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WHO Expert Committee on Biological Standardization

Geneva, 13–20 October 1992

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Introduction

The WHO Expert Committee on Biological Standardization met in Geneva from 13 to 20 October 1992. The meeting was opened on behalf of the Director-General by Dr Hu Ching-Li, Assistant Director-General.

Dr Hu emphasized the importance of WHO's biological standardization programme for countries with developing health programmes and stressed the need for the Committee, in making recommendations, to take account of the procedures essential for assuring the safety and efficacy of biological products, but to avoid specifying unnecessarily stringent or restrictive conditions.

General

Needs for expert advice

The Committee identified an urgent need for additional expert assistance to enable it to fulfil its obligations in view of the rapid development of new biologicals, such as vaccines and therapeutic products prepared by recombinant DNA technology. It therefore requested the Secretariat to explore the organizational and financial means of establishing expert advisory groups for this purpose.

The Committee was informed that, in response to requests made in its thirty-eighth and fortieth reports (WHO Technical Report Series, No. 771, 1988, pp. 11-12, and No. 800, 1990, pp. 11-12), the WHO Secretariat had evaluated the need for reference materials for clinical diagnostic tests. It had concluded that a large number of such materials were required to ensure the accuracy and comparability of tests used for diagnosis, in the treatment of patients and for collecting epidemiological data.

In view of the vast number of diagnostic assays available and the need to conserve resources and avoid duplication of effort, the Committee recommended that WHO should consider convening a meeting of relevant units within the Organization and professional groups with expertise in the field, to advise the Committee on priorities for action and to recommend procedures for establishing reference materials for such tests.

The Committee was also informed that the number of cytokines and growth factors of clinical importance had continued to increase. Since 1988 an ad hoc committee of international experts has been assessing the need for, and overseeing provision of, international reference materials for these products. The Committee recommended the more formal establishment of an expert advisory group to review the results of international collaborative studies and to make recommendations to the Committee.

Distribution of International Biological Standards and Reference Reagents

The Committee noted the distribution of international reference materials by the International Laboratories for Biological Standards and other collaborating laboratories during 1991 (Table 1, BS/92.1693[1]) as well as the product categories and the categories of organization receiving materials. The Committee agreed that the programme was essential for the establishment of standards for biological products, which both national control authorities and manufacturers relied on to fulfil their responsibilities.

The Committee noted with concern the reductions made by WHO to the budgets of the International Laboratories and emphasized the continuing and increasing importance of their work. The Committee recognized with gratitude the efforts that the International Laboratories were making, from their own resources, to maintain their contribution to the biological standardization programme and the considerable support that they received from individual governments.

The Committee strongly recommended that the programme for International Biological Standards and Reference Reagents should be among WHO's priorities, but was also of the opinion that manufacturers and control authorities should be encouraged to contribute to its support.

Bovine spongiform encephalopathy

The Committee noted a report on a WHO meeting on public health issues related to animal and human spongiform encephalopathies (*Bulletin of the World Health Organization*, 1992, 70(2):183-190), which reviewed existing knowledge of the spongiform encephalopathies and evaluated the pathways of transmission and associated hazards. The possible implications of the animal diseases, especially bovine spongiform encephalopathy, with regard to the use of animal tissues as animal feed and human food and in the preparation of medicinal and other products for human use were discussed in the report, and recommendations made to national health authorities on appropriate measures to minimize the consequences of bovine spongiform encephalopathy for public and animal health.

The Committee agreed that bovine thromboplastin posed a theoretical risk to manufacturers and users in laboratories, and recommended that its use should be discouraged since rabbit thromboplastin was available as an alternative. The Committee further recommended that, wherever satisfactory alternatives existed, the use of products of bovine origin as international reference materials should be discouraged.

[1] References prefixed "BS/..." and "BLG/..." are to unpublished working documents of the World Health Organization. They are not issued to the general public, but a limited number of copies may be available to professionally interested persons on application to Biologicals, World Health Organization, 1211 Geneva 27, Switzerland.

Table 1

International Biological Standards and Reference Reagents distributed in 1991 to laboratories in WHO regions by the International Laboratories for Biological Standards and other collaborating laboratories

Recipient WHO region	International Laboratories		Other collaborating laboratories		Total	% of total for all regions
	Number of items distributed	% of total for all regions	Number of items distributed	% of total for all regions		
Africa	101	0.8	14	2.0	115	0.8
Americas	1 939	15.1	79	11.6	2 018	14.9
Eastern Mediterranean	147	1.1	0	0	147	1.1
Europe	8 988	69.9	444	65.0	9 432	69.6
South-East Asia	318	2.5	80	11.7	398	2.9
Western Pacific	1 144	8.9	66	9.7	1 210	8.9
Total	12 863[a]		683		13 546[a]	

[a] Includes 226 items distributed by the Central Veterinary Laboratory, Weybridge, Surrey, England, without identification of the recipient WHO region.

Laboratory methods for the potency testing of diphtheria, tetanus, pertussis and combined vaccines

The Committee noted a document (BLG/92.1) prepared by the Secretariat, in collaboration with a group of experts, which is intended to give guidance to vaccine manufacturers and national control laboratories for the potency testing of diphtheria, tetanus, pertussis and combined vaccines. In addition to describing the existing three-dilution methods, the document details alternative methods requiring the use of fewer laboratory animals yet capable of ensuring that the potencies of the vaccines meet requirements published by WHO. These alternative methods are based on immunization with single dilutions of test and reference vaccines, followed either by a challenge or by the assessment of serological responses by *in vitro* methods.

Proposal to modify the requirements for rabies vaccine to permit the release of inactivated vaccines on the basis of tests for antigen content

The Committee noted a report (BS/92.1688) prepared by the Secretariat in response to a proposal that the requirements for rabies vaccine should be modified to permit release of rabies vaccine on the basis of *in vitro* estimates of antigen content. The Committee was informed that the proposal was not universally welcomed because the antigen quantification tests, unlike the conventional NIH mouse test, did not seem to be able to detect the decrease in potency of rabies vaccines partially degraded by heat (BS/91.1654).

The Committee concluded that, although a simple *in vitro* test was desirable, it would be inappropriate at this time to replace the current NIH test. However, it requested the Secretariat to examine whether rabies vaccines could be released on the basis of another suitable test.

Antibiotics

Gramicidin

The Committee noted that, in accordance with the request made at its forty-second meeting (WHO Technical Report Series, No. 822, 1992, pp. 5–6), manufacturers had been approached for the supply of suitable materials to replace the International Reference Preparation of Gramicidin, stocks of which were depleted. It was anticipated that the dispensing into ampoules, assessment of homogeneity and characteristics, and short-term stability study of the proposed replacement preparation would be completed within a few months (BS/92.1694/5). A collaborative study would be arranged in association with the European Pharmacopoeia.

Gentamicin

The Committee noted that, in accordance with the request made at its forty-second meeting (WHO Technical Report Series, No. 822, 1992, p. 6), manufacturers had been approached for the supply of suitable materials to replace the International Reference Preparation of Gentamycin (International Nonproprietary Name: gentamicin), stocks of which were depleted. It was anticipated that the dispensing into ampoules, assessment of homogeneity and characteristics, and short-term stability study of the proposed replacement preparation would be completed within a few months (BS/92.1694/5). A collaborative study would be arranged in association with the European Pharmacopoeia.

Antibodies

In vitro methods for estimating antibodies to diphtheria and tetanus toxoid

The Committee noted a report on an inter-laboratory collaborative study of *in vitro* methods for estimating antibodies to diphtheria and tetanus toxoid (BS/92.1689). The study confirmed that *in vitro* assays such as enzyme-linked immunosorbent assays (ELISA) are unreliable for detecting levels of antibody close to the generally regarded minimal protective level of 0.01 International Units (IU) per ml.

The results of this limited study indicated that the antibody titration methods investigated could be used for determining the potency of toxoid vaccines by assessment of antitoxin responses in animals. The Committee noted that differences had been observed between certain methods when primary and booster immunization sera were compared.

In considering these issues, the Committee was concerned by the implications of a review (BS/92.1691) that questioned the validity of the current assay methods, and therefore proposed that the WHO Secretariat should consider convening a group of experts to review the situation and to make recommendations.

Anti-toxoplasma IgG and IgM antibodies

The Committee noted that, as requested in its forty-second report (WHO Technical Report Series, No. 822, 1992, p. 7), a proposed replacement preparation (TOXM 1-85) had been obtained for the second International Standard for Anti-Toxoplasma Serum, Human, and had been shown to have adequate stability (BS/92.1692). The Committee therefore requested the State Serum Institute, Copenhagen, to organize a collaborative study.

Rabies immunoglobulin

The Committee noted that, in accordance with the request made in its forty-second report (WHO Technical Report Series, No. 822, 1992, p. 7), a

proposed replacement preparation had been obtained for the International Standard for Rabies Immunoglobulin (BS/92.1690). A collaborative assay had been arranged to compare the International Standard with the proposed replacement preparation in mouse neutralization tests and rapid fluorescent-focus inhibition tests.

Tetanus immunoglobulin

The Committee noted that the collaborative study of the candidate reference material referred to in its forty-first report (WHO Technical Report Series, No. 814, 1991, p. 8) had been completed (BS/92.1696 & Addendum 1). The Committee established the preparation, a human immunoglobulin coded 26/488, as the International Standard for Tetanus Immunoglobulin, Human, and assigned a potency of 120 International Units of Tetanus Immunoglobulin, Human, to the contents of each ampoule.

Antigens

Inactivated poliovirus vaccine

The Committee noted an interim report (BS/92.1697) on the proposal to establish an International Reference Reagent for inactivated poliovirus vaccine to meet the need for improved standardization of potency assays for this vaccine. Two candidate reference materials had been donated by manufacturers and a two-phase collaborative study, including *in vitro* and *in vivo* potency assays, had been designed to assess the suitability of these materials.

Proposed international reference materials for measles, mumps and rubella vaccines

The Committee noted that candidate reference materials had been acquired for assays of mumps vaccines, measles vaccines and rubella vaccines. The Committee was informed that, although different strains were included in these vaccines, current information indicated that potency could be satisfactorily determined using either homologous or heterologous reference strains.

For mumps and rubella vaccines, the candidate reference materials contained the Urabe AM9 and RA-27/3 strains respectively, and had been lyophilized in sealed glass ampoules at the National Institute for Biological Standards and Control, Potters Bar. Preliminary tests had indicated that the materials were suitable in principle, and accelerated stability tests were in progress.

The Committee noted that the candidate material for measles vaccine was the Schwarz strain and that, because it had been lyophilized in vials, it did not comply with the "Guidelines for the preparation, characterization and

establishment of international and other standards and reference reagents for biological substances" (WHO Technical Report Series, No. 800, 1990, Annex 4).

HIV-1 p24 antigen

The Committee was informed of the need for an international reference material to permit the comparison of assays of the p24 antigen of human immunodeficiency virus type 1 (HIV-1). These assays have a role in establishing the diagnosis and prognosis for individuals infected with HIV-1 and in monitoring the effects of antiviral therapy. The Committee noted the results of a collaborative study (BS/92.1699).

The Committee established a preparation of HIV-1 p24 antigen isolated from detergent-treated HIV, in ampoules labelled 90/636, as the International Reference Reagent for HIV-1 p24 Antigen and assigned a potency of 1000 units to the contents of each ampoule.

Blood products and related substances

Plasma fibrinogen

The Committee noted that the preparation of human plasma fibrinogen referred to in its forty-first report (WHO Technical Report Series, No. 814, 1991, p. 10) had been shown to possess adequate stability (BS/92.1700). The Committee established the preparation, in ampoules labelled 89/644, as the International Reference Reagent for Plasma Fibrinogen, Human, with a content of 2.4 mg of fibrinogen per ampoule.

Ferritin

The Committee noted the results of the limited collaborative study (BS/92.1715) requested at its forty-second meeting (WHO Technical Report Series, No. 822, 1992, p. 12), which aimed at comparing the proposed replacement reference material and the existing International Standard for Liver Ferritin Protein, Human. The Committee established the preparation, in ampoules coded 80/578, as the second International Standard for Ferritin, Human, with a content of 9.1 µg per ampoule.

The Committee acknowledged the assistance of the International Council for Standardization in Haematology in the preparation of this material.

Blood coagulation factor VIII and von Willebrand factor in plasma

The Committee noted that, in accordance with its earlier request (WHO Technical Report Series, No. 822, 1992, p.11), the National Institute for Biological Standards and Control, Potters Bar, had obtained a suitable material for replacement of the second International Standard for Factor VIII and von Willebrand Factor in Plasma and that a collaborative study had been completed (BS/92.1709).

The Committee established the material studied, in ampoules coded 91/666, as the third International Standard for Blood Coagulation Factor VIII and von Willebrand Factor in Plasma, and assigned activities to the contents of each ampoule in terms of the four assay systems calibrated against the second International Standard: factor VIII clotting activity, 0.80 IU; factor VIII antigen, 0.90 IU; von Willebrand antigen, 0.96 IU; and von Willebrand ristocetin cofactor activity, 0.93 IU.

Noting concerns about the stability of the new International Standard, the Committee recommended that a small number of ampoules should be stored at -196 °C to provide baseline data for long-term stability studies.

The Committee also requested information about the preparation of the source plasma pool 91/666 (number of donors, distribution of ABO blood groups) that might be relevant for the use of the International Standard.

The Committee noted that the International Council for Standardization in Haematology (ICSH), the International Society of Hematology and the Steering Committee on Standardization in Coagulation Testing of the International Society on Thrombosis and Hemostasis, ICSH and the International Federation of Clinical Chemistry stipulated that "Manufactured reference preparations used in coagulation tests must always give the potency of the material in International Units of activity (per ml or per ampoule), related to the relevant WHO IRP [international reference preparation] where one exists. Manufacturers may additionally indicate the equivalent concentration in mol or mg/ml if they so wish."

Plasminogen-activator inhibitor-1 (PAI-1)

The Committee was informed that the collaborative study of a candidate reference material referred to in its forty-second report (WHO Technical Report Series, No. 822, 1992, p.10) had been completed. However, since only a small number of ampoules of preparation 87/512 remained, the National Institute for Biological Standards and Control, Potters Bar, now proposed to obtain a recombinant preparation with human plasma as a carrier and to organize a further collaborative study.

Activated factor VII concentrates

The Committee noted the results of a collaborative study to calibrate an international reference material for activated factor VII concentrates (BS/92.1701) and agreed to reconsider the matter at its next meeting after comments had been obtained from the participants.

Vitamin B_{12} in human serum

The Committee noted the report (BS/92.1703) of a collaborative study of a candidate reference material for vitamin B_{12} in human serum performed by the International Council for Standardization in Haematology. The Committee established the material studied, in ampoules marked 81/563,

as the first International Reference Reagent for Vitamin B_{12} in Human Serum, with a content of 320 pg of vitamin B_{12} per ampoule, based on the estimate provided by the ICSH.

Single-chain urinary-type plasminogen activator

The Committee noted that the International Standard for High Molecular Weight Urokinase referred to in its forty-second report (WHO Technical Report Series, No. 822, 1992, p.11) appeared to be unsuitable for immunological assays of single-chain urinary-type plasminogen activator (BS/92.1705). Further studies were in progress.

Apolipoprotein A-1 in human serum

The Committee noted that the International Federation of Clinical Chemistry Committee on Lipoproteins had organized a collaborative study of candidate reference materials for standardizing apolipoprotein A-1 measurements (BS/92.1706). The Committee established a material in screw-capped vials labelled SP1-01 as the International Reference Reagent for Apolipoprotein A-1 in Human Serum and noted that the contents of each container had been estimated at 1.66 mg of apolipoprotein A-1.

Thromboplastin

The Committee was informed of the need for an international reference material for recombinant human thromboplastin with an International Sensitivity Index. It would be necessary to decide whether species specificity was applicable to this material as it was to the natural materials of bovine (OBT/79), rabbit (RBT/79) and human (BCT/253) origin in the existing International Reference Preparations. The Committee asked the Secretariat to investigate the situation.

Haemoglobin F and haemoglobin A_2

At its thirty-eighth meeting (WHO Technical Report Series, No. 771, 1988, pp. 24-25), the Committee recognized the need for international reference materials for normal haemoglobin F and normal haemoglobin A_2. At its present meeting, the Committee reviewed previously uncirculated data from international collaborative studies of candidate materials coordinated by the International Council for Standardization in Haematology.

The Committee expressed concern about the possible need to establish several reference materials for haemoglobins F and A_2 for quality-assurance procedures; such materials might need to be related to primary standards. It requested the WHO Secretariat to seek additional information, including stability data, so that the matter could be considered at its next meeting. The Committee noted that the candidate reference materials for haemoglobin F and haemoglobin A_2 were available from the National Institute for Biological Standards and Control, Potters Bar.

Cytokines and growth factors

Basic fibroblast growth factor

The Committee noted that the collaborative study referred to in its forty-second report (WHO Technical Report Series, No. 822, 1992, p.15) had been completed and that the results were being analysed (BS/92.1708).

Nerve growth factor

The Committee was informed that a candidate reference material for nerve growth factor had been obtained and that a collaborative study was being arranged by the National Institute for Biological Standards and Control, Potters Bar, as requested in its forty-second report (WHO Technical Report Series, No. 822, 1992, p.15).

Epidermal growth factor

The Committee was informed that several candidate reference preparations of epidermal growth factor had been obtained and that a collaborative study was in progress to assess their suitability as reference materials for a range of different bioassays and immunoassays.

Platelet-derived growth factor

The Committee was informed that, because of differences in biological properties, separate international reference materials might be required for each of the three isoforms of platelet-derived growth factor. Attempts were being made to identify sources of suitable materials for dispensing into ampoules so that collaborative studies could be organized.

Granulocyte colony-stimulating factor

The Committee noted the results of the collaborative study of a candidate reference material for granulocyte colony-stimulating factor (BS/92.1711). The Committee established the material studied, in ampoules marked 88/502, as the International Standard for Granulocyte Colony-Stimulating Factor, Recombinant Human, for Bioassay, and assigned a potency of 10 000 International Units of Granulocyte Colony-Stimulating Factor, Recombinant Human, for Bioassay, to the contents of each ampoule.

Granulocyte/macrophage colony-stimulating factor

The Committee noted the results of the collaborative study of a candidate reference material for granulocyte/macrophage colony-stimulating factor (BS/92.1711). The Committee established the material studied, in ampoules marked 88/646, as the International Standard for Granulocyte/Macrophage Colony-Stimulating Factor, Recombinant

Human, for Bioassay, and assigned a potency of 10 000 International Units of Granulocyte/Macrophage Colony-Stimulating Factor, Recombinant Human, for Bioassay, to the contents of each ampoule.

Macrophage colony-stimulating factor

The Committee noted the results of the collaborative study of a candidate reference material for macrophage colony-stimulating factor (BS/92.1712). The Committee established the recombinant human material studied, in ampoules coded 89/512, as the International Standard for Macrophage Colony-Stimulating Factor, Recombinant Human, for Bioassay, and assigned a potency of 60 000 International Units of Macrophage Colony-Stimulating Factor, Recombinant Human, for Bioassay, to the contents of each ampoule.

The Committee was informed that the collaborative study had emphasized that this material could not be used as a standard for immunoassays without appropriate calibration of the system. In addition, since two similar recombinant materials had shown different specific activities, it had been concluded that similar products of the same mass might have different biological activities.

Interleukin-6

The Committee noted the report of an international collaborative study of a candidate reference material for interleukin-6 (BS/92.1713) previously referred to in its fortieth report (WHO Technical Report Series, No. 800, 1990, p. 24). The Committee established the recombinant human interleukin-6 studied, in ampoules labelled 89/548, as the International Standard for Interleukin-6, Recombinant Human, and assigned a potency of 100 000 International Units of Interleukin-6, Recombinant Human, to the contents of each ampoule.

Interleukin-9, interleukin-10 and interleukin-11

The Committee was informed that, in view of the need for international reference materials for interleukin-9, interleukin-10 and interleukin-11, the National Institute for Biological Standards and Control, Potters Bar, had obtained several candidate preparations and, subject to the demonstration of their stability, would organize collaborative studies.

Endocrinological and related substances

Follicle-stimulating hormone

The Committee was informed that a clinical trial of recombinant follicle-stimulating hormone was in progress and that there was a need for an international reference material. A candidate preparation had been

obtained by the National Institute for Biological Standards and Control, Potters Bar, and a collaborative study was being organized.

Inhibin

The Committee was informed that, in accordance with the request made in its forty-first report (WHO Technical Report Series, No. 814, 1991, p.11), the National Institute for Biological Standards and Control, Potters Bar, had obtained a candidate international reference material for recombinant human inhibin and that a collaborative study was under way.

Requirements for biological substances[1]

Requirements for Vi polysaccharide typhoid vaccine

After making some amendments to a draft prepared by the Secretariat (BS/92.1684), the Committee adopted the text as the Requirements for Vi Polysaccharide Typhoid Vaccine, and agreed that it should be annexed to its report (Annex 1).

Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives

The Committee studied a proposed revision of the Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives (BS/92.1685) that had been formulated by the participants at a consultation in 1991. After making a number of modifications, the Committee adopted the revised Requirements and agreed that they should be annexed to its report (Annex 2).

Requirements for measles, mumps and rubella vaccines and combined vaccine (live)

The Committee noted that the WHO Secretariat had prepared a revised draft of the proposed Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live), which had originally been considered at its forty-second meeting (WHO Technical Report Series, No. 822, 1992, p. 5). The revised document (BS/91.1653 Rev. 2) had been circulated to members of the WHO Expert Advisory Panel on Biological Standardization and a number of comments received.

After making some amendments, the Committee adopted the revised text as the Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live), and agreed that it should be annexed to its report (Annex 3).

[1] For a summary list of all the requirements for biological substances and other sets of recommendations, see Annex 8.

Requirements for rabies vaccine for human use

The Committee endorsed the recommendation of the WHO Expert Committee on Rabies (WHO Technical Report Series, No.824, 1992, p.12) that the minimum potency of inactivated rabies vaccines for human use derived from suckling-mouse brain tissues should be 1.3 IU per single human dose.

The Expert Committee on Rabies had also recommended that the WHO Expert Committee on Biological Standardization should consider revising the requirements for rabies vaccines to permit the release of cell-culture vaccines with potencies below the recommended minimum 2.5 IU per human dose, provided that these vaccines had been shown to elicit an adequate level of virus-neutralizing antibodies in clinical trials in humans. The Committee decided, however, to maintain its previous recommendation that purified cell-culture vaccines should contain at least 2.5 IU per single human dose. This level of vaccine potency has been found effective in the past for post-exposure treatment and the Committee considered that vaccines containing less than 2.5 IU per single human dose would not necessarily be protective.

A draft amendment (BS/92.1683) for the Requirements for Rabies Vaccine for Human Use (WHO Technical Report Series, No.658, 1981, Annex 2) had been prepared by the Secretariat, taking into account the recommended change in the minimum potency of suckling-mouse brain vaccines as well as other recent developments. After making some changes, the Committee adopted the amendment and agreed that it should be annexed to its report (Annex 4).

Requirements for rabies vaccine (inactivated) for human use produced in continuous cell lines

The Committee noted that a draft amendment (BS/92.1687) for the Requirements for Rabies Vaccine for Human Use Produced in Continuous Cell Lines (WHO Technical Report Series, No.760, 1987, Annex 9) had been prepared by the Secretariat to maintain consistency with the amended Requirements for Rabies Vaccine for Human Use. After making some changes, the Committee adopted the amendment and agreed that it should be annexed to its report (Annex 5).

Requirements for rabies vaccine for veterinary use

The Committee noted that a draft amendment (BS/92.1686) for the Requirements for Rabies Vaccine for Veterinary use (WHO Technical Report Series, No.658, 1981, Annex 3) had been prepared by the Secretariat. After making some changes, the Committee adopted the amendment and agreed that it should be annexed to its report (Annex 6).

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Annex 1
Requirements for Vi polysaccharide typhoid vaccine (Requirements for Biological Substances No. 48)

Introduction

Typhoid fever is still a common disease, causing significant morbidity and mortality in countries that have not yet achieved satisfactory control of drinking-water, food and sewage disposal (*1, 2*). Control of typhoid fever may be aided by immunization, but widespread protection by this method has not been achieved for various reasons, including the limitations and insufficient use of available vaccines (*3, 4*).

Typhoid fever is a septicaemic disease caused by a capsulated bacterium, *Salmonella typhi* (*S. typhi*), whose capsular polysaccharide is called the Vi polysaccharide. The success of immunization with purified capsular polysaccharides against invasive diseases caused by other capsulated bacteria such as *Neisseria meningitidis* groups A and C, pneumococci and *Haemophilus influenzae* type b provided background for the development of a Vi capsular polysaccharide vaccine for the prevention of typhoid fever.

Two controlled, double-blind, randomized field trials of the Vi polysaccharide typhoid vaccine (*5, 6*) have demonstrated protection against typhoid fever in areas with high endemicity.

The Vi polysaccharide elicits serum antibodies in mice (*7*) and in adult humans (*5, 6*). In clinical studies 25 μg or 50 μg of the Vi polysaccharide elicited a fourfold or greater rise in the level of serum antibodies in 95% of adults from France and the United States. The vaccine was less immunogenic in children and adults from Nepal (*5*) and the eastern Transvaal, South Africa (*6*), where a fourfold or greater rise in serum levels of anti-Vi antibodies was observed in only about 75% of the recipients.

The serum antibody responses to individual polysaccharides in multivalent vaccines consisting of four meningococcal polysaccharides (A, C, Y and W-135) or 23 pneumococcal polysaccharides (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F) are the same as those elicited by each polysaccharide administered alone (*8*). Manufacturers wishing to combine such multivalent polysaccharide vaccines with the *S. typhi* Vi polysaccharide should demonstrate, during the validation procedure required by the licensing process, that the serum antibody response to each polysaccharide component is not adversely affected by the presence of the other components. Furthermore, such multivalent products should meet the appropriate manufacturing and control requirements for each bacterial component.

As the Vi vaccine is already licensed and being used in several countries, it is appropriate to issue international Requirements for the manufacture of this product.

General considerations

Development of vaccines for typhoid fever has been hindered because the causative agent, *S. typhi*, infects and is pathogenic in humans only. Vaccines composed of whole, inactivated *S. typhi* have been available since the turn of the century. Their development followed the principle set forth by Pasteur, i.e. isolate, inactivate and then inject the pathogenic agent.

In the 1950s and 1960s, WHO sponsored trials of cellular vaccines manufactured according to protocols developed at the Walter Reed Army Institute of Research, USA. *S. typhi* was inactivated by one of two procedures: (1) heat and then treatment with phenol (L vaccines); or (2) treatment with acetone (K vaccines). In direct comparisons, both vaccines conferred protection in clinical trials, but the K-type was more effective (*9*). Later it was shown that potency assay, by mucin-enhanced intraperitoneal challenge in mice, could reliably predict the effectiveness of whole-cell vaccines (*10, 11*). Subsequently, experimental data showed that the K-type vaccine contained more Vi polysaccharide than the L-type, and that the resistance to challenge was mediated by the induction of

anti-Vi antibodies (*7, 11*). A vaccine containing the Vi polysaccharide was prepared for clinical evaluation. Because methods of purification at that time were not sufficient to remove contaminating lipopolysaccharide, which produced unwanted side-reactions, an investigation lot of Vi polysaccharide was prepared by heating in 1.0 mol/l acetic acid at 100 °C for 24 hours (*9*). The resultant product was less pyrogenic, reacted with Vi polysaccharide antiserum and elicited anti-Vi antibodies in mice and adult humans. It did not, however, confer protection on adult volunteers challenged with *S. typhi.* This method of preparation was later shown to remove *O*-acetyl and *N*-acetyl moieties and to reduce the molecular mass of the Vi antigen (*11*).

In 1985, two randomized, double-blind controlled clinical trials were conducted (*5, 6*) on a Vi vaccine manufactured by a method similar to that used for meningococcal polysaccharide vaccine. A single injection of the Vi or the control vaccine was administered. The first trial was conducted in Nepal in five villages outside Kathmandu, with participants aged from 5 to 44 years. The control group received the pneumococcal 23-valent vaccine. The protection rate for typhoid was about 70% for the two years of surveillance. The second randomized and controlled trial was conducted in eastern Transvaal, South Africa, with participants aged from 5 to 16 years. The control vaccine was the meningococcal groups A and C polysaccharide. A protection rate of about 60% for typhoid was shown for the ensuing 21 months. In both trials, an attack rate of typhoid fever of about 1% per year in the controls was recorded during the study. Following injection of the Vi vaccine, local reactions were slight and there were no serious systemic reactions such as high fever.

In terms of vaccine efficacy, these results are similar to those obtained with whole-cell inactivated vaccines and live Ty21a vaccines. Direct comparisons of efficacy in large-scale field trials have not been made.

The Vi polysaccharide is a linear homopolymer of (1→4)-linked *N*-acetyl-α-D-galactosaminuronic acid, acetylated at O-3 (*12*). It is present on almost all strains of *S. typhi* isolated from the blood of patients (*13*). Non-capsulated variants of *S. typhi* may be detected in stool cultures and from blood isolates after several passages of the strain in the laboratory. The Vi polysaccharide may also be detected in some strains of *S. paratyphi C, S. dublin* and *Citrobacter freundii* (*14*). Because of its chemical structure, the Vi polysaccharide vaccine is not normally susceptible to depolymerization; its integrity is maintained when the polysaccharide is kept at 25 °C for several months as a powder or in solution at pH 6.0–7.8.

As a consequence of the polysaccharide nature of the Vi antigen, the requirements for vaccine production and control contained in this document have many similarities with those included in the Requirements for Biological Substances No. 23 (Requirements for Meningococcal Polysaccharide Vaccine) (*15, 16*).

National control authorities should satisfy themselves that a Vi vaccine is

suitable for clinical use by reviewing safety and immunogenicity studies on the first production lots manufactured in accordance with the present Requirements. Such studies should be conducted whenever significant changes in production methods occur. To obtain statistically valid data on reactivity and immunogenicity, national control authorities should ensure that an adequate number of subjects are studied.

Each of the following sections constitutes a recommendation. The parts of each section printed in normal type have been written in the form of requirements, so that, if a health administration so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments or recommendations for guidance. Detailed descriptions of technical methods that may be used for the manufacture and control of Vi polysaccharide typhoid vaccine are given in the Appendix.

Should individual countries wish to adopt these Requirements as the basis for their national regulations concerning Vi capsular polysaccharide typhoid vaccine, it is recommended that modifications be made only on condition that the modified requirements ensure at least an equal degree of safety and potency of the product. It is desirable that the World Health Organization should be informed of any such changes.

Part A. Manufacturing requirements

A.1 Definitions

A.1.1 *International and proper name*

The international and proper name of the vaccine shall be "Vi polysaccharide typhoid vaccine", translated into the language of the country of use. The use of this name shall be limited to vaccines that satisfy the requirements formulated below.

A.1.2 *Descriptive definition*

Vi polysaccharide typhoid vaccine shall consist of purified Vi polysaccharide. The polysaccharide shall be prepared from a strain of *Salmonella typhi* that satisfies the requirements of section A.3.1.1, and shall be purified by the methods outlined in section A.3.3 of these Requirements. The Vi polysaccharide typhoid vaccine shall satisfy all the requirements formulated in sections A.3–A.5 (inclusive).

A.1.3 *Reference materials*

The Vi polysaccharide typhoid vaccine is controlled by examination of physical and chemical characteristics. An international reference material is therefore not needed. In-house standards are required for determining identity and comparing immunochemical activity, and should be stored desiccated at a temperature below -20 °C to prevent degradation.

A.1.4 *Terminology*

Master seed lot: A bacterial suspension of living *S. typhi* organisms, stored as aliquots derived from a strain that has been processed as a single lot and has a uniform composition. The master seed lot is used for preparing working seed lots. It shall be maintained in the freeze-dried form or at a temperature not higher than -45 °C.

Working seed lot: Living *S. typhi* organisms derived from the master seed lot by growing the organisms and maintaining them in aliquots in the freeze-dried form or at a temperature not higher than -45 °C.

Single harvest: The material obtained from one batch of cultures that have been inoculated with an inoculum derived from the working seed lot, harvested and processed together.

Purified Vi polysaccharide lot: The material obtained after final purification. The lot of purified Vi polysaccharide may be derived from a single harvest or a pool of single harvests processed together.

Final bulk: The homogeneous material derived from a purified Vi polysaccharide lot or pool and present in a single container from which the final containers are filled. The final bulk may be used to produce one or more final lots.

Final lot: A collection of sealed final containers that have been filled from a single container in a single continuous working session, are uniform in their contents, and are homogeneous with respect to the risks of contamination during filling and, if appropriate, freeze-drying.

Stability: The capacity of Vi polysaccharide typhoid vaccine to remain unmodified during storage under specified conditions, so that its composition, characteristics and immunological properties do not change. The stability of the vaccine is checked by recording the molecular size and *O*-acetyl content of the polysaccharide from at least three final lots (each derived from a different final bulk) stored at the recommended temperature for a specified period of time (see section A.6). This information is used to ensure that the product meets the validity-period specification (see section A.9).

Validity period and expiry date: The validity period is the maximum time permitted by the national control authority for storage and use of the product after the final containers have been filled. The expiry date is set after stability studies have been performed on the vaccine (see section A.6); it is normally the date after which it is no longer possible to guarantee that the vaccine meets each of the manufacturing and control requirements.

A.2 General manufacturing requirements

The requirements of Good Manufacturing Practices for Pharmaceutical Products (*17*) and Good Manufacturing Practices for Biological Products

(*18*) shall apply to establishments manufacturing Vi polysaccharide typhoid vaccine. Other biological products, in particular other polysaccharide vaccines, shall not be manufactured at the same time in the same area. If other polysaccharide vaccines are manufactured at other times in the same area, the rooms and equipment shall be cleaned and decontaminated by methods shown to be effective in removing polysaccharides whenever production is switched to a different vaccine. The Vi polysaccharide shall be purified under clean, but not necessarily sterile, conditions.

Details of the standard operating procedures adopted by the manufacturer for the preparation and testing of Vi polysaccharide typhoid vaccine, with appropriate validation of each production step, shall be submitted for approval to the national control authority. Data on stability at different stages of production shall be obtained so that "intermediate" products are stored under adequate conditions.

Proposals for modifications, if any, of the manufacturing or control methods shall also be submitted for approval to the national control authority.

Personnel in the production and control facilities shall be adequately trained and protected, including by immunization, against accidental infection with *S. typhi.*

A.3 Production control

A.3.1 *Control of source of materials*

A.3.1.1 Strains of S. typhi

The strain of *S. typhi* used for production of the Vi polysaccharide shall be approved by the national control authority. The strain shall have been shown to be capable of producing the Vi polysaccharide.

> *S. typhi* Ty2 has been shown to be a suitable strain.

The cultures shall have the following characteristics: (1) stained smears made from a culture shall be typical of *S. typhi*; (2) the cultures shall utilize glucose without production of gas; (3) the colonies on agar shall be oxidase-negative; (4) a suspension of a culture shall be agglutinated specifically with an appropriate anti-Vi antiserum or colonies shall form haloes on an antiserum-containing agar plate (*19*).

A.3.1.2 Seed lot system

The production of Vi polysaccharide typhoid vaccine shall be based on a seed lot system. The master seed lot used shall be identified by a record of its history, including the source from which it was obtained, and by its biochemical and serological characteristics. Cultures derived from the working seed lot shall have the same characteristics as cultures of the strain from which the master seed lot was derived. Seed lots shall comply with the requirements of section A.3.2.

Ultrafiltered tryptic soya-bean medium may be used in the preparation of working seed lots. A medium containing 50 g/l monosodium glutamate and 50 g/l bovine plasma albumin is suitable for drying or preserving master or working seed lots in the frozen state. If bovine albumin is used it should come from herds certified free from bovine spongiform encephalopathy and bovine leukosis. In some countries bovine albumin is replaced by skimmed milk.

A.3.1.3 Culture media for vaccine production

The fluid culture medium used for vaccine production shall be free from ingredients that form a precipitate upon addition of hexadecyltrimethylammonium bromide at the concentration used by the manufacturer. It shall also be free from adventitious substances that may give rise to sensitization in humans, such as blood-group substances, unless such substances are removed by the purification process.

A semi-synthetic medium (*15, 16, 20, 21*) supplemented with 5 g/l dialysate of yeast extract may be used. The basal medium may be autoclaved but the supplement, consisting of yeast extract dialysate, magnesium sulfate and glucose, should be sterilized by filtration.

A.3.2 ***Single harvests***

The growth of *S. typhi* shall be shown to be consistent by monitoring the growth rate of the bacteria, the pH of the culture and the yield of Vi polysaccharide.

Cultures will normally grow satisfactorily between 35 °C and 37 °C. The working seed lot may be plated on solid medium, and after 12–18 hours of incubation the culture may be used as an inoculum for liquid precultures. As the precultures reach the stationary phase, they may be used as an inoculum for the production tanks, and the production cultures may be harvested during the early stationary phase.

A.3.2.1 Control of bacterial purity

Samples of the culture taken before inactivation shall be tested for contamination by microscopic examination of Gram-stained smears and by inoculation into appropriate media. Several microscopic fields shall be examined at high magnification such that at least 10 000 organisms are examined. If any contaminants are found, the culture and any product derived from it shall be discarded.

A.3.3 ***Purification of Vi polysaccharide***

After inactivation with formalin, the organisms are separated from the culture and the Vi polysaccharide is precipitated from the culture fluid by hexadecyltrimethylammonium bromide.

All steps in the purification shall be carried out in containers compatible with the solvents in use. Analytical-grade reagents, or their equivalent, shall be employed, and aqueous solutions of salts shall be sterilized, for example by filtration through a membrane of pore size not greater than 0.22 µm. All purification steps shall be carried out in the cold with chilled

reagents, except for phenol treatment, which is conducted at room temperature. Either the time intervals between purification steps shall be kept to the minimum, or the products shall be kept frozen at or below -20 °C between steps. After addition of hexadecyltrimethylammonium bromide, the precipitate shall be collected.

The Vi polysaccharide shall be purified from its complex with hexadecyltrimethylammonium bromide. The material resulting from this preliminary purification is referred to as the "intermediate product".

The dry product obtained after final purification constitutes a purified Vi polysaccharide lot. It shall be handled with sterile precautions and stored at a temperature below 8 °C.

> The cold phenol method (*21*), as described in the Requirements for Biological Substances No. 23 (Requirements for Meningococcal Polysaccharide Vaccine) (*15, 16*), has been found to be suitable.

The method of purification and the limits on residual chemicals in the purification process shall be approved by the national control authority.

The following tests shall be applied to each purified Vi polysaccharide lot; measurements shall be based on the dry weight of the polysaccharide in its salt form.

A.3.3.1 Moisture content

The moisture content of the purified polysaccharide shall be determined.

> Thermogravimetric analysis at 100 °C is generally used. In laboratories lacking facilities for thermogravimetric analysis, the moisture content may be determined by the Karl Fischer method, or by drying the polysaccharide under vacuum over phosphorus pentoxide at 37 °C until a constant weight is reached.

A.3.3.2 Protein content

Each purified Vi polysaccharide lot shall contain less than 10 mg of protein per gram of polysaccharide as determined by the method of Lowry et al. (*22*) using bovine plasma albumin as a reference.

A.3.3.3 Nucleic acid content

Each purified Vi polysaccharide lot shall contain less than 20 mg of nucleic acid per gram of polysaccharide as determined by ultraviolet spectroscopy, on the assumption that, at a wavelength of 260 nm, the absorbance of a 1 g/l solution of nucleic acid contained in a cell 1 cm wide is 20 (*15, 16*).

A.3.3.4 O-acetyl content

The *O*-acetyl content of each purified Vi polysaccharide lot shall be not less than 2.0 mmol/g of Vi polysaccharide as determined by a method based on that described by Hestrin (*23*) (see Appendix).

A.3.3.5 Molecular size

The molecular size of each purified Vi polysaccharide lot shall be estimated by gel filtration (*15, 16*).

Sepharose CL-4B, with 0.2 mol/l sodium chloride as eluent, is suitable for this purpose.

At least 50% of the Vi polysaccharide from the column shall elute before a distribution constant (K_D) of 0.25 is reached (see Appendix). The concentration of Vi polysaccharide in eluted fractions is measured by the method of Hestrin (*23*).

As there is a relationship between immunogenicity and molecular size, manufacturers are encouraged to produce Vi polysaccharide with even lower K_D values (higher molecular masses) (*15, 16, 24*).

A.3.3.6 Identity

The identity of the Vi polysaccharide lot with an in-house standard Vi polysaccharide conforming to the purity requirements of sections A.3.3.2–3.3.5 shall be established by immunoprecipitation.

Hyperimmune serum prepared by multiple intravenous injections of *Citrobacter freundii*, a non-pathogenic organism that has a Vi polysaccharide identical to that of *S. typhi*, has proved reliable for serological assays.

A.3.4 ***Final bulk***

The final bulk shall be prepared from either a single lot of purified Vi polysaccharide or several pooled lots. If multi-dose vials are used, a preservative shall be added. The amount of preservative in the final bulk shall have been shown to have no deleterious effect upon the polysaccharide or upon other vaccine components with which the Vi polysaccharide may be combined. The preservative and its concentration shall be approved by the national control authority.

The final bulk shall be dissolved under aseptic conditions in a solution free of pyrogenic substances and shall be sterilized by filtration through a membrane.

Membranes with a pore size of 0.22 µm have been found to be satisfactory.

The osmolality of the final product shall be such that vaccines to be injected are isotonic with serum.

A.3.4.1 Sterility

The final bulk shall be tested for bacterial and mycotic sterility according to the requirements given in Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*25*), or by a method approved by the national control authority. If a preservative has been added to the product, appropriate measures shall be taken to prevent any interference by the preservative in the sterility test.

A.3.4.2 Vi polysaccharide concentration

The final bulk may be assayed for its polysaccharide content by a quantitative immunochemical test approved by the national control authority.

A.3.4.3 Identity

The identity of the final bulk shall be established by a serological assay based on immunoprecipitation, as described in section A.3.3.6.

A.4 Filling and containers

The requirements concerning filling and containers given in Good Manufacturing Practices for Biological Products (*18*) shall apply.

A.5 Control tests on final lot

The following tests shall be carried out on each final lot of Vi polysaccharide typhoid vaccine.

A.5.1 *Identity*

An identity test shall be performed by a serological method (see section A.3.3.6) on at least one labelled container.

A.5.2 *Vi polysaccharide content*

At least three final containers, taken at random, shall be assayed for Vi polysaccharide content by a quantitative immunochemical test in comparison with a reference material conforming to the purity requirements of sections A.3.3.2 and A.3.3.5 and approved by the national control authority. The national control authority shall approve the extent of deviation from the target value that is acceptable for each of the three estimates of Vi polysaccharide content.

> The target value is 25 µg of Vi polysaccharide per single human dose (*5, 6*). In one country, the maximum authorized deviation of each estimate is 30% from the target value.

A.5.3 *Sterility*

Each final lot shall be tested for sterility as specified in section A.3.4.1.

A.5.4 *Pyrogenicity*

Each final lot shall be tested for pyrogenicity by intravenous injection into rabbits. The national control authority shall define acceptable pyrogen levels.

The test shall be conducted as specified in *The international pharmacopoeia* (*26*) or in the national pharmacopoeia.

> A test that has been found suitable for the current vaccine involves injection into the ear vein of rabbits of 1 ml per kg of body weight of a dilution of vaccine containing 25 ng per ml.

A.5.5 *Abnormal toxicity*

Each final lot shall be tested for abnormal toxicity by the intraperitoneal injection of one human dose into each of five mice (weighing 17–22 g)

and at least one human dose into each of two guinea-pigs (weighing 250–350 g). The tests shall be approved by the national control authority. The final product shall be considered satisfactory if the animals survive for at least seven days without weight loss.

A.5.6 *Preservative content*

If preservative has been added to the bulk, the preservative content of each final lot shall be determined. The method used shall be approved by the national control authority.

A.5.7 *Residual moisture*

If the vaccine is freeze-dried, the moisture content of the dried material shall be determined as indicated in section A.3.3.1. The method used shall be approved by the national control authority.

The test shall be performed on 1 vial per 1000 up to a maximum of 10 vials, but on not less than 5 vials, taken at random from throughout the final lot. The average residual moisture shall be not greater than 2.5%, and no vial shall be found to have a residual moisture content of 3% or greater.

A.5.8 *pH*

The pH of each final lot shall be determined.

> A pH of 7 ± 0.5 measured at ambient temperature has been found to be compatible with vaccine stability.

A.5.9 *Inspection of final containers*

Every container in each final lot shall be inspected, and those showing abnormalities, such as improper sealing, lack of integrity and, if applicable, clumping or the presence of particles, shall be discarded.

A.6 Stability studies

Stability studies are needed for determining the expiry date of the Vi polysaccharide typhoid vaccine. They shall be based on tests for *O*-acetyl content and molecular size (see sections A.3.3.4 and A.3.3.5) and shall be conducted on at least three final lots, each derived from a different purified Vi polysaccharide lot, stored under the conditions to be recommended in the leaflet of instructions to the user. In the molecular-size assay, at least 50% of the Vi polysaccharide from the column shall elute before a K_D of 0.25 is reached. The tests for stability shall be approved by the national control authority.

> Liquid Vi polysaccharide vaccine may require concentration before the molecular-size assay is performed.

Whenever significant changes are made in the production of Vi polysaccharide typhoid vaccine, further stability studies shall be conducted to determine the proposed validity period of the product.

A new validity period may be approved by the national control authority on the basis of the results of validated accelerated-degradation tests.

A suitable method for conducting accelerated-degradation tests on certain biological products has been described by Kirkwood (*27*).

A.7 Records

The requirements of Good Manufacturing Practices for Biological Products (*18*, pages 27-28) shall apply.

A.8 Samples

The requirements of Good Manufacturing Practices for Biological Products (*18*, page 29, paragraph 9.5) shall apply.

A.9 Labelling and package insert

The requirements of Good Manufacturing Practices for Biological Products (*18*, pages 26-27) shall apply, with the addition of the following:

The statements concerning storage temperature and expiry date shall be based on experimental evidence of stability obtained as specified in section A.6, and shall be submitted for approval to the national control authority.

The expiry date on the label shall be based upon experimental data but shall in any case not be more than three years from the date the final containers are filled.

Storage at a temperature of 2–8 °C has been found to be suitable by one manufacturer.

The label on the carton or the leaflet accompanying the container shall indicate:

- the amount of Vi polysaccharide in each single human dose;
- the volume and nature of the reconstituting fluid, if applicable.

Furthermore, the leaflet accompanying the container shall contain:

- a statement that, once a freeze-dried product has been reconstituted or a multi-dose container of liquid product opened, the vaccine should be used within a working day;
- a statement of the temperature at which the Vi polysaccharide typhoid vaccine should be stored.

A.10 Distribution and shipping

The requirements of Good Manufacturing Practices for Biological Products (*18*) shall apply.

Part B. National control requirements

B.1 General

The Guidelines for National Authorities on Quality Assurance for Biological Products (*28*) shall apply.

The national control authority shall approve:

- the strain(s) of *S. typhi* from which the Vi polysaccharide typhoid vaccine is produced;
- the method of manufacture;
- the methods of purification;
- the preservative and its concentration, if applicable;
- the Vi polysaccharide content of each single human dose and the method of assay; and
- the tests for stability, the validity period and the expiry date.

B.2 Release and certification

A vaccine lot shall be released only if it fulfils the national requirements and/or Part A of the present Requirements. A summary protocol for vaccine production and testing, signed by the responsible official of the manufacturing establishment, shall be prepared and submitted to the national control authority in support of a request for release of vaccine for use.

At the request of the manufacturing establishment, the national control authority shall provide a certificate that states whether the vaccine meets all national requirements and/or Part A of the present Requirements. The certificate shall also state the number under which the lot was released by the national controller, and the number appearing on the labels of the final containers.

> The purpose of the certificate is to facilitate the exchange of Vi polysaccharide typhoid vaccine between countries. The certificate may be prepared along the lines of those required for the release of vaccines acquired by United Nations agencies (*29*).

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Appendix

Examples of suitable methods and tests for standardizing Vi polysaccharide typhoid vaccines[1]

1. Determination of the *O*-acetyl group in the Vi polysaccharide

The method described in this section is based on that described by Hestrin (*1*).

Instrumentation

A spectrophotometer for the visible region of the spectrum is required.

Mechanism

O-Acetyl groups react with hydroxylamine in alkali to form hydroxamic acid. The hydroxamic acid formed is measured by the formation of a purple-brown complex with Fe^{3+} in acid solution.

Reagents

The following reagents are needed:

1. Hydroxylamine hydrochloride, 2 mol/l, which should be stored in the cold.
2. Sodium hydroxide (NaOH), 3.5 mol/l.
3. Concentrated hydrochloric acid (HCl), relative density 1.18, diluted with twice its volume of water.
4. Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), 0.37 mol/l, in 0.1 mol/l hydrochloric acid.
5. Sodium acetate, 0.001 mol/l, pH 4.5.
6. A standard solution of acetylcholine chloride (relative molecular mass 181.7).
7. Vi polysaccharide solution.

Preparation of standard dilutions and blanks

To prepare the standard solution of acetylcholine chloride (reagent 6), dissolve 150 mg of acetylcholine chloride in 10.0 ml of 0.001 mol/l sodium acetate. Transfer 1 ml of this solution to a test-tube and mix it with 9 ml of 0.001 mol/l sodium acetate. From this solution, accurately transfer 0.1, 0.2, 0.3, 0.4 and 0.5 ml quantities in duplicate to 10 test-tubes, and add respectively 0.9, 0.8, 0.7, 0.6 and 0.5 ml of 0.001 mol/l sodium acetate to make the volume in each tube 1.0 ml. The *O*-acetyl concentrations in these standard tubes are respectively 0.83, 1.66, 2.48, 3.32 and 4.15 µmol/ml. The duplicate at each concentration is used as a standard blank.

[1] Draft prepared by Dr C. Frasch, Chief, Laboratory of Bacterial Polysaccharides, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA.

After addition of reagents, as detailed in the procedure below, the absorbances of the standard solutions at 540 nm are about 0.1, 0.25, 0.4, 0.5 and 0.65. The precise wavelength at which the readings should be made is the wavelength at which the net absorbance between 520 and 540 nm (i.e. that of the standard minus that of the corresponding blank) is the highest.

Test procedure

The test procedure is carried out at room temperature. The *O*-acetyl content of the Vi polysaccharide sample is determined by reference to the standard dilutions of acetylcholine chloride. All samples, standard dilutions and blanks are tested in duplicate.

1. Prepare the sample for analysis by making up a 1.0 g/l solution of the polysaccharide in water. For each duplicate test polysaccharide use 1 ml of this solution.
2. To 1 ml of the polysaccharide sample and to each of the standard dilutions add 2 ml of a freshly prepared (within three hours) mixture of equal parts of reagents 1 and 2 and mix.
3. After exactly four minutes at room temperature, add 1 ml of reagent 3 to bring the pH to 1.2 ± 0.2 and mix.
4. Add 1 ml of reagent 4 and mix.
5. Swirl the contents of the test-tubes rapidly during the addition of each of the above reagents.
6. For the blank solutions (one blank for each concentration of the standard and a sample blank of 1 g/l Vi polysaccharide solution) carry out step 3 before step 2, i.e. add the hydrochloric acid before the NaOH-hydroxylamine solution. In Hestrin's method the blank determination is called the "test for non-specific colour".
7. Promptly read the absorbance of the solution in each tube at the appropriate wavelength.
8. Subtract the sample blank reading from that of the test sample and subtract the standard blank readings from those of the standard dilutions.
9. For the standard dilutions, plot a curve of absorbance against *O*-acetyl concentration in µmol/ml. From the values of absorbance obtained for the sample, the corresponding number of micromoles of *O*-acetyl per milligram of Vi polysaccharide can then be read off the reference curve (since the test was conducted on 1 ml of the Vi polysaccharide solution, which contains 1 mg of Vi polysaccharide).

The weight of Vi polysaccharide used in the 1 g/l polysaccharide solution is corrected for its moisture content and the final result is expressed in "millimoles of *O*-acetyl per gram of dry polysaccharide".

Example: Moisture content = 10%. Thus 1 mg of "wet" polysaccharide comprises 0.1 mg of water and 0.9 mg of "dry" polysaccharide. If for

every milligram of "wet" polysaccharide there are 1.95 micromoles of *O*-acetyl, then:

$$\frac{1.95\ \mu\text{mol } O\text{-acetyl}}{1\ \text{mg "wet" polysaccharide}} = \frac{1.95\ \mu\text{mol } O\text{-acetyl}}{0.9\ \text{mg "dry" polysaccharide}}$$

$$= 2.17\ \text{mmol } O\text{-acetyl per gram of "dry" polysaccharide.}$$

2. Molecular sizing of Vi polysaccharide by Sepharose CL-4B gel permeation

The method used for molecular sizing is based on the protocol described in the Requirements for Meningococcal Polysaccharide Vaccine (*2, 3*).

Reagents

The following reagents are needed:
- Sepharose CL-4B gel
- sodium chloride, 0.2 mol/l, as eluent
- sodium azide
- Blue Dextran 2000
- reagents for *O*-acetyl determination by the method of Hestrin (*1*).

Equipment

The following equipment is required:
- column (1.5 × 90 cm)
- automatic fraction collector and test-tubes
- gel and eluent reservoir
- refractometer or other appropriate monitoring device and recorder (optional).

Procedure

Preparation of the gel-filtration column

Prepare the column according to the recommendations of the supplier.

Calibration of the column

The void volume (V_0) is determined with Blue Dextran and the total volume (V_t) with sodium azide. Dissolve 200 mg of Blue Dextran in 100 ml of 0.2 mol/l sodium chloride solution containing 500 mg of sodium azide. Apply 1 ml of this solution to the column and collect fractions of about 2 ml each. Determine V_0 and V_t either by refractometry or by ultraviolet absorption at 206 nm or 260 nm.

Assay of the purified Vi polysaccharide

Dissolve 5 mg of Vi polysaccharide in 1 ml of eluent. Apply this solution to the column and collect 2-ml fractions. Make two pools of fractions:

– pool I: fractions eluted before a distribution constant (K_D) of 0.25 is reached,

– pool II: fractions eluted after a K_D of 0.25 is reached.

Quantify the Vi polysaccharide in each pool by determining the *O*-acetyl content by the method of Hestrin (*1*).

At least 50% of the recovered Vi polysaccharide shall be eluted before a K_D of 0.25 is reached.

References

1. Hestrin S. The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine and its analytic applications. *Journal of biological chemistry,* 1949, 180:249–261.

2. Molecular sizing of capsular polysaccharide antigens by Sepharose 4B column chromatography. In: *WHO Expert Committee on Biological Standardization. Twenty-seventh Report.* Geneva, World Health Organization, 1976:67–69 (WHO Technical Report Series, No. 594).

3. Requirements for Meningococcal Polysaccharide Vaccine (Requirements for Biological Substances No. 23, addendum 1980). In: *WHO Expert Committee on Biological Standardization. Thirty-first Report.* Geneva, World Health Organization, 1981, Annex 6 (WHO Technical Report Series, No. 658).

WHO Technical Report Series, No. 840, 1994

Annex 2
Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (Requirements for Biological Substances No. 27, revised 1992)

Introduction

In 1976, a WHO Working Group on the Standardization of Human Blood Products and Related Substances (*1*) considered the 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 determining the quality of the final products, such requirements should cover all the stages in the process, from the collection of the source materials to the quality control of the final product. In response to the Working Group's recommendations, the Requirements for the Collection, Processing and Quality Control of Human Blood and Blood Products were published in 1978 (*2*). These Requirements were updated and revised in 1988 (*3*), and WHO recommendations concerning testing for antibodies to human immunodeficiency virus (HIV, *4*) were taken into account. This Annex contains a further revision of the Requirements, applicable to the quality control of blood, blood components and plasma derivatives.

A number of other WHO publications have dealt with whole blood and its components, among them guidelines intended mainly for blood transfusion services (*5*). Guidelines of a more general nature, such as the Guidelines for National Authorities on Quality Assurance for Biological Products, have also been published (*6*). The latter call for a quality-assurance system based on the existence of a national structure that is independent of the manufacturer and is responsible for granting licences for biological products, defining procedures for product release and setting up a post-marketing surveillance system. These Guidelines should be followed by any country having or wishing to set up an organization for the collection and fractionation of blood and blood components.

The names of the many experts who provided advice and data taken into account in this revision of the Requirements are listed in the Acknowledgements section, page 96.

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 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. A logical developmental sequence for a comprehensive organization starts with the collection and distribution of whole blood, progressing later to the separation of whole blood into components and then the fractionation of plasma pools. 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, in particular for whole blood and component cells. In addition, 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 may be able to help in arranging such training.

One of the basic questions to be answered by a country considering whether to start fractionation of plasma is whether there is 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. However, 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 continuous operation.

In a comprehensive organization, the greatest expense is that involved in setting up the fractionation plant, but it is also possible to regard the collection of source material and its fractionation as quite separate operations. A country may wish to establish collection centres for separating the cell components and then send the plasma to an established fractionation plant in another country, from where the products could be returned to the original country. The costs of such an operation might be less than those involved in establishing and operating a fractionation plant.

The general prevalence of certain infectious diseases, such as various forms of hepatitis and parasitic diseases, and of HIV infection differs so markedly in different geographical regions that each national authority must decide for itself whether it is cost-effective to apply the most sensitive test to each blood donation and whether it is feasible to collect suitable source material. A brief protocol for the collection of source material is in any case mandatory (see Appendix). Great emphasis should be placed on the production of fractions by a process that experience has shown results in the least risk of contamination. For example, immunoglobulin prepared by the cold ethanol fractionation method of Cohn has a well established

clinical record of being free from contamination with HIV and hepatitis B virus (HBV), as have albumin products prepared by the same method, stabilized and heated for 10 hours at 60 °C (*5*). Nevertheless, extreme care is required in manufacture to ensure that these products are free from infectious viruses, and it cannot be assumed that different fractionation methods will be equally effective. When a fractionation process is introduced or significant modifications are made to an existing production process, the process or the modifications should be validated or revalidated by appropriate procedures, including the use of marker viruses and, where applicable, special *in vitro* and *in vivo* testing.

Blood can harbour a number of different viruses, and the use of medicinal products derived from human blood has led to transmission of viruses such as HBV and HIV. The risk of virus transmission by blood and blood products can be diminished by the testing of all individual donations. Policies for mandatory testing shall be determined by the national control authority, and should be reviewed regularly and modified according to the current state of knowledge.

Special care and appropriate measures approved by the national control authority must be taken to protect the health of the staff of blood collection and fractionation facilities.

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

In these Requirements, the word "human" has been omitted from the names of products derived from human blood. Products of animal origin are immunogenic, and their administration to humans should be avoided whenever equivalent products of human origin can be used instead. The proper name of any blood product of non-human origin should include the species of origin.

These Requirements consist of four parts:

- Part A. Requirements for the collection of source materials
- Part B. Requirements for single-donor and small-pool products
- Part C. Requirements for large-pool products
- Part D. National control requirements.

Each deals with a separate aspect of collection, processing and quality control, but all the parts are intended to be taken together to constitute a single document. It will not be possible to rely on any blood product unless the relevant requirements for each step are complied with, and any attempt

to make them less stringent may have serious consequences for the safety of the final product.

Parts A–D are divided into sections, each of which constitutes a recommendation. The parts of each section printed in normal type have been written in the form of requirements, so that, if a health administration so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments or 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 modifications be made only on condition that the modified requirements ensure at least an equal degree of safety and potency of the products. It is desirable that the World Health Organization should be informed of any such changes.

Increasing demand for blood products is resulting in the extensive movement of such products from one country to another. Internationally accepted requirements are therefore necessary so that countries without any regulations on blood products and related substances may refer to them when importing such products.

International Biological Standards and Reference Reagents

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

The activity of blood products must be expressed in International Units where an International Standard exists. WHO publishes a list of such standards (revised from time to time and most recently in 1990) under the title *Biological substances: International Standards and Reference Reagents.*

Definitions

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

Blood collection: a procedure whereby a single donation of blood is collected in an anticoagulant and/or stabilizing solution.

Processing: any procedure that takes place after the blood is collected.

Plasmapheresis, apheresis and cytapheresis: procedures whereby whole blood is separated by physical means into components and one or more of them returned to the donor.

Closed blood-collection and processing system: a system for collecting and processing blood in containers that have been connected together by the manufacturer before sterilization, so that there is no possibility of bacterial or viral contamination from outside after collection of blood from the donor.

Donor: a person who gives blood or one of its components.

Single-donor materials

Whole blood (sometimes referred to as "blood"): blood collected in an anticoagulant solution with or without the addition of nutrients such as glucose or adenine. Whole blood is collected in units of 450 ml.

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

Plasma: the liquid portion remaining after separation of the cellular elements from blood collected in a receptacle containing an anticoagulant, or separated by continuous filtration or centrifugation of anticoagulated blood in an apheresis procedure.

Plasma, frozen: a plasma separated more than 8 h after collection of the blood and stored below -20 °C.

Plasma, fresh-frozen: a plasma separated within 8 h of donation, frozen rapidly and stored below -20 °C (and preferably below -30 °C).

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

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

Plasma, recovered: plasma recovered from a whole blood donation.

Cryoprecipitated factor VIII: a crude preparation containing factor VIII that is obtained from single units (or small pools) of plasma derived either from whole blood or by plasmapheresis, by means of a process involving freezing, thawing and precipitation.

Serum: the liquid part of coagulated blood or plasma.

Red cells: whole blood from which most of the plasma has been removed and having an erythrocyte volume fraction greater than 0.7.

Red cells suspended in additive solution: red cells to which a preservative solution, for example containing adenine, glucose and mannitol, is added to permit storage for longer periods; the resulting suspension has an erythrocyte volume fraction of approximately 0.6-0.7.

Red cells, washed: red cells from which most of the plasma has been removed by one or more stages of washing with an isotonic solution.

Red cells, leukocyte-depleted: a unit of a red-cell preparation containing fewer than 1.2×10^9 leukocytes.

Red cells, leukocyte-poor: a unit of a red-cell preparation containing fewer than 5×10^6 leukocytes.

Red cells, frozen: red cells that have been stored continuously at -65 °C or below, and to which a cryoprotective agent such as glycerol has been added before freezing.

Red cells, deglycerolized: frozen red cells that have been thawed and from which glycerol has been removed by washing.

Platelets: platelets obtained either by separation of whole blood, buffy coat or platelet-rich plasma or by apheresis and suspended in a small volume of plasma from the same donation.

Leukocytes: leukocytes obtained either by the separation of whole blood or by apheresis and suspended in a small volume of plasma from the same donation.

Large-pool products

Bulk material: plasma, powder, paste or liquid material prepared by the fractionation of pooled plasma.

Final bulk: a sterile solution prepared from bulk material and bearing the corresponding batch number. It is used to fill the final containers.

> In some countries, the final bulk is distributed into containers through a sterilizing filter. If the total final bulk is not distributed into containers in one session, each of the filling lots is given a sub-batch number.

Filling lot (final lot): a collection of sealed final containers that are homogeneous with respect to composition and the risk of contamination during filling and (where appropriate) drying or other further processing such as heat treatment. A filling lot must therefore have been filled and (where appropriate) dried in one working session.

Part A. Requirements for the collection of source materials

1. 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 requirements of Good Manufacturing Practices for Pharmaceutical (*7*) and Biological (*8*) Products and in addition provide adequate space, lighting and ventilation for the following activities where applicable:

- The medical examination of individuals in private to determine their fitness as donors of blood and/or blood components and to provide an opportunity for the confidential self-exclusion of unsuitable potential donors.
- The withdrawal of blood from donors and, where applicable, the re-infusion of blood components with minimum risk of contamination and errors.
- The care of donors, including the treatment of those who suffer adverse reactions.
- The storage of whole blood and blood components in quarantine pending completion of processing and testing.
- The laboratory testing of blood and blood components.
- The processing and distribution of whole blood and blood components in a manner that prevents contamination and loss of potency.
- The performance of all steps in apheresis procedures, if applicable.
- The performance of labelling, packaging and other finishing operations in a manner that prevents errors.
- The storage of equipment.
- The separate storage of quarantined and finished products.
- The documentation, recording and storage of data on the donor, the donated blood and the ultimate recipient.

Mobile teams can be used for the collection of blood. Although the premises used by such teams may not comply with the more stringent requirements for centres built specially for the purpose, they must be adequate to ensure the safety of the donor, the collected blood or blood components and the staff participating in blood collection. The safety of the subsequent users of the premises should also not be forgotten.

2. Equipment

The equipment used in the collection, processing, storage and distribution of blood and blood components shall be calibrated, tested and validated before initial use, and shall be kept clean and maintained and checked regularly. The requirements of Good Manufacturing Practices for Pharmaceutical (*7*) and Biological (*8*) Products shall apply in every particular.

The equipment employed to sterilize materials used in the collection of blood or blood components or for the disposal of contaminated products shall ensure that contaminating microorganisms are destroyed and shall be validated for this purpose. The effectiveness of the sterilization procedure shall be not less than that achieved by a temperature of 121.5 °C maintained for 20 min by means of 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 2 h with dry heat.

All contaminated material should be made safe before disposal. Disposal should comply with the relevant local laws.

Tests for sterility are given in the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (9, pp. 40–61).

3. Personnel

An organization for the collection of blood or blood components shall be under the direction of a designated and appropriately qualified person who shall be responsible for ensuring that all operations are carried out properly and competently. The director shall have 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 such components by apheresis.

The director shall be responsible for ensuring that employees are adequately trained and acquire practical experience and that they are 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 relevant 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, for review of the procedures manual and for the quality-control programme, including techniques, equipment, procedures and staff.

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

4. Donors

4.1 *Donor selection*

The provision of blood, blood components and plasma derivatives from voluntary, non-remunerated donors should be the aim of all countries.

In selecting individuals for blood donation, it is most important to determine whether the person is in good health, in order to protect the donor against damage to his or her own health and to protect the recipient against exposure to diseases or to medicinal products from the blood or blood products. It should be recognized that the donor selection process contributes significantly to the safety of blood products derived from large plasma pools. The following provisions apply to donations of blood or blood components not intended for autologous use.

The health of a donor shall be determined by a licensed physician or a person under the direct supervision of a licensed physician, and the donor shall be free from any disease transmissible by blood transfusion in so far as can be determined by history-taking and examination (see section 4.3). Donors shall be healthy persons of either sex between the ages of 18 and 65 years.

> In some countries, there is no upper limit to the age of the donor. With parental consent the minimum age may be lowered to 16 years.
>
> Red blood cells 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 donor should be considered for plasmapheresis only where the procedures involved result in products or services shown to serve accepted medical purposes, including prophylaxis, therapy and diagnosis, as verified by valid scientific evidence. All donors should be certified as acceptable, at the time of each plasmapheresis procedure, by a registered physician or by trained personnel under the direct supervision of the physician.

Those eligible for apheresis donation include: (a) healthy persons who fulfil the general criteria for blood donors; (b) persons with antibody levels that have been increased, either naturally or by immunization; (c) subject to (a) above, persons with plasma that is of value for diagnostic or reference purposes; and (d) persons whose blood may be used in the preparation of certain vaccines.

> When a potential donor does not fulfil the general criteria for blood donation, the acceptance of her or him as a donor for a specific component of blood should be at the discretion of the responsible physician. Where appropriate, the physician should have access to an ethical committee.

Donor education and selection programmes are intended to prevent potentially infectious units of blood and plasma from being collected. It is essential that such programmes are comprehensible and readily accessible to all potential donors.

To reduce the likelihood of transmitting infections, all potential donors should be informed of factors in their history or behaviour that may increase their risk of being infected. The national control authority must determine the appropriate exclusion criteria for the country concerned.

Persons in the following categories shall be excluded from acting as donors:

- those with clinical or laboratory evidence of infectious disease, e.g. infection with hepatitis viruses, HIV-1 or HIV-2;
- past or present intravenous drug abusers;
- men who have had a sexual relationship with another man;

- men and women who have engaged in prostitution;
- those with haemophilia or other clotting-factor defects who have received clotting-factor preparations;
- sexual partners of any of the above.

In some countries, the sexual partners of those at risk of transmitting infections are excluded from acting as donors for only one year.

Persons who have received blood transfusions should be excluded from acting as donors for at least one year.

Donors should be made aware before donating blood that it will be tested for the presence of serological markers of infection. It is advisable that the right to test donations and the legal implications of testing donations should be clarified by the appropriate authority.

4.2 *Donation frequency and volume*

4.2.1 *Whole blood*

The frequency of whole-blood donations shall not exceed once every two months, with a maximum volume in any consecutive 12-month period of 3 l.

A standard donation should not be collected from persons weighing less than 50 kg.

A *standard donation* is 450 ml; an optimum blood/anticoagulant ratio is 7 to 1.

The frequency of donation may have to be modified on an individual basis. In general, premenopausal women should not donate blood as frequently as men.

4.2.2 *Plasma*

Plasma donors can be divided into three groups: those who donate at a frequency comparable to that allowed for whole-blood donations; those who donate two to three times as frequently as whole-blood donors; and those who donate at a maximum of twice a week. The first group shall be accepted on the basis of the general criteria for blood donors.

The maximum volume of plasma that may be removed from a donor during one plasmapheresis procedure shall be determined by the national health authority, and shall depend on whether the plasma is obtained by manual or automated plasmapheresis.

In some countries, the volume of plasma collected during a manual procedure is the quantity obtained from 1.0–1.2 l of whole blood. The volume of plasma collected during an automated procedure depends on the equipment used.

It is difficult to specify 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. The limits imposed in different countries vary, and depend on the nutritional status of the donor.

If a plasma donor donates a unit of whole blood or if the red blood cells are

not returned in an apheresis procedure, the next donation shall be deferred by eight weeks unless special circumstances warrant approval by the responsible physician of plasmapheresis at an earlier date.

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

4.3 *Medical history*

4.3.1 *General*

Before each donation, questions shall be asked so as to ensure that the donor is in normal health and has not suffered, or is not suffering, from any serious illness.

A 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, postmenopausal hormone therapy or oral contraceptives, shall not be accepted unless approved by a physician.

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

4.3.2 *Infectious diseases*

Potential donors with a history that places them at increased risk of transmitting infection shall not donate blood or plasma for an appropriate time period. A donor shall be permanently excluded if one of his or her previous blood donations was believed to be responsible for transmitting disease.

> In most countries, questions concerning the signs and symptoms of HIV infection will be part of the routine assessment of medical history and appropriate monitoring for HIV, as defined by the national control authority, will be included. As a result of this assessment, a potential donor may be disqualified.

Donors shall not have a history of: positive laboratory test results for hepatitis or corresponding symptoms and signs; close contact with an individual with hepatitis within the previous year; receipt within the previous year of human blood or any blood component or fraction that might be a source of transmission of infectious agents; or tattooing, scarification or ear piercing (unless performed under sterile conditions) within the previous year.

> Acupuncture within the previous year may also present a risk if not carried out under sterile conditions.
>
> In some countries, potential donors with a history of viral hepatitis or of a positive test for hepatitis B surface antigen (HBsAg) or antibodies to hepatitis C virus (anti-HCV) are permanently excluded. In others, such donors are accepted providing that recovery occurred more than one year previously and that the reaction for HBsAg and anti-HCV in a sensitive test is negative.

The requirements concerning viral hepatitis may be varied, at the discretion of the national control authority, according to the local epidemiological circumstances.

> The collection both of single-donor products (whole blood and its components) and of plasma for pooling for the manufacture of plasma fractions capable of transmitting hepatitis or HIV should be avoided if a group of potential donors shows a prevalence of acute or chronic hepatitis B, hepatitis C or HIV infection higher than that found in the general donor population. Specific approval may be given by national control authorities for the use of donations from such populations to provide plasma for the manufacture of hepatitis B vaccine or hepatitis B immunoglobulin.
>
> In areas with a low incidence of transfusion-transmitted disease, whole blood or blood components should not be used for transfusion if obtained from source material collected in an area where there is a high incidence of blood-borne infectious disease.

Blood and plasma shall be tested for the presence of HBsAg, anti-HIV and anti-HCV by the methods described in Part B, section 7.2; the tests used should be approved by the national control authority or other appropriate authority.

Anyone whose blood has been shown to be reactive for infectious disease markers by approved screening tests shall be excluded as a donor. Selection as a donor may later be permitted if sufficient data are available from tests approved by the national control authority to indicate that the original results were non-specific.

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

> In countries where malaria is not endemic, donors of cellular blood products should have a negative history of malaria exposure during the previous six months and a negative history of clinical malaria, or a history of malaria prophylaxis if they have resided in, or visited, an endemic area within the three years preceding the donation. Such restrictions may be less important in countries where the prevalence of endemic malaria is high among both donors and recipients, except when blood products are required by visitors from non-endemic areas. Malaria history is not pertinent to plasma donation for source material that will be fractionated.

Particular attention should be paid to skin decontamination procedures before blood collection.

> Many parasitic, bacterial and viral diseases, including trypanosomiasis, toxoplasmosis, syphilis and brucellosis, can be transmitted by blood. Precautions shoud be taken to avoid blood collection during the viraemic phase of viral diseases like measles and rubella. Potential donors who have lived in or recently travelled to areas where human T-cell lymphotropic virus infections and haemorrhagic fever are endemic should be investigated for evidence of such infections.

Anyone who has received pituitary hormones of human origin should be permanently excluded as a donor because of possible infection with the agent causing Creutzfeldt-Jakob disease, although transmission of this agent through blood products has not been proved.

4.3.3 *Minor surgery*

Donors shall not have undergone tooth extraction or other minor surgery during a period of 72 h before donation.

4.3.4 *Pregnancy and lactation*

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

> The interval before blood donation is permissible after pregnancy may be shorter in some cases, e.g. six weeks after an abortion during the first trimester.
>
> In some countries, donors are accepted when pregnant or during the period of lactation if their blood contains certain blood-group antibodies or is needed for autologous transfusion. The volume to be taken should be determined by the physician responsible.

4.3.5 *Prophylactic immunization*

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

- Those receiving attenuated vaccines for measles, 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 four weeks after the last injection.
- Those receiving rabies vaccine for post-exposure treatment shall be excluded until one year after the last injection.
- Those receiving passive immunization with animal serum products shall be excluded until four weeks after the last injection.
- Those receiving hepatitis B vaccine need not be excluded unless the vaccine is being given because of exposure to a specific risk, in which case the donor shall be disqualified for at least 12 months after the last such exposure. If hepatitis B immunoglobulin has been administered, the period of deferral shall be at least 12 months because disease onset may be delayed.

4.4 *Physical examination*

As determined by the national control authority, physical examination of donors may include measurement of weight, blood pressure, pulse rate and temperature. If these are measured and the results lie outside the ranges recommended below, the donor concerned shall be accepted only if approved by the licensed physician in charge.

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

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

4.5 *Additional requirements applicable to donors for plasmapheresis*

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

4.5.1 *First-time plasma donors*

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

The following information shall be permanently recorded:

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

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

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

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

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

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

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

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

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

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

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

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

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

4.5.3 *Tests for plasma donors*

The following tests shall be performed at each donation:

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

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

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

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

4.6 *Donors for platelet and leukocyte apheresis*

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

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

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

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

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

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

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

4.7 *Donor immunization and plasma for special purposes*

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

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

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

The following are examples of medically useful plasma:

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

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

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

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

4.7.3 *Immunization of donors*

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

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

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

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

Potential donors should be:

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

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

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

4.7.4 *Immunization with human erythrocytes*

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

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

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

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

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

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

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

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

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

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

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

Prospective erythrocyte recipients in whom antibody screening tests demonstrate the presence of erythrocyte antibodies (other than those deliberately stimulated through immunization by the plasmapheresis centre) should be asked whether they have ever been pregnant or had a

transfusion, a tissue graft or an injection of erythrocytes for any reason. This history should form part of the permanent record and should identify the cause of immunization as clearly as possible. Recipients should be notified in writing of any specific antibodies developed after injection of erythrocytes. The national control authority should be notified annually in writing of unexpected antibodies induced by immunization, and the immunized donor should carry a card specifying the antibodies.

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

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

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

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

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

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

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

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

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

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

5. Collection of blood and plasma

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

5.1 *Blood collection and apheresis procedures*

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

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

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

5.1.1 *Summary of minimum general requirements for apheresis*

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

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

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

5.1.2 *Adverse reactions*

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

5.1.3 *Types of adverse reaction*

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

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

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

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

5.2 Containers

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

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

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

5.3 *Anticoagulants*

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

> Commonly used anticoagulant solutions are acid–citrate–glucose, citrate–phosphate–glucose and citrate–phosphate–glucose–adenine; the amount of adenine used varies in different countries. Solutions of adenine, glucose and mannitol used for red cell preservation may be added after removal of the plasma.
>
> For plasmapheresis, sodium citrate as a 40 g/l solution is widely used as an anticoagulant.

5.4 *Pilot samples*

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

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

5.5 *Identification of samples*

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

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

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

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

6. General considerations

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

It is important to note that single-donor and small-pool products have certain specialized uses other than therapeutic application to correct deficits in patients. Although not dealt with further in these Requirements, these uses include the stimulation of plasma donors with red blood cells in order to raise antibody levels for the preparation of anti-D (anti-Rh_0) immunoglobulin (*12*) and special blood-grouping reagents. It is of the utmost importance that the donors of cells and plasma for such purposes be carefully studied both initially and on a continuing basis to minimize the likelihood of the transmission of infectious diseases to recipients. The use of red cells, stored frozen, that have been demonstrated to be free from infectious agents by retesting the donor 12 months after the initial collection reduces the risk of such transmission to volunteers for immunization.

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

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

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

7. Production and control

7.1 *General requirements*

Single-donor and small-pool products shall comply with any specifications established by the national control authority. Cellular blood components and certain plasma components may deteriorate during separation

or storage. Whatever the method of separation (sedimentation, centrifugation, washing or filtration) used for the preparation of cell components, therefore, it is important that a portion of plasma protein sufficient to ensure optimum cell preservation be left with the cells, except when a cryoprotective substance is added to enable them to be stored for long periods in the frozen state, or additive solutions (for example containing adenine, glucose and mannitol) are used for the same purpose for liquid storage.

> The methods employed for component separation should be checked before they are introduced. The characteristics assessed might include yield of the component, purity, *in vivo* recovery, biological half-life, functional behaviour and sterility.
>
> The nature and number of such checks should be determined by the national control authority.

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

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

7.2 *Testing of whole blood and plasma*

7.2.1 *Sterility*

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

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

7.2.2 *Laboratory tests*

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

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

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

7.2.3 *Tests for infectious agents*

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

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

In some countries plasma pools are also tested.

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

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

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

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

7.3 Blood-grouping

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

In countries where polymorphism for the D (Rh_o) antigen is present, each unit of blood shall be classified according to Rh blood type on the basis of

the results of testing for the D (Rh_o) red cell antigen. The D (Rh_o) type shall be determined with anti-D (anti-Rh_o) reagents.

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

7.4 *Red cells*

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

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

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

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

7.4.1 *Methods and timing of separation*

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

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

All surfaces that come into contact with the blood cells shall be sterile, biocompatible and pyrogen-free. If an open plastic-bag system is used, i.e. the transfer container is not integrally attached to the blood container and the blood container is opened after blood collection, the plasma shall be separated from the cells under conditions such that the original container is kept under positive pressure until it has been sealed. If the separation

procedure involves a vented system, i.e. if an airway is inserted into the container for withdrawal of the plasma, the airway and vent shall be sterile and constructed so as to exclude microorganisms.

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

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

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

> Separation should preferably be effected within 8 h of blood donation.
>
> When platelets and coagulation factors are to be prepared, it is especially important that the venepuncture be performed in such a way as to cause minimal tissue damage so as to prevent the initiation of coagulation. The blood should flow freely without interruption and as rapidly as possible, and be mixed thoroughly with the anticoagulant.

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

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

> Sedimentation is the least expensive method for separation of red blood cells and does not require special equipment.
>
> Repeated washing with saline and centrifugation and filtration are used to reduce the number of leukocytes and platelets and the volume of trapped plasma in red cells. Frozen red cells after thawing are also repeatedly washed with special solutions to remove cryoprotective agents while also preventing haemolysis.

7.4.2 *Expiry date*

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

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

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

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

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

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

7.4.3 *Modified red cells*

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

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

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

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

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

Centrifugation should be carried out in refrigerated centrifuges. If such

equipment is not available, the washing solution should have a temperature of 5 ± 3 °C.

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

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

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

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

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

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

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

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

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

7.5 *Plasma*

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

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

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

7.5.1 *Plasma, fresh-frozen*

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

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

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

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

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

7.5.2 *Plasma, frozen*

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

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

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

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

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

Plasma may be pooled at any time after collection.

7.5.3 *Plasma, freeze-dried*

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

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

7.5.4 *Plasma, recovered*

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

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

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

7.5.5 *Plasma, platelet-rich*

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

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

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

7.6 *Platelets*

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

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

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

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

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

Licensed artificial suspension media may be used to replace plasma.

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

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

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

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

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

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

7.6.1 *Monitoring the quality of platelets*

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

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

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

7.6.2 *Expiry date*

The expiry date of platelets processed in a closed system shall be 72 h after the original whole blood was collected, unless they are stored in a plastic container approved by the national control authority for a longer storage period.

Platelets prepared in an open system should be used within 4 h of preparation if stored at 22 ± 2 °C, unless the procedure used has been shown to allow a longer storage period.

Single-donor platelet concentrates may be pooled for one recipient under aseptic conditions before issue. Such small pools should be used as soon as possible, and within 4 h of preparation if stored at room temperature.

7.7 *Leukocytes*

Leukocytes are obtained by the separation of whole blood or by apheresis, and may contain a large number of platelets and red blood cells, depending on the method of preparation. When leukocytes are obtained from units of whole blood, such units shall comply with the requirements of Part A, section 5, and Part B, section 7.2.

The methods used to process leukocytes shall comply with the requirements and recommendations given in section 7.4.1 for the separation of red cells.

The label on the final container shall bear, in addition to customary data, instructions to use the leukocytes as soon as possible and in any case not more than 4 h after the container has been opened for pooling. The temperature of storage and transport shall be 22 ± 2 °C.

Leukocytes can be separated from blood by centrifugation, sedimentation or leukapheresis. To obtain a sufficient number, the leukocytes from units obtained from several healthy donors may have to be pooled.

Leukapheresis by continuous-flow filtration or centrifugation is the most efficient way of obtaining leukocytes, since it gives large numbers of high-quality cells from a single donor.

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

Approximately 90% of the leukocytes present in 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.

Leukocytes should be negative for cytomegalovirus.

The product should be ABO typed and, in countries where D (Rh_o) is polymorphic, D (Rh_o) typed; it may also be desirable to determine the HLA type. If not HLA typed, leukocytes should be irradiated.

The large number of red cells present in products prepared by some methods makes compatibility testing before transfusion necessary.

7.7.1 *Testing of leukocytes*

The number of units to be tested and the leukocyte yield (number) required shall be specified by the national control authority.

7.7.2 *Expiry date*

The expiry date of leukocytes shall be 24 h after collection of the original whole blood.

7.8 *Cryoprecipitated factor VIII*

Cryoprecipitated factor VIII is a crude preparation of factor VIII. It shall be obtained from single units or small pools of plasma derived either from units of whole blood that comply with the requirements of Part A, section 5, and Part B, section 7.2, or by plasmapheresis.

> The product may be prepared as a pool from a small number of donations, usually four to six but not exceeding ten. It may be freeze-dried. However, preparations of cryoprecipitated factor VIII carry the risk of viral transmission unless they have undergone specific virucidal procedures during manufacture.

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 7.8.1).

In procuring source material for coagulation factors, the following technical considerations should be borne in mind:

- In order to prevent coagulation, venepuncture should performed in such a way that tissue damage is minimal. The blood should flow freely without interruption, and be mixed thoroughly with anticoagulant during collection.
- Microbial contamination should be avoided during separation of the plasma by using multiple-plastic-bag closed systems or laminar-flow cabinets if an open procedure is used.
- The recovery of factor VIII depends on the interval between venepuncture and freezing of the plasma, the temperature at which the plasma is held and the freezing method. While a useful product may be obtained with plasma frozen as late as 18–24 h after phlebotomy, freezing the plasma as early and as rapidly as possible is strongly recommended.
- Ideally, fresh-frozen plasma should be prepared by rapid freezing using a combination of solid carbon dioxide and an organic solvent such as ethanol. Fresh-frozen plasma should be stored at or below -20 °C. Contamination of the plasma by the solvent or leaching of substances from the container into the plasma should be avoided.
- If the temperature of the thawed plasma exceeds 2 °C, a high proportion of the factor VIII is lost in the supernatant. During thawing or separation of the supernatant plasma, therefore, the temperature should not be allowed to exceed 2 °C. The plasma may be separated while there is still a small quantity of the ice present in the plasma

container. Increasing the speed of thawing by circulating air or water at a temperature of 0 °C is believed to increase the yield of factor VIII.

7.8.1 *Testing of cryoprecipitated factor VIII*

Randomly selected units shall be tested for potency and sterility on a regular basis. The number of units to be tested shall be specified by the national control authority. The freeze-dried preparation shall dissolve without any signs of precipitation in the solvent recommended by the manufacturer within 30 min when held at a temperature not exceeding 37 °C.

The potency of cryoprecipitated factor VIII shall be compared with that of an appropriate plasma or intermediate-purity standard, by measuring its ability to correct the prolonged activated partial thromboplastin time of haemophilia A plasma or by another suitable method.

When cryoprecipitated factor VIII is produced from fresh-frozen plasma (frozen within 8 h of donation), the yield should be greater than 400 IU/l of starting plasma. Plasma frozen after this time will yield less cryoprecipitated factor VIII.

> In many laboratories, the average yield of factor VIII is 400 IU/l of starting plasma. The average yield of factor VIII as freeze-dried cryoprecipitate is then at least 300 IU/l of starting plasma. Whether this yield can be obtained elsewhere will depend on local technical possibilities. In some countries, the yields will be much lower, and the national control authority should decide as to the yield that is acceptable.

7.8.2 *Expiry date*

The frozen product shall be stored at or below −20 °C (if possible below −30 °C) and shall have an expiry date one year from the date of collection. The freeze-dried product shall be stored at 5 ± 3 °C and shall also have an expiry date one year from the date of collection. After thawing or reconstitution, cryoprecipitated factor VIII should be kept at 20–24 °C. It shall be used as soon as possible and in any case not more than 4 h after its container has been opened for pooling or reconstitution.

7.9 *Labelling*

After having been tested and before being issued for transfusion, units of single-donor and small-pool products shall be identified by means of container labels that clearly state at least the following information:

- the proper name of the product;
- the unique number or symbol identifying the donor(s);
- the expiry date, and when appropriate, the expiry time after reconstitution;
- any special storage conditions or handling precautions that are necessary;
- a reference to a package insert containing instructions for use, warnings and precautions;

- the name and address of the blood donor centre and, where applicable, the manufacturer and distributor;
- the average content in International Units of activity, where appropriate.

The results of red cell grouping shall be stated on the label of whole blood, red cells, fresh-frozen plasma (for clinical use), platelets and leukocytes but not necessarily on that of cryoprecipitated factor VIII.

Part C. Requirements for large-pool products

8. Introduction

A number of requirements common to albumin, plasma protein fraction, immunoglobulin preparations and coagulation-factor concentrates are given in Parts A and B, sections 3-7. However, for clarity, it has proved convenient to bring together in Part C certain specific requirements applicable to these products when manufactured on a large scale.

The source material for the large-scale preparation of blood products should comply with the relevant provisions of Parts A and B.

9. 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 the requirements of Good Manufacturing Practices for Pharmaceutical (*7*) and Biological (*8*) Products. They shall comply with the Guidelines for National Authorities on Quality Assurance for Biological Products (*6*) and in addition provide adequate space, lighting and ventilation for the activities listed below.

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

9.1 *Storage of whole blood and plasma*

Whole human blood and plasma shall be stored frozen or refrigerated in separate facilities that are used only for this purpose. The source materials shall remain in quarantine until the results of testing show that they are suitable for introduction into the fractionation premises.

9.2 *Separation of cells and fractionation of plasma*

Cells shall be separated and plasma fractionated in a building isolated from those where non-human proteins or microbiological materials, such as vaccines, are manufactured or processed and separate from the animal house.

> In some countries, cell constituents are separated in an area separate from that where plasma is fractionated.

9.3 *Supply and recovery of ancillary materials*

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

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

9.4 *Viral inactivation*

A separate area shall be provided for all processing subsequent to the completion of viral inactivation procedures when these are carried out at a stage in production before aseptic dispensing and filling (see section 9.5).

9.5 *Freeze-drying, filling, packaging, labelling and storage*

Separate facilities shall be used for the freeze-drying, filling, labelling and packaging of containers. A separate area shall be provided for the storage of labels, package inserts and packages. Another separate area shall be used for the storage of final containers before dispatch.

9.6 *Keeping of records*

Adequate provision shall be made for keeping records of all donors, materials, fractionation steps, quality-control procedures and results, of the distribution of the final products and of the disposal of potentially infectious materials. Records should be retained for at least two years beyond the expiry date of the products to which they relate.

Some manufacturers might wish to extend this period to cover any future legal disputes.

9.7 *Quality control*

Separate facilities shall be provided for quality control, including haematological, biochemical, physicochemical, microbiological, pyrogen and safety testing.

9.8 *Disposal of infective material*

Provision shall be made for the suitable disposal of potentially infectious materials by autoclaving or incineration according to good manufacturing practices.

The disposal of these materials should comply with local legislation.

10. Equipment

Equipment used for the collection, processing, storage and distribution of source materials and large-pool blood products shall comply with the requirements of Good Manufacturing Practices for Pharmaceutical (*7*) and Biological (*8*) Products.

Particular attention shall be paid to:

- The maintenance, monitoring and recording of the operation of continuously operating equipment, the validation of its reliability and the provision of stand-by equipment.
- 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 surfaces of some materials can denature certain proteins or activate certain coagulation factors.

- The ease and efficiency with which equipment can be cleaned and, where necessary, sterilized. Any bactericidal agent used shall be capable of being completely eliminated before the equipment is used.

  Caution should be exercised in the use of detergents because of their possible effects on the final product; tests should be made to ensure that they do not have any adverse effect on it.

- The provision of suitable facilities for decontamination and for the disposal of potentially infective materials and equipment.

11. Provision of support services

A number of support services are essential for the fractionation of source materials.

11.1 *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 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 endotoxin content and conductance. The water system should be a continuously circulating one and should have no dead ends.

Water for injections is generally used for the preparation of final products (*14*).

11.2 *Steam supply*

An adequate supply of steam shall be provided for the operation of sterilizing and cleaning equipment. The steam shall be clean and have the quality of water for injections.

11.3 *Other support facilities*

Other support facilities required are:

- A supply of electrical and thermal energy.

- A means of refrigeration for:
  - storing various source materials and fractions;
  - keeping the various fractionation areas at the correct temperature;
  - keeping the process equipment at the correct temperature;
  - storing final products under test;
  - storing final products awaiting dispatch.
- A system of ventilation providing the following two grades of filtered air:
  - air filtered to remove particles of 5 μm or greater in diameter, which shall be supplied to the entire work area; and
  - air passed through a filter with a retention capacity of more than 99.95% for particles greater than 0.5 μm in diameter, which shall be supplied at a positive pressure to areas where aseptic dispensing is to take place.

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

Proteinaceous sewage from a plasma processing plant is highly nitrogenous and has a high biological oxygen demand; it should therefore not be discharged untreated.

These support facilities shall be located separately from the main process areas and in a place where the 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 its operation so as to ensure the safety both of the material being processed and of the process operators. In this way a proper record of the operations of support facilities can be kept and, where necessary, entered into the process record of the product batches.

The equipment should be such as to ensure that both the fractionation process and the proteins are protected if the support services are interrupted. To this end, adequate spare equipment and emergency reserve systems should be available, serviced by engineering staff skilled in the maintenance and repair of such equipment.

12. 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. The director shall have a good working knowledge of the scientific principles involved and shall be responsible for ensuring that employees are adequately trained, have adequate practical experience and are aware that accepted good practices should be applied in their work.

The personnel involved in quality-control functions shall be separate from those involved in production. The head of the quality-control department shall be responsible only to the director.

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

Personnel should be medically examined at regular intervals. Those known to be carriers of specific pathogenic organisms that may adversely affect the product shall be excluded from the production area.

Vaccination against hepatitis B is strongly recommended for employees routinely exposed to blood or blood products.

13. Production control

13.1 *Fractionation of source materials*

The general conditions for the large-scale fractionation of source materials to prepare prophylactic or therapeutic blood products shall comply with Good Manufacturing Practices for Pharmaceutical (*7*) and Biological (*8*) Products 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 they yield protein preparations that have previously been shown to be safe and effective.

The fractionation procedures used shall give a good yield of products meeting the quality requirements of international or national authorities. Fractionation shall be carried out in such a manner that the risk of microbiological contamination and protein denaturation is minimized.

> The safety of fractionation steps may be increased by using protected or closed systems. Reproducibility may be increased by the use of automation.
>
> The biological characteristics of the products (such as antibody activity, biological half-life and *in vivo* recovery of the proteins) should not be affected by the fractionation procedures to the extent that they are unacceptable for clinical use.

Methods shall be used that exclude or inactivate pathogenic organisms, in particular hepatitis viruses and human retroviruses, from the final products intended for clinical use. Manufacturers shall validate the ability of their manufacturing processes to inactivate and/or remove potential contaminating viruses by the use of relevant model viruses.

There is increasing evidence that certain manufacturing procedures, coupled with strict control to ensure that the final product complies with precise specifications, result in a product free from HIV, hepatitis B and hepatitis C infectivity.

For coagulation products, viral inactivation and removal methods such as chromatography or treatment with dry heat, wet heat, steam under pressure, heated organic solvents or solvents/detergents shall be used, in combination with other methods that have been shown to be successful in reducing or eliminating the risk of HIV and hepatitis virus transmission.

Donor screening and viral inactivation procedures used in manufacturing plasma coagulation concentrates have significantly improved the safety of these products.

Fibrinogen prepared from plasma pools continues to carry a risk of infection unless it is treated to remove or inactivate viruses. Where large-pool, virally inactivated fibrinogen concentrates are not available, cryoprecipitated factor VIII prepared from individual units or small pools of plasma is preferred as a source of fibrinogen. Approximately 150 mg of fibrinogen is contained in the cryoprecipitate from one unit of plasma (200 ml) frozen within 8 h of collection from the donor.

The operating manual for the fractionation procedure shall specify the times of sampling of the products 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, all materials used for fractionation shall be tested for microbiological contamination, identity, purity, endotoxin content and toxicity in accordance with *The international pharmacopoeia* (*14, 15*) or national pharmacopoeia.

Certain procedures, equipment and materials may introduce contaminants into the final product that can induce allergenic or immunogenic responses in recipients. The quantities of such contaminants in the final product shall be minimized. For example, where monoclonal antibodies are used for product purification, the residual concentration in the final product must be below clinically reactive levels.

> It is advisable to use air filtration under positive pressure during fractionation, to exclude airborne allergenic dust.

13.1.1 *Preservatives and stabilizers*

No preservatives shall be added to albumin, plasma protein fraction, intravenous immunoglobulin or coagulation-factor concentrates either during fractionation or at the stage of the final bulk solution. Antibiotics shall not be used as preservatives or for any other purpose in the fractionation of plasma.

To prevent protein denaturation, stabilizers may be added. Such substances shall have been shown to the satisfaction of the national control authority not to have any deleterious effect on the final product in the amounts present and to cause no untoward reactions in humans.

> Stable solutions of immunoglobulins may be prepared in approximately 0.3 mol/l glycine or 0.15 mol/l sodium chloride. In some countries, thiomersal and sodium timerfonate are not permitted as preservatives in intramuscular immunoglobulins.

13.2 *Storage and control of source materials*

At all stages of the manufacturing process, the source materials and resulting fractions shall be stored at temperatures and under conditions

shown to prevent further contamination and the growth of micro-organisms, to protect the identity and the integrity of the proteins and to preserve the biological activity and safety of the products.

If similar materials are stored together, the places allocated to them shall be clearly demarcated.

All source materials and resulting fractions shall be fully identified at all times; such identification shall include the batch number of all in-process fractions and final containers awaiting labelling.

13.2.1 *In-process control*

Source materials are subject to biological variability and the products resulting from protein separation will contain various amounts of other protein components of plasma. It is essential, therefore, to establish a monitoring system such that the safe operating limits of each process are maintained.

The main information collected is on variations in physical conditions (temperature, pH, ionic strength, timing, etc.) and in the number and species of contaminating organisms.

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

13.2.2 *Record-keeping*

Records shall be kept of the performance of all steps in the manufacture, quality control and distribution of large-pool blood products and related substances (*7, 8*).

These records shall:

- be original (not a transcription), indelible, legible and dated;
- be made at the time that the specific operations and tests are performed;
- identify the person recording the data as well as the person checking them or authorizing the continuation of processing;
- be detailed enough to allow all the relevant procedures performed to be clearly reconstructed and understood;
- permit the tracing of all successive steps and identify the relationships between dependent procedures, products and waste materials;
- be maintained in an orderly fashion that will permit the retrieval of data for a period consistent with shelf-lives and the legal requirements of the national control authority and, if necessary, allow a prompt and complete recall of any particular lot;
- show the lot numbers of the materials used for specified lots of products;
- indicate that processing and testing were carried out in accordance with procedures established and approved by the designated responsible authority.

14. Control of albumin and plasma protein fraction

Source materials should be processed in such a manner that the albumin in the solutions manufactured will be changed as little as possible and will not cause undesirable reactions in the recipients. Source materials may contain either vasoactive substances or substances capable of generating or releasing endogenous vasoactive substances. Such substances may also be formed in the course of fractionation, and consequently contaminate the albumin and plasma protein fraction. To guard against this possibility, adequate in-process controls and the testing before release for prekallikrein activator activity are mandatory for albumin solutions of purity less than 95% (such as plasma protein fraction) containing 35–50 g of protein per litre. Such testing is also recommended for highly purified albumin products (purity greater than 95%).

Within 24 h of the start of filling, albumin and plasma protein fraction in solution shall be heated in the final container to 60 ± 0.5 °C and maintained at that temperature for not less than 10 h but not more than 11 h by a method that ensures uniform heat distribution throughout the batch. Although pasteurization at the final bulk stage may be possible, this approach requires careful validation before use.

Special attention should be given to microbial contamination of source material and intermediates, since soluble microbial substances, especially endotoxins, may accumulate in the finished albumin solution. In addition, it is possible that small amounts of endotoxin, present even in products for which satisfactory results have been obtained in tests for pyrogens, may have a cumulative effect in recipients receiving large product volumes in relatively short periods of time, as, for example, in therapeutic plasma exchange.

> In some countries, information is being collected about the usefulness of quantitative *Limulus* assays for the presence of endotoxin.

The in-process controls should be capable of detecting contamination with bacteria and moulds. In addition, care should be taken to ensure, by a method that shall be validated, that all equipment and reagents used in the manufacturing process are scrupulously clean and free from toxic materials.

14.1 *Stability of albumin solutions*

The stability of solutions of albumin and plasma protein fraction (that have been heated for 10–11 h at 60 °C) shall be tested by heating adequate samples at 57 °C for 50 h. The test solutions shall remain visually unchanged when compared to control samples that have been heated for only 10–11 h at 60 °C.

The thermal stability of albumin solutions shall be taken into consideration by the national control authority in determining the expiry dates.

The physicochemical quality of stored albumin solutions, as measured by the formation of dimers and particularly polymers, is influenced by:

— the quality of the starting plasma;
— the quality of the fractionation, particularly with respect to the degree of purity achieved and the number of reprecipitation and reheating procedures involved; and
— the storage conditions with respect not only to temperature and time but also to the physical state and concentration of the solutions.

With regard to the thermal stability of albumin solutions, the following general statements may be made:

- The addition of stabilizing chemicals is necessary. Commonly used products are sodium octanoate and sodium acetyltryptophanate.
- Albumin prepared from aged liquid or dried plasma is less stable than albumin made from fresh-frozen plasma.
- Reprocessing steps, such as reprecipitation and reheating, may reduce the stability of albumin solutions.
- On long-term storage, albumin solutions are more stable at 5 ± 3 °C than at 32–35 °C. Long-term storage above 30 °C should be avoided.

14.2 *Control of bulk material*

14.2.1 *Tests on bulk material*

Tests on the 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 material. Tests shall be carried out on a specially dissolved sample processed to a stage equivalent to the final product, after sterilization by filtration. The tests shall be those listed in sections 14.3.2 to 14.3.7 inclusive.

14.2.2 *Storage*

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

14.3 *Control of the final bulk solution*

14.3.1 *Preparation*

The final bulk solution shall be prepared from bulk powder or by the dilution of concentrates by a method approved by the national control authority. It shall meet all of the requirements of sections 14.3.2 to 14.3.7 inclusive.

14.3.2 *Concentration and purity*

The albumin concentration in final bulk albumin solutions shall be between 35 and 265 g/l. Not less than 95% of the proteins present shall be albumin, as determined by a suitable electrophoretic method after the sample has been heated for 10–11 h at 60 °C.

The protein concentration in final bulk solutions of plasma protein fraction shall be at least 35 g/l. Plasma protein fraction shall contain at least 83% albumin and not more than 17% globulins. Not more than 1% of the protein in plasma protein fraction shall be γ-globulin.

14.3.3 *Hydrogen ion concentration*

The final bulk solution, diluted with 0.15 mol/l sodium chloride to give a protein concentration of 10 g/l, shall, when measured at a temperature of 20–27 °C, have a pH of 6.9 ± 0.5 (albumin) or 7.0 ± 0.3 (plasma protein fraction).

In some countries, different ranges of pH values and temperatures are permitted.

14.3.4 *Sterility and safety*

The final bulk shall be sterile. If required by the national control authority, it shall be tested for sterility; samples shall be taken for such testing in a manner that does not compromise the sterility of the bulk material. Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*9*, p. 48) shall apply.

14.3.5 *Sodium content*

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

14.3.6 *Potassium content*

The final bulk solutions of albumin and plasma protein fraction shall have a maximum potassium concentration of 2.0 mmol/l.

14.3.7 *Aluminium content*

The final bulk solutions of albumin and plasma protein fraction shall have a maximum aluminium concentration of 7.5 μmol/l (200 μg/l).

14.4 *Filling and containers*

The requiremens concerning filling and containers given in Good Manufacturing Practices for Biological Products (*8*) shall apply.

Special attention shall be paid to the requirement that solutions of albumin and plasma protein fraction in the closed final containers shall be heated to inactivate any infectious agents that may be present (see section 14, paragraph 2). In order to prevent protein denaturation, a stabilizer shall be added to albumin solution before heating (see section 13.1.1).

In some countries, the national control authority may authorize an interval longer than 24 h between filling and heating to 60 °C.

14.5 *Control tests on the final product*

The tests specified below shall be performed on representative samples from every filling lot. If the product is processed further after filling, e.g. by freeze-drying, the tests shall be performed on samples from each drying chamber.

14.5.1 *Identity test*

An identity test shall be performed on at least one labelled container from each filling lot to verify that the preparation is of human origin. The test shall be one approved by the national control authority. Additional tests shall be made to determine that the protein is predominantly albumin or plasma protein fraction as appropriate. The tests mentioned in section 14.3.2 shall be used.

14.5.2 *Protein concentration and purity*

The protein concentration and purity of each filling lot shall be within the limits prescribed in section 14.3.2.

Tests to determine the concentration of additives (such as polyethylene glycol, porcine enzymes and reducing and alkylating agents) used during production shall be carried out if required by the national control authority.

14.5.3 *Sterility test*

Each filling lot 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) (*9*, p.48) shall apply. Samples for sterility testing shall be taken from final containers selected at random after heating at 60 °C for 10–11 h.

In one country, the sterility test is carried out at least 10 days after heating at 60 °C for 10 h. In some countries, the sterility test is carried out both before and after heating at 60 °C for 10 h.

14.5.4 *General safety test*

In some countries a general safety test may be required, whereby each filling lot is tested for extraneous toxic contaminants by appropriate tests involving injection into mice and guinea-pigs. The injection shall cause neither significant untoward reactions nor death within an observation period of seven days. The tests shall be approved by the national control authority.

The tests generally used are the intraperitoneal injection of 0.5 ml into each of at least two mice weighing approximately 20 g and the injection of 5.0 ml into each of at least two guinea-pigs weighing approximately 350 g. In some countries, if one of the animals dies or shows signs of ill-health, such as weight loss, during a specified period, 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, such as weight loss, during that period.

14.5.5 *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 proportionally, on a rabbit body-weight basis, to the maximum single human dose recommended, but not more than 10 ml/kg of body weight. For albumin at concentrations of 200 g/l and 250 g/l, the test dose for each rabbit shall be at least 3 ml/kg of body weight, and for albumin at concentrations of 35 g/l and 50 g/l and plasma protein fraction, 10 ml/kg of body weight.

A filling lot shall pass the test if it satisfies the requirements specified by the national control authority.

14.5.6 *Moisture content*

The residual moisture content shall, where appropriate, be determined by a method approved by the national control authority.

> The methods in use are: (a) drying over phosphorus pentoxide for at least 24 h at a pressure not exceeding 2.7 Pa (0.02 mmHg); and (b) the Karl Fischer method.

The acceptable moisture content shall be determined by the national control authority.

14.5.7 *Prekallikrein activator*

An assay shall be performed for prekallikrein activator. The product shall contain not more than 35 IU of prekallikrein activator per ml.

14.5.8 *Hydrogen ion concentration*

The final product, reconstituted if necessary and diluted with 0.15 mol/l sodium chloride to give a protein concentration of 10 g/l, shall, when measured at a temperature of 20–27 °C, have a pH of 6.9 ± 0.5 (albumin) or 7.0 ± 0.3 (plasma protein fraction).

> In some countries, different ranges of pH values are permitted.

14.5.9 *Absorbance*

A sample taken from the final solutions of albumin and plasma protein fraction, when diluted with water to a concentration of 10 g/l of protein and placed in a cell with a 1-cm light path, shall have an absorbance not exceeding 0.25 when measured in a spectrophotometer set at 403 nm.

14.5.10 *Inspection of filled containers*

All final containers shall be inspected for abnormalities, such as non-uniform colour, turbidity, microbial contamination and the presence of atypical particles, after storage at 20–35 °C for at least 14 days following heat treatment at 60 °C for 10 h. Containers showing abnormalities shall not be distributed.

The normal colour of albumin solutions may range from colourless to yellow or green to brown.

When turbidity or non-uniform colour raises the possibility of microbial contamination, testing should be done to isolate and identify the microorganisms.

14.6 *Records*

The requirements of Good Manufacturing Practices for Biological Products (*8*, pages 27–28) shall apply.

14.7 *Samples*

The requirements of Good Manufacturing Practices for Biological Products (*8*, page 29, paragraph 9.5) shall apply.

14.8 *Labelling*

The requirements of Good Manufacturing Practices for Biological Products (*8*, pages 26–27) and the national control authority's requirements for parenteral solutions shall apply.

In addition, the label on the container should state:

- the type of source material,
- the protein concentration,
- the oncotic equivalent in terms of plasma,
- that preservatives are absent
- the warning "Do not use if turbid",
- the sodium and potassium concentrations.

14.9 *Distribution and shipping*

The requirements of Good Manufacturing Practices for Biological Products (*8*) shall apply.

14.10 *Storage and shelf-life*

The requirements of Good Manufacturing Practices for Biological Products (*8*, pages 26–27) shall apply.

Final containers of albumin solution shall have a maximum shelf-life of three years if they are stored at or below 30 °C, and of five years if they are stored at 5 ± 3 °C.

Other storage conditions and shelf-lives may be approved by the national control authority.

Final containers of plasma protein fraction solution shall have a maximum shelf-life of three years if they are stored at or below 30 °C, and of five years if they are stored at 5 ± 3 °C.

Other storage conditions and shelf-lives may be approved by the national control authority.

15. Control of immunoglobulins

The final bulk solution of normal immunoglobulin shall be made from material from at least 1000 donors. If normal immunoglobulin is to be used for preventing or treating a particular infection, the titre of specific antibody should be measured.

> For normal immunoglobulins, a large number of donors are needed if the final product is to contain adequate amounts of the various desired antibodies.
>
> For specific immunoglobulins, whether intended for intravenous or intramuscular injection, the number of donors represented is less important because the requirement for specific antibody in the final product will be defined.

The immunoglobulin concentration in the final bulk of normal and specific immunoglobulin preparations for intramuscular use shall be 100–180 g/l. Concentrations lower than 100 g/l shall require the approval of the national control authority.

The immunoglobulin concentration in the final bulk of intravenous immunoglobulin shall be at least 30 g/l. If, in a specific immunoglobulin preparation, the concentration is lower than 30 g/l, it shall require the approval of the national control authority.

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

Tests shall be conducted on each filling lot of immunoglobulin solution to determine the proportion of aggregated and fragmented immunoglobulin. The recommended distribution shall be that at least 90% of the protein, other than proteins added as stabilizers to intravenous immunoglobulins, shall have the molecular size of immunoglobulin monomer and dimer. Not more than 10% shall consist of split products together with aggregates (oligomers of relative molecular mass equal to or greater than that of immunoglobulin trimer). This requirement shall not apply to products deliberately fragmented. The tests and limits shall be approved by the national control authority. Of the material having the molecular size of immunoglobulin monomer and dimer, most will consist of monomer. If a minimum level of monomer *per se* is to be established, the time and temperature at which samples must be incubated before analysis shall be specified.

> Gel-permeation chromatography and high-performance exclusion chromatography are useful techniques for determining molecular size distribution and can be standardized for making these measurements.

For intravenous immunoglobulin, the following tests shall be performed on a sample from each filling lot:

- A test for hypotensive activity.

An appropriate test is that for prekallikrein activator content. In some countries the kallikrein test is also used.

- A test for anticomplement activity.

  Several methods are available. The test method used and the maximum level of anticomplement activity permitted should be approved by the national control authority.

- A test for haemagglutinins by the antiglobulin (Coombs) technique.

  In such tests, group O D(Rh_0)-positive cells should be used to test for anti-D (anti-Rh_0); group A and group B D(Rh_0)-negative cells should be used for anti-A and anti-B, respectively.

  The purpose of the test is to ensure that the use of the product will not give rise to haemolytic reactions. The upper limit of activity should be specified by the national control authority.

15.1 *Potency of normal immunoglobulins*

A 160 g/l solution of normal immunoglobulin shall be prepared from final bulk solution by a method that has been shown to be capable of concentrating, by a factor of 10 from source material, at least two different antibodies, one viral and one bacterial, for which an international standard or reference preparation is available (*16*) (e.g. antibodies against poliomyelitis virus, measles virus, streptolysin O, diphtheria toxin, tetanus toxin, staphylococcal α-toxin).

For immunoglobulins formulated at an immunoglobulin concentration lower than 16%, the concentrating factor for antibodies from source material may be proportionally lower.

The immunoglobulin solution shall be tested for potency at the concentration at which it will be present in the final container.

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

15.2 *Potency of specific immunoglobulins*

The potency of each final lot of specific immunoglobulin shall be tested with respect to the particular antibody that the preparation has been specified to contain. For intramuscular immunoglobulins, the following levels shall apply:

- For tetanus immunoglobulin, at least 100 IU/ml of tetanus antitoxin, as determined by a neutralization protection test in animals or by a method shown to be equivalent.
- For rabies immunoglobulin, at least 100 IU/ml of anti-rabies antibody,

as determined by an appropriate neutralization test in animals or by a method shown to be equivalent.

- For hepatitis B immunoglobulin, at least 100 IU/ml of anti-hepatitis antibody.
- For varicella zoster immunoglobulin, at least 100 IU/ml of anti-varicella zoster antibody, as measured by a comparative enzyme-linked immunosorbent assay or by a method shown to be equivalent.
- For anti-D (anti-Rh_0) immunoglobulin, the estimated potency 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 estimated potency.

The national control authority shall specify the antibody limits for other immunoglobulins.

After the potency tests, a test for immunoglobulin subclass may be performed. Different manufacturing steps have been shown to reduce the concentration of specific immunoglobulin subclasses (e.g. IgG1, IgG2, IgG3 and IgG4) in immunoglobulin preparations. The distribution of the four subclasses of IgG may be a factor in the efficacy of intravenous immunoglobulin preparations, since specific antibodies belonging to particular subclasses have been identified as being important in several infectious diseases.

In some countries the distribution of IgG subclasses has been measured by radial immunodiffusion. Enzyme-linked immunosorbent assays have also been described, and may be used if properly validated. Assays should be calibrated against the appropriate international reference materials.

15.3 *Sterility and safety*

Each filling lot 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) (*9*, p. 48) shall apply.

In some countries a general safety test may be required, whereby each filling lot is tested for extraneous toxic contaminants by appropriate tests involving injection into mice and guinea-pigs. The injection shall cause neither significant toxic reactions nor death within an observation period of seven days. The tests shall be approved by the national control authority.

The tests generally used are the intraperitoneal injection of 0.5 ml into each of at least two mice weighing approximately 20 g and the injection of 5.0 ml into each of at least two guinea-pigs weighing approximately 350 g. In some countries, if one of the animals dies or shows signs of ill-health, such as weight loss, during a specified period, 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, such as weight loss, during that period.

15.4 *Identity test*

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

Additional tests shall be made to determine that the protein is predominantly immunoglobulin.

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

15.5 *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 proportionally, on a rabbit body-weight basis, to the maximum single human dose recommended, but not more than 10 ml/kg of body weight. The recommended test doses are 1 ml/kg and 10 ml/kg of body weight for intramuscular and intravenous preparations, respectively.

A filling lot shall pass the test if it satisfies the requirements specified by the national control authority.

15.6 *Moisture content*

The residual moisture content of a sample from each filling lot shall, where appropriate, be determined by a method approved by the national control authority.

The methods in use are: (a) drying over phosphorus pentoxide for at least 24 h at a pressure not exceeding 2.7 Pa (0.02 mmHg); and (b) the Karl Fischer method.

The acceptable moisture content shall be determined by the national control authority.

15.7 *Hydrogen ion concentration*

The final product, reconstituted if necessary and diluted with 0.15 mol/l sodium chloride to give a protein concentration of 10 g/l, should, when measured at a temperature of 20–27 °C, have a pH of 6.9 ± 0.5.

In some countries, a different range of pH values is permitted for intravenous immunoglobulins.

15.8 *Stability*

For immunoglobulin solutions, a stability test shall be performed on each filling lot by heating an adequate sample at 37 °C for four weeks. No gelation or flocculation shall occur.

Alternatively (or in addition), the molecular size distribution of the immunoglobulin or assays of enzymes such as plasmin (fibrinolysin) may be used, when shown to predict stability reliably and when approved by the national control authority.

15.9 *Records*

The requirements of Good Manufacturing Practices for Biological Products (*8*, pages 27–28) shall apply.

15.10 *Samples*

The requirements of Good Manufacturing Practices for Biological Products (*8*, page 29, paragraph 9.5) shall apply.

15.11 *Labelling*

The requirements of Good Manufacturing Practices for Biological Products (*8*, pages 26–27) shall apply.

In addition, the label on the container shall state:

- the type of source material;
- the protein concentration;
- the concentration of preservative, if any;
- "For intramuscular use only" (if the immunoglobulins are not specially prepared for intravenous use);
- "For intravenous use", when appropriate;
- for specific immunoglobulin, the content of specific antibody expressed in International Units or equivalent national units;
- for freeze-dried preparations, the name and volume of reconstituting liquid to be added.

The label on the package or the package insert shall show:

- the approximate concentration of electrolytes and excipients and, for intravenous preparations, the approximate osmolality;
- the buffering capacity when the pH of the diluted product is lower than that specified in section 15.7;
- the concentration of preservative, if any;
- the recommended dose for each particular disease or condition;
- the warning "Do not use if turbid";
- the sodium and potassium concentrations (if the immunoglobulin is intended for intravenous use).

15.12 *Distribution and shipping*

The requirements of Good Manufacturing Practices for Biological Products (*8*) shall apply.

15.13 *Storage and shelf-life*

The requirements of Good Manufacturing Practices for Biological Products (*8*, pages 26–27) shall apply.

Liquid immunoglobulin shall be stored at 5 ± 3 °C and shall have a shelf-life of not more than three years. Freeze-dried preparations shall be stored below 25 °C and shall have a shelf-life of not more than five years.

Other storage conditions and shelf-lives may be approved by the national control authority.

16. Control of preparations of coagulation-factor concentrates (factor VIII, factor IX and fibrinogen)

Factor VIII preparations are available as both frozen products and freeze-dried concentrates. The frozen products are usually derived from a single donation and consist of the cryoprecipitated factor VIII from the donor concerned prepared in a closed separation system. The control of this product and the freeze-dried product from fewer than 10 plasma donations is covered in Part B, section 7.8.1.

Generally, the small-pool product undergoes little or no purification and is handled and subdivided in such a way that many control tests are inappropriate. However, freeze-dried factor VIII concentrates prepared from more than 10 donations may be purified.

Source material for factor VIII preparations shall meet the general criteria for donor selection and testing for disease markers as specified in Parts A and B. It shall preferably be plasma frozen within 8 h of collection or frozen cryoprecipitate. Such material shall be kept frozen at such a temperature that the activity of the factor VIII is maintained.

16.1 *Tests on final containers*

16.1.1 *Sterility and safety*

Each filling lot 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) (*9*, p. 48) shall apply.

In some countries a general safety test may be required, whereby each filling lot is tested for extraneous toxic contaminants by appropriate tests involving injection into mice and guinea-pigs. The injection shall cause neither significant toxic reactions nor death within an observation period of seven days. The tests shall be approved by the national control authority.

> The tests generally used are the intraperitoneal injection of 0.5 ml into each of at least two mice weighing approximately 20 g and the injection of 5.0 ml into each of at least two guinea-pigs weighing approximately 350 g. In some countries, if one of the animals dies or shows signs of ill-health, such as weight loss, during a specified period, 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, such as weight loss, during that period. For factor VIII and factor IX concentrates, the test dose should not exceed 500 IU of the coagulation factor per kg of body weight of the test animal.

16.1.2 *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

proportionally, on a rabbit body-weight basis, to the maximum single human dose recommended, but not more than 10 ml/kg of body weight.

The following test doses are suggested: factor VIII, 10 IU/kg of body weight; factor IX, 50 IU/kg of body weight; and fibrinogen, 30 mg/kg of body weight.

16.1.3 *Solubility and clarity*

Factor VIII preparations shall dissolve in the solvent recommended by the manufacturer within 30 min when held at a temperature not exceeding 37 °C. Factor IX preparations shall dissolve in the solvent recommended by the manufacturer within 15 min when held at 20–25 °C. The solutions, when kept at room temperature, shall not show any sign of precipitation or gel formation within 3 h of dissolution of the coagulation factors.

16.1.4 *Protein content*

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

16.1.5 *Additives*

Tests to determine the concentration of additives (such as heparin, polyethylene glycol, sodium citrate and glycine) used during production shall be carried out if required by the national control authority.

16.1.6 *Moisture content*

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

> The methods available are: (a) drying over phosphorus pentoxide for 24 h at a pressure not exceeding 2.7 Pa (0.02 mmHg); and (b) the Karl Fischer method.

16.1.7 *Hydrogen ion concentration*

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

> In some countries, different pH values are approved.

16.2 *Test applicable to factor VIII concentrates*

Each filling lot shall be assayed for factor VIII activity by a test approved by the national control authority, using a standard calibrated against the International Standard for Blood Coagulation Factor VIII: Concentrate.

> 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.

The specific activity shall be at least 500 IU/g of protein. The estimated potency shall be not less than 80% and not more than 125% of the stated potency. The confidence limits of error shall be not less than 64% and not more than 156% of the estimated potency.

16.3 *Tests applicable to factor IX concentrates*

16.3.1 *Potency*

Each filling lot shall be assayed for factor IX activity by a test approved by the national control authority, using a standard calibrated against the International Standard for Human Blood Coagulation Factors II, IX, and X in Concentrates.

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

16.3.2 *Presence of activated coagulation factors*

A test for the presence of activated coagulation factors shall be carried out by a method approved by the national control authority.

> In some countries, the non-activated 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 carried out by mixing equal volumes of the product under test and fibrinogen solution. The mixture is held at 37 °C and should not coagulate within 6 h. The usual range of concentrations of fibrinogen solution is 3–10 g/l.

16.3.3 *Alloantibodies*

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

> It is not possible to be specific about the tests for alloantibodies or to specify an upper limit for the titre.

16.4 *Test applicable to fibrinogen*

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

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

16.5 *Identity test*

An identity test shall be performed on at least one labelled container from each filling lot of coagulation-factor concentrate to verify that the preparation is of human origin. The test shall be one approved by the national control authority.

For albumin and plasma protein fraction, additional tests shall be made to determine that the protein is predominantly albumin.

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

16.6 ***Records***

The requirements of Good Manufacturing Practices for Biological Products (*8*, pages 27–28) shall apply.

16.7 ***Samples***

The requirements of Good Manufacturing Practices for Biological Products (*8*, page 29, paragraph 9.5) shall apply.

16.8 ***Labelling***

The requirements of Good Manufacturing Practices for Biological Products (*8*, pages 26–27) shall apply.

In addition, the label on the container shall state:

- the content of the coagulation factor expressed in International Units, where they exist;
- the amount of protein in the container;
- the volume of diluent needed for reconstitution;
- a reference to a package insert giving instructions for use, warnings about the possible transmission of infectious agents and precautions.

16.9 ***Distribution and shipping***

The requirements of Good Manufacturing Practices for Biological Products (*8*) shall apply.

16.10 ***Storage and shelf-life***

The requirements of Good Manufacturing Practices for Biological Products (*8*, pages 26–27) shall apply.

Final containers of freeze-dried preparations of factor VIII and factor IX shall have a maximum shelf-life of two years if they are stored at 5 ± 3 °C. Final containers of fibrinogen shall have a maximum shelf-life of five years if they are stored at 5 ± 3 °C.

> Other storage conditions and shelf-lives may be approved by the national control authority provided that they are consistent with the data on the stability of the products.

Part D. National control requirements

17. General

The general requirements for control laboratories in the Guidelines for National Authorities on Quality Assurance for Biological Products (*6*) shall apply.

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

products. Where appropriate, these standards should be calibrated against the relevant International Standard.

The national control authority shall have authority to approve the production and control methods used and settle all matters left for its decision or approval in Parts A, B and C.

The national control authority shall also have authority to approve the use of materials that carry potential risk and shall approve any new method of production and the preparation of any new product.

> New products or products prepared by new production methods may be monitored to confirm their efficacy and safety.

18. Release and certification

Human blood and blood products shall be released only if they satisfy the requirements of Parts A, B and C, wherever applicable.

A certificate signed by the appropriate official of the national control authority shall be provided at the request of the manufacturing establishment and shall state whether the product in question meets all national requirements as well as Parts A, B and C (whichever is relevant) of the present Requirements. The certificate shall also state the date of the last satisfactory potency test performed by the manufacturer, 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 document shall be attached.

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

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Appendix

Summary protocol for collection of source material

1. Name and address of collecting centre _____

   _____

2. Source material _____

3. Details of single donations, where applicable:
   (a) Donor identification _____
   (b) Date of collection _____
   (c) Volume in container _____
   (d) Results of tests for HBsAg _____
   (e) Results of tests for anti-HIV _____
   (f) Results of tests for anti-HCV _____
   (g) If applicable, results of tests for antibody to hepatitis B core antigen _____
   (h) If applicable, results of tests for alanine aminotransferase _____

4. Special information:
   (a) Anticoagulant used _____
   (b) Was the material collected for special purposes (e.g. as a source of specific antibodies)? _____
   (c) Precautions to be taken when using the material _____

5. Conditions of storage _____

6. Does the donation comply with existing agreements between the supplier and manufacturer? _____

7. Does the donation comply with the Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives published by WHO? _____

Name and signature of responsible person _____

Date _____