is involved in the process and obtaining their informed consent, shall be performed under the direct supervision of a licensed physician.

There are two groups of plasmapheresis donors: those who donate at a frequency comparable to that allowed for whole blood donations and those who donate more frequently. The former group shall be accepted on the basis of the above criteria for donors of whole blood.

In addition to these criteria, donors participating in a more frequent plasmapheresis programme shall be examined by a licensed physician on the day of the first donation, or no more than one week prior to the first donation. This examination shall include urine analysis and blood sampling for liver function tests, a serological test for syphilis, and determination of plasma proteins by electrophoresis or another suitable method.

On the day of each donation, in addition to meeting the requirements for whole blood donors, plasmapheresis donors shall be shown to have a total serum protein of no less than 60 g/l.

The medical evaluation of plasmapheresis donors shall be repeated at regular intervals, as specified by national control authorities. The interval between physical and laboratory examinations shall not exceed four months.

Whenever a laboratory value is found outside the established normal limits or a donor exhibits any important abnormalities of history or on physical examination, the donor shall be removed from the programme. The donor shall not return to the programme until the abnormal finding has returned to normal and the responsible physician has given approval.

In the event that a plasmapheresis donor donates a unit of whole blood or does not have the red blood cells returned from a unit taken during the procedure, the donor shall be deferred for eight weeks unless special circumstances warrant approval by the responsible physician of earlier plasmapheresis.

In general, plasma collected by therapeutic plasmapheresis shall not be used for fractionation.

There may be individual exceptions to this last requirement—e.g., plasma collected by intensive plasmapheresis during pregnancy of patients with high levels of anti-Rh_0 (anti-D) immunoglobulin.

It is difficult to state the maximum volumes of plasma that can be safely collected from donors until more definitive data are available on the effects of plasmapheresis on donors. In 1967 the Subcommittee of Specialists on Blood Problems, of the Council of Europe, recommended that no more than 8 single units of plasma (each of approximately 300 ml) should be removed in one month, and not more than 50 single units should be removed in one year.[1] However, different limits are imposed in certain countries, e.g., USSR and France: 10 litres per year; USA: 50 and 60 litres per year for donors weighing respectively below and above 80 kg.

A.5.7 Donors for plateletpheresis and leukopheresis

In general, plateletpheresis and leukopheresis donors shall meet the criteria for whole blood and plasmapheresis donors.

The optimum conditions for performing plateletpheresis and leukopheresis to assure donor safety and satisfactory quality of the products are under active investigation in many countries. The following recommendations may be useful for guidance.

On the day of each donation, plateletpheresis donors should have an absolute platelet number concentration ("count") of not less than 100×10^9/l and leukopheresis donors should have an absolute granulocyte number concentration of not less than 1.5×10^9/l. Both types of donor should have a normal leukocyte type number fraction ("differential count").

Recovery of circulating platelet and leukocyte levels occurs promptly in donors, but data are not at present available to define the maximum numbers of platelets and leukocytes that can be safely collected from donors.

Leukopheresis may entail the administration of drugs to donors and their exposure to colloidal agents in order to enhance the yield of granulocytes. Appropriate precautions should be taken to protect the donors, such as investigation for latent diabetes by a glucose tolerance test on those who are to be given corticosteroids.

[1] WHO Technical Report Series, No. 468, 1971, p. 11.

Where leukopheresis is carried out for the treatment of a patient with chronic myeloid leukaemia it should be done only if approved by his attending physician. It is generally considered inadvisable to use the leukocytes from such patients.

A.5.8 Donors for immunization

Immunization of donors shall be carried out only when sufficient supplies of material of suitable quality cannot be obtained by selection of appropriate donors or from donations selected by screening. 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. Donors of blood and those undergoing plasmapheresis shall if necessary undergo investigations that may reveal hypersensitivity to a proposed antigen.

When immunization is intended, the donor should be :

(1) informed of the procedures by a licensed physician and encouraged to take part in a free discussion, which in some countries is achieved by informing potential donors initially in small groups of people ;

(2) encouraged to seek advice from his family doctor before agreeing to immunization ;

(3) informed that any licensed physician of his choice will be sent all information about the proposed immunization procedure ; and

(4) required to indicate his agreement by signing an informed-consent form.

A.6 COLLECTION OF BLOOD

In the collection of blood several precautions must be taken, as described in the following sections.

A.6.1 The taking of the blood

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. The collection of blood into a container shall be done using 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 (see section B.1.1).

A.6.2 The containers

The original blood container or a satellite attached in an integral manner shall be the final container for whole blood and red cell concentrates, with the exception of modified red cell concentrates. Containers shall be uncoloured and transparent and the labelling shall be placed to allow visual inspection of the contents. Closures shall maintain a hermetic seal and prevent contamination of the contents. The container material shall not interact with the contents under the prescribed conditions of storage and use since such interaction may have an adverse effect on the safety or efficacy of the products.

The specifications for containers should be approved by the national control authority.[1]

Wherever possible it is desirable to use sterilized non-detachable satellite containers to prepare components in a closed system in order to minimize the likelihood of microbial contamination.

A.6.3 Anticoagulants

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

Commonly used anticoagulant solutions are acid citrate dextrose and citrate phosphate dextrose.

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

A.6.4 Volume of blood

The national control authority shall determine the quantity of anticoagulant to be used in each container of whole blood and the volume of blood to be collected. Provision shall be made to ensure that only those units meeting the requirements are issued for use.

---

[1] Much useful information is contained in the following publications : COOPER, J. *Plastic containers for pharmaceuticals : testing and control*. Geneva, World Health Organization, 1974 (WHO Offset Publications, No. 4). *Specifications for the quality control of pharmaceutical preparations : second edition of the International Pharmacopoeia*. Geneva, World Health Organization, 1967, p. 869. WHO Technical Report Series, No. 614, 1977 (WHO Expert Committee on Pharmaceutical Preparations : twenty-sixth report), p. 25, Annex 3.

A.6.5 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. They shall be marked before collection to be identical with that of the unit of whole blood.

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

Laboratory samples used in testing the blood may be collected in addition to the pilot samples. They should meet the above requirements.

A.6.6 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 at the time of determination of donor fitness as well as at the time of blood collection.

When source material is transferred to a fractionation plant the appropriate details shall accompany such material.

An example of a protocol that may be useful for such purposes is included in Appendix 3.

PART B:
REQUIREMENTS FOR SINGLE-DONOR AND SMALL-POOL PRODUCTS

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 pheresis, starting with the testing of the units and proceeding to the separation of the various cell and plasma protein components. Among the products are those that may be prepared in small pools (12 donors or fewer), such as cryoprecipitated Factor VIII and platelet concentrates. 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 replace deficits in patients. Although not dealt with further in these requirements, these uses include: (1) stimulation of plasma donors with red blood cells in order to raise antibody levels for the preparation of anti-D immunoglobulin [1] and special blood grouping reagents; and (2) preparation of *in vivo* diagnostic products such as radiolabelled fibrinogen for the diagnosis of deep vein thrombosis. It is of the utmost importance that the donors of cells and plasma for such purposes be carefully studied both initially as well as on a continuing basis to minimize the likelihood of transmitting viral hepatitis to recipients. Red cells, stored frozen, which have been demonstrated to be free from hepatitis are valuable for the immunization of volunteers to reduce the risk of transmitting viral hepatitis.

Plasma donors may be immunized also 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 knowledgeable about the antigens being used and especially about reactions or complications that may arise. Donors being immunized shall have been fully informed of all known hazards and shall have given their written informed consent to the procedures.

B.1. TESTING OF WHOLE BLOOD

B.1.1 Sterility

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

National control authorities may require that tests for sterility shall be carried out at regular intervals on final containers, taken at random and at the end of the storage period. The purpose of this test is to check on the aseptic technique used for taking and processing the blood as well as on the conditions of storage.

Each donation of whole blood shall be visually inspected immediately before issue. It shall not be issued if there is any evidence of leakage or

[1] WHO Technical Report Series, No. 468, 1971, pp. 7–12.

suspicion of microbial contamination such as unusual turbidity, haemolysis, or change of colour.

B.1.2 Laboratory tests

Laboratory tests are made on laboratory samples taken at the time of collection or from the pilot samples accompanying the final container, labelled as required in section A.6.

In some countries the national control authorities require that test reagents, particularly those used for blood grouping and detection of hepatitis B surface antigen, should be approved by the authorities.

Results of the tests are used for ensuring the safety and proper labelling of all components prepared from units of whole blood.

B.1.3 Tests for infectious agents

B.1.3.1 *Test for syphilis*

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

B.1.3.2 *Test for viral hepatitis*

A test for hepatitis B surface antigen shall be done on each unit of blood or plasma collected and only those giving a negative result shall be used.[1] Units giving a positive result shall be so marked, segregated, and disposed of by a method approved by the national control authorities, unless designated for reagent or experimental vaccine production in an area designed and segregated for such production.

The test should preferably be done by a highly sensitive method such as radioimmunoassay, reversed passive haemagglutination, or enzyme-linked immunoassay.

With respect to plasma intended for pooling, all donations entering the pool should be tested and found nonreactive for hepatitis B surface antigen. In some countries small subpools and the final products are tested by highly sensitive methods, but this is considered to be inferior to the testing of single donations.

[1] WHO Technical Report Series, No. 602, 1977 (*Advances in viral hepatitis*), pp. 42–45 and 59.

As with other infectious diseases, there may be areas of such high levels of hepatitis B virus endemicity in donors and immunity in recipients that national control authorities may wish to modify this testing requirement to meet their special needs and conditions.

The label on the container or the direction circular should indicate the geographical source of the material as well as whether and how the material has been tested for hepatitis B surface antigen.

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

B.1.4 Red blood cell grouping

Each unit of blood collected shall be classified according to ABO blood group by testing the red blood cells with anti-A and anti-B sera and by testing the serum or plasma with known type A (or a single subtype A_1) cells and known type 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 with the donor records, they shall be resolved prior to labelling the units.

Each unit of blood shall be classified according to Rh blood type based on the results of testing for the Rh_0 (D) red cell antigen. The Rh_0 (D) type shall be determined with anti-Rh_0 (anti-D) typing serum.

Before labelling blood as Rh_0(D)-negative it should have been tested using a technique designed to detect Rh_0(D) variants (D^u).

Some national authorities require further analysis of Rh phenotypes.

In many countries anti-(A + B) is included in order to detect weak subgroups of A.

B.2. SEPARATION OF RED CELL CONCENTRATES

The preparation of red cell concentrates shall be performed under aseptic conditions and wherever possible in a closed system. The sterility of all components shall be maintained during processing by using aseptic techniques and sterile pyrogen-free equipment. The methods shall be those approved by the national control authority, and a written description of the procedures shall be prepared for each product, describing each step in production and testing. Proposals for any procedural modifications shall be submitted to the national authorities for approval before they are implemented.

Whole blood for preparation of all components shall be collected as described in section A.6 and tested as described in section B.1.

The following red cell concentrates may be prepared for therapeutic purposes :

(1) whole blood, plasma-reduced

(2) red cell concentrate

(3) modified red cell concentrates
   (*a*) red cell concentrate, leukocyte-poor
   (*b*) red cell concentrate, washed
   (*c*) red cell concentrate, frozen and deglycerolized.

B.2.1 Methods of separation

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

> Multiple plastic bag systems are preferable because they minimize the risk of microbial contamination by providing completely closed systems. They are easy to handle and are disposable. They have two drawbacks : their high cost and the leaching into the blood and its components of substances such as plasticizers from poly(vinyl chloride) bags. The use of glass bottles is less costly but has the disadvantage of being an open or vented system ; thus separation steps must be carried out under strictly aseptic conditions in sterile rooms or laminar flow cabinets and with microbiological monitoring. The same conditions apply also 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 and pyrogen-free. If an open plastic bag system is used—i.e., the transfer container is not integrally attached to the blood containers and the blood container is broached after blood collection—the plasma shall be separated from the cells with positive pressure on the original container and maintained until it has been sealed. If the separation procedure involves a vented system—i.e., an airway is inserted in 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 plasma reduced blood and red cell concentrates (but not modified red cell concentrates) shall be the container in which the blood was originally collected or a satellite container attached in an integral manner. If pilot samples are detached from the blood container during removal of any component, the pilot samples shall be reattached to the container of plasma-reduced blood or red cell concentrate. The removal and reattachment of the pilot samples shall be recorded conspicuously and signed on the label of the unit. The final containers for all other components shall meet the requirements for blood containers described in section A.6. At the time when the final container is filled and if a different container is used, it shall be given a number or other symbol to identify the donor(s) of the source blood. Whenever appropriate the secondary container shall be similarly labelled while attached to the primary container.

B.2.2 Time of separation

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

> It is preferable to effect the separation within 4–6 hours of the blood donation.
>
> When platelet concentrates and coagulation factors are to be prepared, it is important that the venepuncture be performed with minimal tissue damage in order to prevent the initiation of coagulation. The blood should flow freely without interruption, as rapidly as possible, and be mixed thoroughly with the anticoagulant.

If platelet concentrate is to be prepared from a whole blood unit, the blood shall be kept at a temperature as close as possible to 20–24°C until the platelet-rich plasma has separated from the red blood cells.

Separation of blood cells by centrifugation shall be done in a manner that will not increase the temperature of the blood. Cells may also be separated by spontaneous sedimentation.

> Sedimentation is the least expensive method for red blood cell separation and there is no need for special equipment.
>
> Repeated washing with saline solutions and centrifugation and filtration are used to reduce the amount of leukocytes, platelets,

and trapped plasma in red cell concentrates. Repeated washings are also used for the removal of glycerol from frozen red cells after thawing.

B.2.3 Plasma-reduced blood and red cell concentrate (red blood cells)

Plasma-reduced whole blood is obtained when sufficient plasma has been withdrawn to yield a product with an erythrocyte volume fraction (" packed cell volume ") of not more than 0.6. Red cell concentrates are formed when more plasma is removed, yielding a product with an erythrocyte volume fraction of approximately 0.7–0.9.

Red cell concentrates may be prepared either by centrifugation or by undisturbed sedimentation prior to the expiry date of the original whole blood.

B.2.4 Expiry date

The expiry date of whole blood and red cell concentrates prepared in a closed system from acid citrate dextrose (ACD) blood as well as from citrate phosphate dextrose (CPD) blood is 21 days after collection in general. The time of removal of plasma is not relevant to the expiry date of the red cell concentrates.

When red cell concentrates are prepared with very high haematocrits, an expiry date of 17 days after collection is recommended in some countries because of glucose deficiency.

The shelf-life of stored blood has been extended to 35 days in some countries by collecting the blood in ACD 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. Recent studies indicate that it may be possible to achieve the same 35-day life of stored blood by collecting it in CPD supplemented with 0.25 mmol/l adenine and extra glucose.

The *in vitro* oxygen transport function of the red cell is substantially decreased after 5–7 days in ACD blood and after 10–14 days in CPD blood.

Provided that sterility is maintained, the expiry date of red cell concentrates is not influenced by the type of separation used. However, if an open system is used, which does not maintain sterility, the expiry date is 24 hours after separation, but the cells should be used as soon as possible. Red cell concentrates shall be stored at 4°C ±2°C and transported at 5°C ±4°C in ice in insulated boxes.

Refrigerated whole blood and red cell concentrates 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 the time when the products are used.

Sodium chloride solution (suitable for intravenous use) may be added immediately prior to use to facilitate both mixing and administration of red cell concentrates.

B.2.5 Modified red cell concentrates

B.2.5.1 *Red cell concentrate, leukocyte-poor*

Leukocyte-poor red cell concentrate is a red cell preparation containing at least 80% of the red cells and less than 25% of the leukocytes of the original whole blood. The number and type of residual leukocytes vary with the method of preparation.

Because of the possibility of reactions some countries require that the red cell concentrate contain less than 5% of the leukocytes of the original whole blood.

The preparation of leukocyte-poor blood can be performed by filtration, by freezing and washing, or by washing alone. It should be performed as soon as possible after the collection of the blood. Filtration is an efficient method for the elimination of the leukocytes if applied within 48 hours of blood collection, but the filtration of heparinized blood should take place within one hour. Freezing of the red cell concentrate should be performed as described below for frozen red cells.

B.2.5.2 *Red cell concentrate, washed*

Washing of red cells can be performed by interrupted or continuous flow centrifugation. By the former method the washing procedure shall be repeated three times.

The centrifugation should be carried out in refrigerated centrifuges. If such equipment is not available the washing solution should have a temperature of 4°C ±2°C.

The washing of red cells can also be performed by reversible agglomeration and sedimentation using sugar solutions.

Washed red cell concentrates should be transfused as soon as possible and not later than 24 hours after processing. They should be stored at all times at 4°C ±2°C.

Requirements for pilot samples, labels, and temperature of storage and transport are the same as those for red cell concentrates.

B.2.5.3 *Red cell concentrate, frozen and deglycerolized*

Frozen red cell concentrates are red cells that have been stored continuously at low temperatures in the presence of a cryoprotective agent. The red cells must be washed to remove the cryoprotective agent prior to use for transfusion. The methods of preparation, storage, thawing, and washing shall ensure a viability of at least 70% of the transfused cells 24 hours after transfusion.

The cryoprotective agent in most common use is glycerol. The temperature of storage should be between −70°C and −160°C depending on the glycerol concentration used.

The storage period of frozen cells is at least 3 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 hours after thawing.

The usual method of shipment of frozen cells is in solid carbon dioxide or liquid nitrogen. Deglycerolized cells should be stored at 4°C ±2°C and shipped at 5°C ±4°C.

Requirements for pilot samples and labels are the same as those for red cell concentrate.

B.3. OTHER SINGLE-DONOR OR SMALL-POOL COMPONENTS

B.3.1 Single-donor plasma

B.3.1.1 *Plasma, fresh frozen*

Fresh frozen plasma shall be separated from the whole blood and frozen solid preferably within 6 hours of collection. It can be kept frozen or it can be freeze-dried.

Freezing may be accomplished in a mechanical freezer at or below −40°C or with a combination of solid carbon dioxide and an organic solvent such as alcohol. The latter procedure should have been shown not to allow penetration of the container by the solvent or leaching of substances from the container into the contents.

Prior to use for infusion, the frozen plasma should be thawep rapidly to 30–37°C.

When stored at or below −20°C (preferably below −30°C), fresh frozen plasma has an expiry date of 1 year from the date of collection. In freeze-dried form the expiry period is 5 years.

Before its expiry date, fresh frozen plasma may be used for preparing cryoprecipitated Factor VIII. It may be used for preparation of other pooled plasma fractions at any time, even after its expiry date.

B.3.1.2 *Plasma, frozen*

Frozen plasma shall be separated from whole blood 6 hours or more after collection of the blood, but the time 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. In addition, it may be combined in small pools before freezing to prepare freeze-dried plasma.

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

If such plasma is intended to be used directly in patients without further processing, the blood shall be collected in such a manner and in such containers as to allow aseptic handling—e.g., by the use of closed systems.

Whenever the container is broached in an open procedure the method used for handling shall avoid microbial contamination, and, 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 to minimize the risk of contamination.

B.3.1.3 *Plasma, freeze-dried*

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

Storage conditions and expiry dates of different forms of freeze-dried plasma shall be approved by the national control authorities.

The intended use for which freeze-dried plasma is suitable and the expiry date are related to the source material, storage conditions, and residual moisture in the product.

B.3.1.4 *Plasma, recovered*

Recovered plasma shall be separated from whole blood at any time up to 5 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. It shall be stored and transported at temperatures not exceeding 10°C.

Recovered plasma is intended to be pooled for fractionation but should not be used directly for transfusion. It may be pooled at any time after collection. To avoid microbial growth in contaminated plasma, recovered plasma should be preferably stored and transported in the frozen state. A preservative should not be added.

B.3.1.4 *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 within 4–6 hours of the collection of whole blood, and the temperature and time of processing as well as of storage shall be consistent with the maintenance of platelet survival and function.

Platelet-rich plasma shall be transfused as soon as possible (but no later than 72 hours) after collection in order to achieve the desired haemostatic effect.

B.3.2 Platelet concentrates

Platelet concentrates can be processed by separation from whole blood, by separation from platelet-rich plasma, or by plateletpheresis.

The whole blood from which platelet concentrates or platelet-rich plasma is derived shall be maintained at 22°C ±2°C until the platelets are separated. The separation shall be performed within 4–6 hours after the collection of whole blood or plasma. The bleeding of the donor shall be performed by a single venepuncture giving an uninterrupted flow of blood with minimum damage to the tissue of the donor. The time and speed of centrifugation must have been demonstrated to produce a suspension without visible aggregation or haemolysis. The suspension shall contain a minimum count of 12.5×10^{10} platelets from each litre of whole blood (i.e., 2.5 units of blood) in at least 75% of the concentrates tested at the maximum storage time.

A pH of 6.0 or higher shall be maintained throughout storage. The volume of original plasma to be used for resuspension of the platelets depends on the storage temperature. Platelets stored at room temperature shall be resuspended in approximately 50 ml of plasma. Platelets stored at 4°C ±2°C shall be resuspended in 20-30 ml of plasma.

If platelet concentrates are stored at room temperature, continuous gentle agitation should be maintained throughout the storage period.

Recent studies indicate that platelet concentrates with high platelet counts and stored at 22°C ±2°C may require up to 70 ml of plasma to maintain the pH above 6.0 throughout the storage period, which may be as long as 72 hours.

The product should be ABO and $Rh_0(D)$ typed and it may be desirable also to know the HL-A type.

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

Requirements for the labelling of the final container are the same as those in section B.3.6. In addition to the customary data the label shall bear the following: (1) the recommended storage temperature; (2) if stored at 20–24°C, instructions to maintain a continuous gentle agitation of the concentrate during storage to obtain maximum haemostatic effectiveness; (3) instructions that the contents shall be used as soon as possible, preferably less than 4 hours after broaching the containers for pooling.

B.3.2.1 *Expiry date*

The expiry date of platelet concentrates processed in a closed system shall be 72 hours after the collection of the original whole blood.

Platelet concentrates prepared in an open system should be used within 4 hours of preparation if stored at 22°C ±2°C and within 24 hours if stored at 4°C ±2°C.

Single-donor platelet concentrates may be pooled under aseptic conditions prior to issue. Such small pools should be used as soon as possible, and no later than 4 hours after preparation if stored at room temperature and 24 hours if stored at 4°C ±2°C.

B.3.3 Leukocyte concentrate

Leukocyte concentrate is a concentrate of separated leukocytes, which may also contain a large number of platelets and red blood cells, depending on the method of preparation.

Methods of processing leukocyte concentrates shall comply with the requirements and recommendations described in sections B.2.1 and B.2.2.

The label of the final container shall bear, in addition to customary data, instructions to use the leukocyte concentrate as soon as possible but not more than 4 hours after the container has been broached for

pooling. Temperature of storage shall be 4°C ±2°C and of transport 5°C ±4°C.

Leukocytes are separated from the blood of healthy donors or, in some centres, from whole blood obtained from patients with chronic myeloid leukaemia.

Leukocytes can be separated from blood by centrifugation, by sedimentation, or by continuous-flow filtration or centrifugation (leukopheresis). The leukocytes from units obtained from several healthy donors may have to be pooled to obtain a sufficient amount of leukocyte concentrate. Leukopheresis by continuous-flow filtration or centrifugation is the most efficient way of obtaining leukocyte concentrates with large numbers of high-quality leukocytes from a single donor.

Leukocytes can also be extracted from blood by filtration of whole blood. Leukocytes adhere to, and can subsequently be recovered from, the surface of the filters. The chemical and physical properties of the filter should be such that the *in vivo* cell survival and cell function of the leukocytes are not impaired.

By centrifugation of whole blood, 30–60% of the leukocytes present in the original whole blood may be recovered.

Approximately 90% of the leukocytes of the original whole blood can be separated by sedimentation of the red cells accelerated by the addition of suitable substances with high relative molecular mass. Continuous-flow filtration (filtration leukopheresis) may give a final yield of 70% of the leukocytes of the donor blood.

The product should be ABO and $Rh_0(D)$ typed and it may be desirable also to know the HL-A type.

B.3.3.1 *Expiry date*

The expiry date of leukocyte concentrates shall be 24 hours after collection of the original whole blood.

B.3.4 Cryoprecipitated Factor VIII

Single-pool cryoprecipitated Factor VIII is a preparation of Factor VIII obtained from a single unit of plasma from whole blood or by plasmapheresis.

The product may also be prepared as a pool from a small number of donations, usually 4–6 and not exceeding 10. It may be freeze-dried.

The plasma shall be separated from red blood cells and frozen solid preferably within 6 hours of collection.

Freezing may be accomplished in a mechanical freezer at −40°C or below or with a combination of solid carbon dioxide and an organic solvent such as alcohol. The latter procedure should have been shown not to allow penetration of the container by the solvent or leaching of substances from the container into the contents.

The method of thawing and harvesting the cryoprecipitate shall have been shown to yield a product containing an adequate activity of Factor VIII (see section B.3.5.4).

B.3.4.1 *Expiry date*

The frozen product shall be stored at or below −20°C (if possible below −30°C) and shall have an expiry date of one year from the date of collection. The freeze-dried product shall be stored at 4°C ± 2°C and shall have an expiry date of one year. It shall be used promptly after thawing or reconstitution. The thawed or reconstituted product should be kept at room temperature (20–24°C) prior to use. It shall be used as soon as possible and not more than 4 hours after its container has been broached for pooling or reconstitution.

B.3.5 Control of single-donor and small-pool products

B.3.5.1 *General*

Single-donor and small-pool products shall comply with any specifications established by the national control authority. Cellular blood components and some plasma components may deteriorate during their separation or storage. Therefore, whatever the method of separation (sedimentation, centrifugation, washing, or filtration) used for the preparation of cell components, it is important that a portion of plasma protein sufficient to assure optimum cell preservation be left with the cells except when a cryoprotective substance is added for prolonged storage in the frozen state.

Methods employed for component separation should be checked before their implementation and at regulated intervals for the quality of the final products. The characteristics assessed should include yield, purity, *in vivo* recovery, biological half-life, functional behaviour, and sterility.

The intervals at which such checks are carried out should be determined by the national control authorities.

Immediately before issue for transfusion the components shall be inspected visually. They shall not be issued for transfusion if abnor-

malities of colour are observed or if there is any other indication of microbial contamination or of defects in the container.

Components shall be stored and transported at the temperature most suitable for the given component. Refrigerator or freezer compartments in which components are stored shall contain only whole blood and blood components.

B.3.5.2 *Red cell concentrate, single-donor plasma, and leukocyte concentrates*

When red cell concentrates and leukocyte concentrates are obtained from units of whole blood, such units shall comply with the requirements in sections A.6 and B.1. Single-donor plasma shall be obtained from units of whole blood that comply with the requirements in sections A.6 and B.1 or by plasmapheresis.

B.3.5.3 *Platelet concentrates*

Platelet concentrates shall be obtained from units of whole blood that comply with the requirements in sections A.6 and B.1 or by plateletpheresis.

Randomly selected units at the end of their shelf-lives shall be tested on a regular basis. They shall be shown to have :

(1) platelet number concentrations (" counts ") of at least 125×10^9/l (i.e., per 2.5 units) of whole blood ;

(2) plasma volumes appropriate to the storage temperature (see section B.3) ; and

(3) a pH between 6.0 and 7.4.

The number of units to be tested shall be specified by the national control authorities.

B.3.5.4 *Cryoprecipitated Factor VIII*

Cryoprecipitated Factor VIII shall be obtained from units of whole blood that comply with the requirements in sections A.6 and B.1 or by plasmapheresis.

Randomly selected units shall be tested on a regular basis within 30 days of their preparation. The number of units to be tested shall be specified by the national control authorities. The freeze-dried Factor VIII preparation shall dissolve completely in the solvent recommended

by the manufacturers within 30 minutes when held at a temperature not exceeding 37°C. The solution kept at room temperature shall not show any signs of precipitation in the first 3 hours after it has been dissolved.

> In many laboratories an average potency of 400 International Units of Factor VIII per litre of starting plasma is reached. The average potency of freeze-dried cryoprecipitate is then at least 300 International Units of Factor VIII per litre of starting plasma. Whether this potency can be reached depends on local technical possibilities. In several countries the yields will be much lower, and the national control authority in the country will have to decide on the level of acceptability.

B.3.6 Labelling of single-donor and small-pool products

When testing is completed and before issue for transfusion, units of single-donor and small-pool products shall be identified with container labels that clearly indicate at least the following information :

(1) the proper name of the product ;

(2) the unique number or symbol identifying the donor(s) ;

(3) the expiry date ;

(4) any special storage conditions or handling precautions that are necessary ;

(5) a reference to a leaflet containing instructions for use, warnings, and precautions ;

(6) the name and address of the blood donor centre and, where applicable, the manufacturer and distributor.

The results of red blood cell grouping shall be on the label of whole blood, red cell concentrates, plasma products, platelet concentrates, and leukocyte concentrates but not necessarily on cryoprecipitated Factor VIII.

B.3.7 Placental source material

Whole placenta, placental blood or serum, and retroplacental blood or serum may all serve as source material for certain plasma fractions.

> This source material should be used only in methods of production and for products that have not been associated with the transmission of hepatitis, such as heat-treated albumin products and immunoglobulins prepared by the Cohn fractionation process. If another fractionation method is used, clinical evidence should prove that no transmission of hepatitis occurs.

Where it is impracticable to test individual source material for the presence of hepatitis B surface antigen the pooled material shall be assumed to be contaminated. Because hepatitis B surface antigen would be diluted in pooled material and may escape detection even with highly sensitive techniques, the label or package insert of the final product shall state whether a test for hepatitis B surface antigen was carried out, and if so, whether it was carried out on the individual source material or after pooling.

It is important that national control authorities assume the responsibility for the method of testing, the production method, and the use of the products obtained from this source material.

PART C:
REQUIREMENTS FOR THE MANUFACTURING OF HUMAN BLOOD PRODUCTS

C.1. BUILDINGS

The buildings used for the fractionation of plasma shall be of suitable size, construction, and location to facilitate their proper operation, cleaning, and maintenance in accordance with general rules of hygiene. They shall comply with the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)[1] and in addition provide adequate space, lighting, and ventilation for the activities listed below.

Each of the activities listed below is an important integral part of the production procedure, and countries wishing to start manufacturing human blood products and related substances should not do so unless adequate provision can be made for all activities.

C.1.1 Storage of whole blood and its components

Whole human blood and its components shall be stored in separate refrigerated rooms in which they may be frozen or refrigerated and which are used only for this purpose. The products shall remain in the rooms until the results of testing show that they are suitable for introduction into the fractionation premises.

[1] WHO Technical Report Series, No. 323, 1966, p. 13.

C.1.2 Separation of cells and fractionation of components

The separation of cells and fractionation of components shall be done in a building isolated from the manufacture or processing of nonhuman proteins or microbiological materials, such as vaccines, and separate from the animal house.

In some countries the separation of cell constituents is carried out in a separate area from that in which components are fractionated.

C.1.3 Supply and recovery of ancillary materials

Adequate facilities shall be provided for the supply of ancillary materials, such as ethanol, water, salts, and poly(ethylene glycol).

Facilities for the recovery of organic solvents used in fractionation may also be provided.

C.1.4 Freeze-drying and filling

The sterile filling of final containers shall be done in a separate area.

A separate area and separate equipment should be used for the freeze-drying of bulk product and of final product.

C.1.5 Packaging, labelling, and storing

Separate facilities shall be used for the labelling and packaging of containers. A separate area shall be provided for the storage of labels, leaflets, and packages. Another separate area shall be used for the storage of final containers prior to despatch.

C.1.6 Keeping of records

Adequate provision shall be made for the keeping of records of all materials, fractionation steps, quality control procedures, results, the distribution of the final product, and the disposal of potentially infective materials.

C.1.7 Provision for control

Provision shall be made for quality control, including haematological, biochemical, physicochemical and microbiological testing as well as pyrogen and safety testing.

It is desirable that those parts of the quality control laboratories that are hazardous to production be separate from the production area.

C.1.8 Disposal of infective material

Provision shall be made for the suitable disposal of potentially infective materials by autoclaving or incineration.

Local laws should be complied with in the disposal of these materials.

C.2. EQUIPMENT

Equipment used for the collection, processing, storage, and distribution of source materials and plasma fractions shall comply with the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories). [1]

Particular attention shall be paid to the following points.

(1) The reliability, maintenance, monitoring, and recording of continuously operating equipment and the provision of reserve facilities.

(2) The suitability and compatibility of the surfaces of all materials (e.g., filter medium, glass, stainless steel, plastic, and rubber) that come into contact with the products.

Metal surfaces that come into contact with proteins should be resistant to scratching. The surface of certain materials can denature certain proteins or activate certain coagulation factors.

(3) The ease and efficiency of cleaning and, where necessary, sterilizing equipment. Any bactericidal agent used shall be capable of being completely eliminated before the equipment is used.

If possible all surfaces with which the plasma and the solvents come into contact should be amenable to visual inspection, and stainless steel tubing should be dismountable.

Washing fluids in common use are sodium hydroxide or soda solutions, which are bactericidal and virucidal. Caution should be exercised in the use of detergents because of a possible effect on the final product; tests should be made to ensure that they do not have any adverse effect.

(4) The provision of suitable facilities for autoclaving and for the disposal of potentially infective materials and equipment.

[1] WHO Technical Report Series, No. 323, 1966, p. 14.

C.3. PROVISION OF SUPPORT SERVICES

The fractionation of source materials requires a number of services essential for the operations involved.

C.3.1 Water supply

An adequate supply of suitable pyrogen-free water shall be provided for use during the fractionation process and for the reconstitution and/or dilution of the plasma fractions before filling and freeze-drying.

The two most commonly used types of water are pyrogen-free distilled water [1] and pyrogen-free deionized water, each of which should be maintained at 80°C. Water preparation and delivery systems should be tested at regular intervals for pyrogenicity and conductance. The water system should be continuously circulating and should have no dead ends.

Water for injection is generally used for the preparation of final products.

C.3.2 Steam supply

An adequate supply of steam shall be provided for the cleaning of equipment and the operation of apparatus used to sterilize the equipment and containers. The steam shall be maintained to a standard of cleanliness such that it does not cause or leave a contaminating deposit on the equipment and containers so cleaned or sterilized.

C.3.3 Other support facilities

Other support facilities are listed below.

(1) A supply of electrical and thermal energy.

(2) A means of refrigeration for the following purposes:

(*a*) storage of various source materials and fractions;

[1] *Specifications for the quality control of pharmaceutical preparations: second edition of the International Pharmacopoeia*. Geneva, World Health Organization, 1967, p. 50.

(*b*) temperature maintenance of various fractionation areas;
(*c*) temperature maintenance of process equipment;
(*d*) storage of final products under test;
(*e*) storage of final products awaiting despatch.

(3) A system of ventilation providing two grades of filtered air:
(*a*) a supply through a filter of pore size 5.0 µm to the entire work area, and
(*b*) a supply through a filter of pore size 0.5 µm at a positive pressure to areas where aseptic dispensing is to take place.

Other support facilities may include the recovery of solvents and a sewage disposal service. Sewage disposal must be carried out in accordance with the sanitary standards of the health authority.[1]

It should be noted that proteinaceous sewage from a plasma processing plant is highly nitrogenous and, having a high biological oxygen demand rating, should not be discharged untreated.

The equipment providing such services shall be located separately from the main process areas and in a place where conditions (light, physical access, etc.) are conducive to the establishment of effective and routine preventive maintenance programmes. The equipment shall incorporate devices capable of monitoring and recording the functioning of the equipment so that the safety of material in process and the safety of the process operators are ensured. In this way a proper record of function can be maintained and, where necessary, entered into the process record of the product batches.

The equipment should be adequate to ensure that the fractionation process as well as the proteins are protected in the event of an interruption in the support services.

In order to ensure this, there should be adequate spare equipment and emergency reserve systems serviced by engineering staff skilled in the maintenance and repair of such equipment.

C.4. PERSONNEL

The plasma fractionation plant shall be under the direction of a designated qualified person who shall be responsible for ensuring that all operations are carried out properly and competently. This director shall have a good working knowledge of the scientific principles involved. The director shall be responsible for ensuring that employees are adequately trained in the work involved and have adequate practical experience and that they are aware of the application of accepted good practices to their respective functions. The director shall have the authority to enforce discipline among employees.

Personnel involved in quality control functions shall be separate from the staff involved in production and shall be responsible only to the director.

Where appropriate, personnel shall wear gowns, masks, boots, gloves, and eye protectors.

Personnel known to be carriers of specific pathogenic organisms (e.g., *Salmonella*, tuberculosis, viral hepatitis) shall be excluded from the production areas.

Personnel should be medically examined at regular intervals.

In view of the occupational hazard of infection with hepatitis virus, employees engaged in plasma fractionation should take special precautions against infection.

C.5. FRACTIONATION OF SOURCE MATERIALS

The general conditions for carrying out the fractionation procedure for preparing prophylactic or therapeutic plasma protein fractions from source material shall comply with good manufacturing practices[1] and shall be approved by the national control authority.

Most physical and chemical techniques of protein separation may be used for the preparation of plasma fractions, provided that the procedures lead to protein preparations that have been shown to be safe and effective.

Only fractionation procedures shall be used that give a good yield of products meeting the quality requirements of the international or national authorities. They shall be carried out in such a manner that the risk of microbiological contamination is reduced to a minimum.

The safety of fractionation steps may be increased by using protected or closed systems. The reproducibility may be increased by the use of automation.

The fractionation procedures used shall not significantly denature the proteins.

[1] WHO Official Records, No. 226, 1975, p. 88 (Annex 12: *Good practices in the manufacture and quality control of drugs*).

[1] WHO Official Records, No. 226, 1975, p. 88.

The biological characteristics (such as antibody activity, biological half-life, and *in vivo* recovery of the proteins) should not be affected to such an extent that the product is unacceptable for clinical use.

When possible, methods shall be used that exclude or inactivate disease-causing agents, particularly hepatitis virus, from the final products intended for clinical use.

Certain plasma fractions, such as Factor VIII and Factor IX concentrates and fibrinogen, cannot yet be manufactured in a manner that ensures their freedom from hepatitis viruses.

In certain geographical regions there may be other known microbial contaminants. Attempts should be made to determine that the fractionation procedure will either remove or inactivate such contaminants.

Placenta-derived source materials require further initial treatment. These materials must initially be handled in rooms separate from those in which other fractionation processes are carried out and using separate equipment.

The operating manual shall specify the times of sampling and the volumes to be taken at each stage of the process as well as the tests to be made on the samples.

Where appropriate, ancillary materials used for fractionation shall be controlled for microbiological contamination, identity, purity, pyrogenicity, and toxicity according to the international or national pharmacopoeia.

Equipment, procedures, and ancillary materials that may introduce allergenic components into the final product shall be avoided.

It is advisable to use air filtration to exclude airborne allergenic dust.

C.6. STORAGE, HEAT TREATMENT, AND INCUBATION OF BLOOD PRODUCTS

At all stages of the manufacturing process, the source materials and resulting fractions shall be stored at temperatures and under conditions shown to be adequate to prevent further contamination and growth of microorganisms, to protect the identity and the integrity of the proteins, and to preserve the biological activities and safety of the products.

There shall be clear demarcation in the storage of similar materials.

There shall be full identification at all times, including batch number of all in-process fractions and unlabelled final containers.

C.6.1 Heat treatment of albumin

Albumin in solution shall be heated in the final container to 60°C ±0.5°C and maintained at that temperature for 10 hours.

C.7. IN-PROCESS CONTROL

It must be recognized that the source materials are subject to biological variability and the products resulting from protein separation are variously contaminated with the other protein components of plasma. It is essential, therefore, to establish a monitoring system such that the safe operating limits of each process be maintained.

The main information collected comprises variations in physical conditions (temperature, pH, ionic strength, timing, etc.) and variations in the number and species of microbiological contaminants.

Owing to the number and interdependence of the factors involved, there are no universally accepted specifications for such in-process quality assurance systems. For this reason, continued information collection should be combined with data from previous experience, using the same process to ensure production control appropriate to the quality requirements of the final product.

C.8. RECORD KEEPING

Records shall be kept of the performance of all steps in the manufacture, quality control, and distribution of blood products and related substances.[1]

These records shall :

— be original (not a transcription), indelible, legible, and dated ;

— be made concurrently with the performance of each operation and test ;

— identify the person recording the data as well as the person checking the data or authorizing continuation of processing ;

[1] WHO Official Records, No. 226, 1975, p. 88.

— be detailed enough to allow a clear reconstruction and understanding of all relevant procedures performed ;
— allow tracing of all successive steps and identify the interrelationships of dependent procedures, products, and waste materials ;
— be maintained in an orderly fashion permitting the retrieval of data for a period consistent with dating periods and legal requirements ;
— indicate that processing and testing were carried out in accordance with procedures established and approved by the designated responsible authority ;
— if necessary, allow a prompt and complete recall of any particular lot ; and
— show the lot numbers of materials used for specified lots of products.

PART D:
REQUIREMENTS FOR THE CONTROL OF PLASMA FRACTIONS

D.1. INTRODUCTION

There are a number of requirements common to albumin, plasma protein fraction, immunoglobulins, and coagulation factor concentrates. It is therefore convenient to produce a single set of requirements, making specific recommendations for each product under the relevant sections.

D.2. TERMINOLOGY

Bulk purified material : powder or liquid material prepared by the fractionation of pooled source material.

Final bulk : a sterile solution or powder of material prepared from bulk purified material. It is used to fill the final containers. The final bulk must be provided with the respective batch number.

In some countries the final bulk is filled through a sterilizing filter.

Filling lot (final lot): a collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling

and (where appropriate) drying or other further processing. A filling lot must, therefore, have been filled and (where appropriate) dried in one working session. If the total final bulk is not filled in one session, it must bear a sub-batch number.

D.3. CONTROL OF BULK PURIFIED MATERIAL

D.3.1 Storage

The bulk material, either as liquid or as powder, shall be stored in sealed containers under conditions that minimize the multiplication of microbial agents as well as denaturation.

Powder free from the precipitating agent and containing less than 50 mg moisture per g of powder can be stored at up to 5°C.

D.3.2 Tests on bulk purified material

Tests on the purified bulk powder or solution shall be made if the manufacturer sends the material to another institution for further processing. Samples for these tests shall be taken under conditions that do not impair the quality of the bulk purified material. Tests shall be carried out on a specially dissolved sample processed to a stage equivalent to the final product, after sterile filtration. The tests shall be those listed in sections D.4.3 to D.4.9 inclusive, except that the tests for sodium and potassium (D.4.8 and D.4.9) are inappropriate for immunoglobulins. The sterility test on the dissolved sample of the bulk powder (section D.4.5) may be excluded.

For the control of immunoglobulins the specially dissolved sample shall be made at a concentration of 100–180 g/l.

D.4. CONTROL OF FINAL BULK SOLUTION

D.4.1 Preparation

The final bulk solution shall be prepared from bulk purified powder or by the dilution of concentrates by a method approved by the national control authority. The final bulk solution shall meet each of the requirements herein described (sections D.4.2 to D.4.10 inclusive) except that the tests for sodium and potassium (D.4.8 and D.4.9) are unnecessary

for immunoglobulins whereas the tests for aggregation and potency (D.4.11, D.4.12, and D.4.13) are applied only to the immunoglobulins.

The final bulk solution of normal immunoglobulins shall be made using material from a large number of donors (approximately 1000).

In the case of normal immunoglobulin a large number of donors is necessary in order to obtain adequate amounts of the various desired antibodies in the final product.

In the case of specific immunoglobulin the number of donors represented is less important because the particular antibody present will be controlled.

D.4.2 Preservatives and stabilizers

No preservative shall be added to the albumin, plasma protein fraction, or coagulation factor concentrates either during fractionation or at the stage of the final bulk solution.

To prevent protein denaturation, stabilizers shall be added. Such substances shall have been shown to the satisfaction of the national control authority to have no deleterious effect on the final product in the amounts present and to cause no untoward reactions in man.

Protein denaturation may be prevented by using, for example, either 0.16 mmol of sodium acetyltryptophanate or 0.08 mmol of sodium caprylate and 0.08 mmol of sodium acetyltryptophanate per g of albumin or plasma protein fraction.

Any stabilizers or preservative added to immunoglobulins either during production or in the final bulk shall have been shown to the satisfaction of the national control authority to have no deleterious effect on the final products in the amount present and to cause no untoward reactions in man. Antibiotics shall not be used as preservatives or for any other purpose in the fractionation of plasma.

Stable solutions of immunoglobulins may be prepared in approximately 0.3 mol/l glycine or 0.15 mol/l sodium chloride. Thiomersal or sodium timerfonate may be used as preservatives.

D.4.3 Concentration and purity

The albumin concentration in the final bulk albumin solutions shall be between 35 and 265 g/l. Not less than 96% of the proteins present shall be albumin as determined by a suitable electrophoretic method. The test shall be carried out on a sample both before and after heating (see section C.6.1).

The protein concentration in the final bulk plasma protein fraction solution shall be at least 35 g/l.

Plasma protein fraction contains at least 83% albumin and not more than 17% globulins. Not more than 1% of the protein in plasma protein fraction shall be gamma globulin.

The immunoglobulin concentration in the final bulk of normal and specific immunoglobulin preparations shall be 100-180 g/l. If in a specific immunoglobulin preparation the concentration is lower than 100 g/l, it shall require the approval of the national control authority.

The immunoglobulin shall be composed of not less than 90% of immunoglobulin G, as determined by a method approved by the national control authority.

The methods in most common use are radial immunodiffusion and electrophoresis.

D.4.4 Hydrogen ion concentration

The final bulk solution, diluted to 1% protein concentration with 0.15 mol/l sodium chloride, shall, when measured at a temperature of 20°C ±2°C, have a pH of 6.9 ±0.5 for albumin and immunoglobulin and 7.0 ±0.3 for plasma protein fraction.

D.4.5 Sterility

The final bulk material shall be tested for sterility. Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) [1] shall apply.

D.4.6 Freedom from undue toxicity and pyrogens

The final bulk material shall be tested for freedom from undue toxicity and absence of pyrogens by the methods described in sections D.6.4 and D.6.5.

A manufacturer introducing a change in an established fractionation procedure is advised to test the albumin products for undesirable hypotensive and hypertensive substances using the most suitable methods available.

[1] WHO Technical Report Series, No. 530, 1973, p. 48.

D.4.7 Stability

Sterile samples of the final bulk albumin and plasma protein fraction solutions, which have been heated for 10 hours at 60°C, shall remain visually unchanged after heating further at 57°C for 50 hours and when compared to a control sample that has been heated for only 10 hours at 60°C.

For the immunoglobulin solutions the test shall be made by heating an adequate sample at 57°C for 4 hours. No gelation or flocculation shall appear.

D.4.8 Sodium content

The final bulk albumin and plasma protein fraction solutions shall have a maximum sodium concentration of 160 mmol/l.

D.4.9 Potassium content

The final bulk albumin and plasma protein fraction solutions shall have a maximum potassium concentration of 2 mmol/l.

D.4.10 "Heme like" content

A sample taken from the final bulk and heated for 10 hours at 60°C shall, when diluted with water to 10 g protein per litre and placed in a cell with 1 cm light path, have an absorbance not exceeding 0.25 when measured in a spectrophotometer set at 403 nm.

In some countries the absorbance limit for this test may not be 0.25. In such countries the national control authority should specify the limit.

D.4.11 Determination of aggregated and fragmented molecules in immunoglobulins

Tests shall be made on the immunoglobulin solutions to determine the proportion of aggregated and fragmented immunoglobulin. The test and limits shall be approved by the national control authority.

Ultracentrifugation or gel filtration chromatography may be used, and other methods are being developed.

D.4.12 Potency test for normal immunoglobulin

Normal immunoglobulin shall be prepared from pooled material by a method that has been shown to be capable of concentrating tenfold from source material at least two different antibodies, one viral and one bacterial, for which an international standard or reference preparation is available [1] (e.g., antibodies against poliomyelitis virus, measles virus, streptolysin O, diphtheria toxin, tetanus toxin, staphylococcal α toxin). The immunoglobulin solution shall be tested at the protein concentration at which it will be present in the final ampoule.

Since preparations of normal immunoglobulin produced in different countries can be expected to differ in their content of various antibodies, depending on the antigenic stimulation to which the general population has been subjected (either by natural infection or by artificial immunization), at least two antibodies should be chosen for the potency test by the national control authority. The final bulk passes the test if it contains at least the minimum antibody levels required by the national control authority.

D.4.13 Potency tests of specific immunoglobulin

The potency of the final bulk shall be tested in respect of the particular antibody that the preparation has been specified to contain.

The final bulk of antitetanus human immunoglobulin passes the test if it contains at least 50 IU of tetanus antitoxin per millilitre, as determined by a neutralization protection test in animals.

The final bulk of antimeasles human immunoglobulin passes the test if it contains at least 50 IU of measles antibody per millilitre, as determined by a tissue culture neutralization test or a haemagglutination inhibition test.

The final bulk of antivaccinia human immunoglobulin passes the test if it contains at least 500 IU of vaccinia antibody per millilitre, in terms of the International Standard for Anti-Smallpox Serum, as determined by a neutralization test in eggs or in tissue culture.

The final bulk of antirabies human immunoglobulin passes the test if it contains at least 50 IU of rabies antibody per millilitre, in terms of the International Standard for Anti-Rabies Serum, as determined by a neutralization protection test in animals.

[1] The list of appropriate international standards and reference preparations is included in Appendix 4.

The estimated potency of the final bulk of anti-Rh_0 immunoglobulin shall be expressed in international units and shall be not less than 90% and not more than 120% of the stated potency, and the fiducial limits of error shall be within 80% and 125% of the stated potency.

The national control authority should specify the limits for other antibody concentrations.

D.4.14 Globulins for intravenous administration

Preparations of immunoglobulins intended for intravenous administration are now being made in several countries. Such preparations shall comply with all the specifications for normal and specific immunoglobulins unless otherwise agreed by the national authority. Certain additional tests will be required, and these should be specified by the national control authority.

D.5. FILLING AND CONTAINERS

The requirements concerning filling and containers given in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] shall apply.

Special attention shall be paid to the requirement that albumin and plasma protein fraction solutions in the closed final containers shall be heated within 24 hours after starting the filling to a temperature of 60°C ± 0.5°C and shall be maintained at that temperature for 10 hours, in order to inactivate any hepatitis B virus that may be present. In order to prevent protein denaturation a stabilizer shall be added to the albumin solution prior to heating (see section D.4.2).

D.6. CONTROL TESTS ON FINAL PRODUCT

D.6.1 Identity test

An identity test shall be performed on at least one labelled container from each filling lot to verify that the preparation contains protein of human origin only. The test shall be one approved by the national control authority.

[1] WHO Technical Report Series, No. 323, 1966, p. 16.

For immunoglobulins, as well as albumins and plasma protein fractions, additional tests shall be made to determine that the protein is predominantly immunoglobulin G or albumin respectively. The tests in section D.4.3 shall be used.

In the case of specific immunoglobulin an additional test shall be made to identify the specific antibody.

D.6.2 Protein concentration and purity

The protein concentration and purity of each filling lot shall be determined as described in section D.4.3.

D.6.3 Sterility

Each filling lot shall be tested for sterility. Part A, section 5, of revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) [1] shall apply. For albumin and plasma protein fraction, samples for sterility testing shall be taken from final containers drawn at random prior to the heating procedure at 60°C for 10 hours.

In some countries the sterility test is applied both before and after heating at 60°C for 10 hours.

D.6.4 Freedom from undue toxicity

Each filling lot shall be tested for freedom from undue toxicity by appropriate tests involving injection into mice and guineapigs. The tests shall be those approved by the national control authority.

The tests generally used are the parenteral injection of 0.5 ml into each of at least two mice weighing approximately 20 g and the intraperitoneal injection of 5.0 ml into each of at least two guineapigs weighing approximately 350 g. In some countries, if one of the animals dies or shows signs of ill health during the time specified, the test is repeated. The substance passes the test if none of the animals of the second group dies or shows signs of ill health in the time interval specified. For coagulation factor concentrates except fibrinogen, the test dose should not exceed 500 IU of the coagulation factor per kilogram of body weight of the test animal.

[1] WHO Technical Report Series, No. 530, 1973, p. 48.

The injection shall cause neither significant untoward reactions nor death within an observation period of 7 days.

D.6.5 Freedom from pyrogenicity

Each filling lot shall be tested for pyrogenicity by the intravenous injection of the test dose into three or more rabbits that have not previously received blood products. In general the dose shall be at least equivalent proportionately, on a rabbit body-weight basis, to the maximum single human dose recommended, but not more than 10 ml/kg body weight. For albumin 200 g/l and 250 g/l, the test dose for each rabbit shall be at least 3 ml/kg of body weight, for albumin 50 g/l and for plasma protein fraction the test dose shall be 10 ml/kg of body weight, and for immunoglobulins the dose shall be 1.0 ml/kg of body weight.

The criteria for passing the test shall be those specified by the national control authority.

Guidance for these criteria may be found in the International Pharmacopoeia.[1]

D.6.6 Determination of moisture

The residual moisture shall where appropriate be determined by a method approved by the licensing authority.

The methods in most common use are drying over phosphorus pentoxide for 24 hours at a pressure not exceeding 2.7 Pa (0.02 mmHg) and the Karl Fischer method. The acceptable level of moisture shall be determined by the national control authority.

D.6.7 Inspection of filled containers

All final containers of albumin and plasma protein fraction shall be stored at 20–35°C for at least 14 days following heat treatment at 60°C for 10 hours. At the end of this incubation period each final container shall be examined. Those showing abnormalities such as abnormal colour, turbidity, microbial contamination, or presence of atypical particles shall not be distributed.

When turbidity raises the possibility of microbial contamination, testing should be done to isolate and identify the micro-organisms.

[1] *Specifications for the quality control of pharmaceutical preparations : second edition of the International Pharmacopoeia.* Geneva, World Health Organization, 1967, p. 746, Appendix 43.

D.7. RECORDS

The requirements given in Part A, section 6, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] shall apply.

D.8. SAMPLES

The requirements given in Part A, section 7, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [2] shall apply.

D.9. LABELLING

The requirements for labelling given in Part A, section 8, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [2] shall apply, with the exception that the recommended human dose need not be specified on the label on the container, and with the addition of the following information.

For albumin and plasma protein fraction the label on the container shall show :

- the type of source material ;
- the protein concentration ;
- the oncotic equivalent in terms of plasma ;
- the absence of preservative ;
- the warning " Do not use if turbid " ;
- a warning to use within 4 hours of broaching ;
- the sodium and potassium concentrations.

For immunoglobulins the label on the container shall show :

- the type of source material ;
- the protein concentration ;
- (in the case of specific immunoglobulin) the content of specific antibody expressed in international units or equivalent national units ;

[1] WHO Technical Report Series, No. 323, 1966, p. 17.
[2] WHO Technical Report Series, No. 323, 1966, p. 18.

— the statement "For intramuscular use only" (if the immunoglobulins are not specially prepared for intravenous use).

The label on the package, or the leaflet in the package, shall in addition show:

— the recommended dose for each particular disease or condition;
— the fact that the preparation fulfils the requirements of this document.

D.10. DISTRIBUTION AND TRANSPORT

The requirements given in Part A, section 9, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] shall apply.

D.11. STORAGE AND EXPIRY DATE

The requirements given in Part A, section 10, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [2] shall apply.

D.11.1 Storage conditions and expiry date

(1) Final containers with albumin solution shall have a dating period of 3 years provided they are stored at a temperature not higher than 30°C.

(2) Final containers with albumin solution shall have a dating period of 5 years provided they are stored at 5°C ± 3°C.

Other storage conditions and expiry dates may be approved by the national authority.

(3) Final containers with plasma protein fraction solution shall have a dating period of 3 years provided they are stored at a temperature not higher than 30°C.

(4) Final containers with plasma protein fraction solution shall have a dating period of 5 years provided they are stored at 5°C ± 3°C.

Other storage conditions and expiry dates may be approved by the national authority.

[1] WHO Technical Report Series, No. 323, 1966, p. 18.
[2] WHO Technical Report Series, No. 323, 1966, p. 19.

(5) Storage of liquid immunoglobulin shall be at 5°C ± 3°C. Storage of freeze-dried preparations shall be below 25°C.

The expiry date of the liquid preparations shall be not more than 3 years from the date of the first satisfactory potency test on material in final containers.

The expiry date of the freeze-dried preparations shall be not more than 5 years from the date of the first satisfactory potency test on material in final containers.

D.12. CONTROL OF PREPARATIONS OF COAGULATION FACTOR CONCENTRATES

Factor VIII concentrates are prepared both as a frozen product and as a freeze-dried concentrate. The frozen products are usually derived from a single donation and consist of the cryoprecipitate from one donor prepared in a closed system of separation. The control of this product is covered in the section on whole blood and related substances (section B.3.5.4 for Factor VIII).

The freeze-dried Factor VIII concentrate may vary both in the number of donors contributing to a pool of cryoprecipitate and in the degree of purification of the product. Generally the small-pool product is subjected to little or no purification and is handled and subdivided in such a way that many control tests are inappropriate. Such preparations of frozen or freeze-dried cryoprecipitate from pools of fewer than 10 plasma donations should be controlled by the national control authority in a manner similar to that applied to the frozen product from single donations (section B.3.5.4).

Plasma source material for Factor VIII concentrates shall preferably be plasma frozen within 6 hours of collection from the donor or from frozen cryoprecipitate. Such material shall be kept frozen at such a temperature that the activity of the Factor VIII is maintained. Processing of the freshly thawed pooled cryoprecipitate material shall be completed within a few hours in order to preserve Factor VIII activity.

D.12.1 Tests on final containers common to other blood fractions

The following tests on the final containers of Factor VIII and Factor IX concentrates and fibrinogen shall apply.

(1) Sterility (see section D.6.3).
(2) Freedom from undue toxicity (see section D.6.4).

(3) Test for pyrogenicity (see section D.6.5). For Factor VIII each rabbit shall be injected with 10 IU per kg of body weight. For Factor IX each rabbit shall be injected with 50 IU per kg of body weight. For fibrinogen each rabbit shall be injected with 30 mg of protein per kg of body weight.

In addition the following requirements shall apply.

(1) Records : as given in section D.7.

(2) Samples : as given in section D.8.

(3) Labelling : the requirements given in section D.9 shall apply, with the exception that the recommended human dose need not be specified and with the addition of the following :

(*a*) content of the factor expressed in international units (IU) ;
(*b*) the amount of protein in the container ;
(*c*) the volume of diluent for reconstitution ;
(*d*) a warning concerning the possible transmission of hepatitis virus.

(4) Distribution and shipping : as given in section D.10.

(5) Storage and expiry date : final containers of the freeze-dried preparation of Factor VIII and Factor IX will have a dating period of not more than 2 years from the date of the first satisfactory potency test, provided they are stored at 5°C±3°C. Storage at ambient temperature during transport and on short trips will not result in loss of potency.

For fibrinogen the shelf-life is usually 5 years at 5°C ± 3°C. Other storage conditions and expiry dates may be approved by the national control authority provided they are consistent with the data on the stability of the products.

D.12.2 Special tests for coagulation concentrates

D.12.2.1 *Identity tests*

Tests for other animal protein. An identity test shall be performed on at least one labelled container from each filling lot to verify that the preparation contains protein of human origin only. The test shall be one approved by the national control authority.

Identity test for activity. An assay for Factor VIII or Factor IX activity or fibrinogen content, whichever is appropriate, shall be made to identify the product.

One of the potency tests described in sections D.12.3.1, D.12.4.1, and D.12.5.1 may serve as the identity test.

D.12.2.2 *Detection of hepatitis B virus*

Each filling lot shall be tested for hepatitis B surface antigen by the tests indicated in section B.1.3.2.

The most sensitive test available should be used for the detection of hepatitis B virus.

D.12.2.3 *Solubility and clarity*

Factor VIII preparations and fibrinogen shall dissolve completely in the solvent recommended by the manufacturer within 30 minutes when held at a temperature not exceeding 37°C. Factor IX preparation shall dissolve completely in the solvent recommended by the manufacturer within 10 minutes when held at 20–25°C. The solutions kept at room temperature shall not show any sign of precipitation or gel formation within 3 hours of dissolution of the coagulation factors.

D.12.2.4 *Protein content*

The amount of protein in a final container shall be determined by a method approved by the national control authority.

D.12.2.5 *Test for additives*

Tests to determine the concentration of additives (such as aluminium, heparin, poly(ethylene glycol), citrate, sodium, and glycine) used during production shall be carried out, if the national control authority requires them.

D.12.2.6 *Determination of moisture*

The residual moisture shall be determined by a method approved by the national control authority.

The methods in most common use are drying over phosphorus pentoxide for 24 hours at a pressure not exceeding 2.7 Pa (0.02 mmHg), and the Karl Fischer method. The products should not lose more than 2% in weight.

D.12.2.7 *Hydrogen ion concentration*

When the product is dissolved in a volume of water equal to the volume of water for injection stated on the label, the pH in the resulting solution shall be 7.2±0.4.

D.12.3 Special tests applicable to Factor VIII concentrates

D.12.3.1 *Potency test*

Each filling lot shall be assayed for Factor VIII activity by a test approved by the national control authority.

The national standard and the manufacturer's house standard should be a concentrate rather than a plasma because the former has better long-term stability and provides more homogeneous assay results, especially when the partial thromboplastin time test is used.

The specific activity shall be at least 100 IU per g of protein. The estimated potency is not less than 80% and not more than 125% of the stated potency. The confidence limits of error are not less than 64% and not more than 156% of the stated potency.

D.12.3.2 *Test for alloagglutinins*

A test shall be made for the presence of alloagglutinins by a method approved by the national control authority.

Although it is not possible to be specific about the tests for alloagglutinins or to specify an upper limit of titre in the coagulation factor preparations, nevertheless a test for alloagglutinins should be made and their presence declared on the leaflet.

D.12.4 Special tests applicable to Factor IX concentrates

D.12.4.1 *Potency test*

Each lot of the final product shall be tested for potency for Factor IX. The method used shall be approved by the national control authority.

Other coagulation factors may also be present in the final product, depending on the method of production, and products shall be tested for the presence of all coagulation factors claimed to be present at a therapeutic level. These may be Factor II, Factor VII, and Factor X. The methods used for their assay shall be approved by the national control authority.

D.12.4.2 *Test for the presence of activated coagulation factors*

The presence of activated coagulation factors shall be determined by a method approved by the national control authority.

In some countries the nonactivated partial thromboplastin times of normal plasma are measured after the addition of an equal volume of a number of different dilutions of the product under test.

In some countries a test for the presence of thrombin is made by mixing an equal volume of the product under test and fibrinogen solution. The mixture is held at 37°C and should not coagulate within 6 hours. The usual range of concentration of fibrinogen solution is 3-10 g/l.

D.12.5 Special tests applicable to fibrinogen

D.12.5.1 *Test for clottable protein*

Each filling lot shall be assayed for clottable protein by a test approved by the national control authority.

Usually the test consists of the addition of thrombin—with or without calcium chloride—to an appropriately diluted solution of fibrinogen and the determination of the clotting time.

The clotting time should be shorter than a given limit—usually 60 seconds—or should occur in not more than twice the time taken for clotting to occur in fresh normal plasma for the addition of the same amount of thrombin.

Not less than 70% of the total protein shall be clottable by thrombin.

PART E:
NATIONAL CONTROL REQUIREMENTS

E.1. GENERAL

The general requirements for control laboratories in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)[1] shall apply.

The national control authority shall provide the standards and reference preparations necessary for the quality control of human blood and blood products.

The national control authority shall approve the production and control methods used and give indications on all points left for its decision or approval in Parts A, B, C and D.

[1] WHO Technical Report Series, No. 323, 1966, p. 19.

The national control authority shall especially approve the use of materials that may carry any potential risk and shall approve any new method of production or the preparation of a new product.

New products or products prepared by new production methods should be monitored to determine their efficacy and safety before they are released.

E.2. RELEASE AND CERTIFICATION

Human blood and blood products shall be released only if they fulfil Parts A, B, C, and D, wherever applicable.

A statement signed by the appropriate official of the national control authority shall be provided at the request of the manufacturing establishment and shall certify whether or not the product in question meets all national requirements as well as Parts A, B, C, and D—whichever is relevant—of the present requirements. The certificate shall further state the date of the last satisfactory test for potency, if applicable, the number under which the lot is released, and the number appearing on the labels of the containers. In addition, a copy of the official national release documents shall be attached.

The purpose of this certificate is to facilitate the exchange of human blood and blood products between countries.

Appendix 1

LIST OF AUTHORS

The Requirements for the Collection, Processing and Quality Control of Human Blood and Blood Products were prepared by the following WHO consultants and staff members :

Dr J. N. Ashworth, Vice-President, Scientific Affairs, Cutter Laboratories, Berkeley, CA, USA (*Consultant*)

Dr D. R. Bangham, Head, Division of Hormones, National Institute for Biological Standards and Control, London, England (*Consultant*)

Dr L. F. Barker, Director, Division of Blood and Blood Products, Bureau of Biologics, Food and Drug Administration, Bethesda, MD, USA (*Consultant*)

Dr H. Eibl, Austrian Institute for Blood Products, Vienna, Austria (*Consultant*)

Dr F. Elsinger, Austrian Institute for Blood Products, Vienna, Austria (*Consultant*)

Dr J. P. Eygonnet, Mérieux Institute, Lyons, France (*Consultant*)

Dr J. Fischer, Behring Company, Marburg an der Lahn, Federal Republic of Germany (*Consultant*)

Dr H. Gunson, Oxford Regional Transfusion Service, Churchill Hospital, Oxford, England (*Consultant*)

Dr A. Hässig, Central Laboratory of the Swiss Red Cross Blood Transfusion Service, Berne, Switzerland (*Consultant*)

Professor S. Hollán, Director, National Institute of Haematology, Budapest, Hungary (*Consultant*)

Dr H. W. Krijnen, Director, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands (*Consultant*)

Dr H. Nevanlinna, Finnish Red Cross Blood Transfusion Service, Helsinki, Finland (*Consultant*)

Dr F. T. Perkins, Chief, Biologicals, World Health Organization, Geneva, Switzerland

Dr R. Plan, Mérieux Institute, Charbonnière-les-Bains, France (*Consultant*)

Dr H. K. Prins, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands (*Consultant*)

Dr J. D. van Ramshorst, Biologicals, World Health Organization, Geneva, Switzerland

Dr H. G. Schwick, Behring Company, Marburg an der Lahn, Federal Republic of Germany (*Consultant*)

Dr D. P. Thomas, Head, Division of Blood Products, National Institute for Biological Standards and Control, London, England (*Consultant*)

Mr J. Watt, Scottish National Transfusion Service, Protein Fractionation Centre, Edinburgh, Scotland (*Consultant*)