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WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Geneva, 11–19 October 1988

Members

Dr J. Furesz, Director, Bureau of Biologies, Drugs Directorate Virus Laboratory, Tunney's Pasture, Ottawa, Ontario, Canada
Dr M. C. Hardegree, Director, Division of Bacterial Products, Center for Biologies Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA (Chairwoman)
Professor D. K. Hazra, Head, Nuclear Medicine and Radio-immunoassay Unit, Post Graduate Department of Medicine, S.N. Medical College, Agra, India
Dr S. L. Jeffcoate, Head, Department of Endocrinology, National Institute for Biological Standards and Control, Potters Bar, Herts, England (Rapporteur)
Mr J. Lyng, Head, Laboratory for Biological Standards, State Serum Institute, Copenhagen, Denmark
Professor T. Matuhasi, Okinawa Memorial Institute for Medical Research, Toranomon Hospital, Tokyo, Japan (Vice-Chairman)
Dr H. Mirchamsy, Associate Director, Razi State Institute of Sera and Vaccines, Teheran, Islamic Republic of Iran
Professor A. N. U. Njoku-Obi, Head, Department of Medical Microbiology, University of Nigeria (College of Medicine), Enugu, Nigeria

Representatives of other organizations

European Pharmacopoeia Commission
Mr P. Castle, European Pharmacopoeia Commission, Council of Europe, Strasbourg, France

International Federation of Pharmaceutical Manufacturers Associations
Dr J. Fischer, IFPMA, Behringwerke AG, Marburg/Lahn, Federal Republic of Germany

Secretariat

Dr M. Beck, Director, Institute of Immunology, Zagreb, Yugoslavia (Temporary Adviser)
Dr C. Guthrie, Commonwealth Serum Laboratories, Parkville, Victoria, Australia (Temporary Adviser)
Professor W. Hennessen, Berne, Switzerland (Temporary Adviser)
Dr J. G. Kreeftenberg, Head, Laboratory for the Control of Bacterial Vaccines, National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands (Temporary Adviser)

* Unable to attend: Dr Zhang Tian-ren, Shanghai Institute of Biological Products, Shanghai, China; Dr T. B. Jablokova, Tarasević State Institute for the Standardization and Control of Medical Biological Preparations, Moscow, USSR.

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Dr H.W. Krijnen, Director, Central Laboratory of the Netherlands Red Cross
Blood Transfusion Service, Amsterdam, Netherlands (Temporary Adviser)
Dr D. Magrath, Chief, Biologicals, WHO, Geneva, Switzerland (Secretary)
Dr R. Netter, Director-General, National Health Laboratory, Ministry of
Solidarity, Health, and Social Protection, Paris, France (Temporary Adviser)
Dr P.S. Norman, Chairman, Allergen Standardization Subcommittee,
International Union of Immunological Societies, The Good Samaritan
Hospital, Baltimore, MD, USA (Temporary Adviser)
Dr R.D.G. Theakston, Liverpool School of Tropical Medicine, Liverpool,
England (Temporary Adviser)
Dr D.P. Thomas, Head, Division of Haematology, National Institute for
Biological Standards and Control, Potters Bar, Herts, England (Temporary
Adviser)
Dr W.W. Wright, Drug Standards Division, United States Pharmacopeia, The
National Formulary, Rockville, MD, USA (Temporary Adviser)
WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Thirty-ninth Report

The WHO Expert Committee on Biological Standardization met in Geneva from 11 to 19 October 1988. The meeting was opened on behalf of the Director-General by Dr D. Magrath, Chief, Biologicals.

GENERAL

Guidelines for the preparation and establishment of international and other standards and reference reagents for biological substances

In 1978, WHO published the Guidelines for the Preparation and Establishment of Reference Materials for Biological Substances (WHO Technical Report Series, No. 626, 1978, Annex 4). These were amended in 1986 and a revised version was published (WHO Technical Report Series, No. 760, 1987, Annex 3). The Committee reviewed these Guidelines, and agreed that some further amendments were needed and that their usefulness would be increased by putting some parts of the text in large print and others in small print (as in the Requirements for Biological Substances) so as to highlight essential practical matters. The Committee therefore requested the WHO Secretariat to investigate means both of updating the Guidelines and of disseminating them as widely as possible.

Units of activity for international reference reagents

The Committee recognized the difficulties in sustaining the distinction between international standards (required for quantitative assays) and international reference reagents, identified in its thirty-seventh report as "generally not used for the quantitative assay of the activity of biological products" (WHO Technical Report Series, No. 760, 1987, p. 42). In its thirty-eighth report, the Committee had asked the WHO Secretariat to review this matter
(WHO Technical Report Series, No. 771, 1988, p. 11). The Committee agreed that in a few instances it is useful and acceptable to assign units of activity to reference materials that do not qualify for international standard status and that these units can be distinguished from international units by designating the method used to assign them (e.g., radioallergosorbent (RAST) inhibition units for allergens, limit of flocculation (LF) units for toxoids).

Reference materials for uses other than biological standardization

The Committee was informed that, in response to the request made in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, pp. 11–12), the WHO Secretariat had investigated the need for reference materials for uses other than biological standardization. The Committee recognized that this subject was complex and needed to be analysed on a case-by-case basis and that it would require continuing evaluation.

Definition of a “production area” for biologicals

The Committee was informed that, in accordance with the request made in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, pp. 12–13), the WHO Secretariat had reviewed the statement that "At any one time, manufacture of each biological product shall take place in a separate area using separate equipment", which appeared in the General Requirements for Manufacturing Establishments and Control Laboratories (WHO Technical Report Series, No. 323, 1966, Annex 1). On the basis of the available information, the Committee concluded that it would not be appropriate to modify this restriction at this time, even if production were taking place in closed-system fermenters. The Committee did, however, recognize that production technologies for biologicals were developing rapidly and requested the WHO Secretariat to arrange for a complete review of the General Requirements for Manufacturing Establishments and Control Laboratories.

The Committee recognized that the guidelines for the National Control of Vaccines and Sera (WHO Technical Report Series, No. 658, 1981, pp. 299–309) needed strengthening to provide additional guidance, especially with respect to the information to be made available to national authorities before a product is licensed,
and requested the WHO Secretariat to arrange for a review of these
guidelines also.

Residual content of animal serum or its constituents in biologicals
prepared in cell cultures

The Committee noted that, in accordance with the request made
in its thirty-eighth report (WHO Technical Report Series, No. 771,
1988, p. 13), the WHO Secretariat had prepared a review of
"methods by which the content of animal serum constituents... can
be assayed" (BS/88.1592). The limit of 1 part per million (1 μl
animal serum per litre of vaccine) cited in some requirements is
approximately equivalent to 50 ng of albumin per ml. Modern
methods (e.g., enzyme-linked immunosorbent assay (ELISA) and
immunoprecipitin tests) can detect as little as 1 ng/ml of albumin; no
information was presented to the Committee on methods which
could be used for the quantification in vaccines of other animal
serum constituents.

The Committee therefore requested the Secretariat to note, when
revising existing requirements and drafting requirements for new
biological products, that the permitted maximum of 1 part per
million of animal serum in viral vaccines and other biological
products could be tested for by measuring serum albumin, but
recognized that in certain situations albumin might not be an
appropriate indicator for other serum proteins.

Distribution of international reference materials by the four
International Laboratories for Biological Standards

The Committee noted a report (BS/88.1601) on the distribution
during 1987 of international reference materials by the four
International Laboratories for Biological Standards. The
Committee considered that it would be helpful in planning future
activities if the custodian laboratories could provide data for its
next meeting showing the proportions of recipient laboratories
in different categories (e.g., national control authorities,
manufacturers, hospitals, university or research laboratories).

1 References prefixed "BS/..." 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.
Procedure for evaluating the acceptability in principle of vaccines proposed to United Nations agencies for use in immunization programmes

The Committee noted a proposed revision of the Procedure for Evaluating the Acceptability in Principle of Vaccines Proposed to United Nations Agencies for Use in Immunization Programmes (WHO Technical Report Series, No. 760, 1987, Annex 1) (BS/88.1583). After making certain modifications, the Committee agreed that the revised procedure should be annexed to this report (see Annex 1). The Committee also agreed with the implied requirement in the revision for an effective national control authority in the country of manufacture of a vaccine, as previously called for by the Committee in its thirty-first report (WHO Technical Report Series, No. 658, 1981, p. 299).

WHO cell banks of continuous cell lines for the production of biologicals

The Committee was informed of the progress made in response to a recommendation by a WHO study group (WHO Technical Report Series, No. 747, 1987) that WHO should establish banks of characterized cell lines that would be of value to national control authorities and manufacturers for the production of biologicals.

The Committee was informed that a bank of Vero cells had been donated to WHO and was being stored at the European Collection of Animal Cell Cultures, Porton Down, England. The Committee was informed also that 12 laboratories were collaborating in the characterization of this cell line, inter alia by means of tests for sterility, adventitious agents, tumorigenicity, reverse transcriptase, and identity (using isoenzymes and DNA probes) (Requirements for Continuous Cell Lines used for Biologicals Production; WHO Technical Report Series, No. 745, 1987, Annex 3) and that data had been received from four laboratories to date.

Cytokines

Cytokines are a heterogeneous group of biologically active polypeptides that regulate cell growth, differentiation, and function. They are secreted by lymphocytes (lymphokines) or monocytes and macrophages (monokines) and are primarily involved in the induction and maintenance of defence mechanisms against
microbial infections and cancer. Clinical investigation of their use in cancer therapy and in the stimulation of haematopoiesis and the immune system has shown their potential, and cytokines produced by recombinant DNA techniques are now available in sufficient quantities for clinical use in a range of disease types. It is therefore essential to establish appropriate international reference materials for use in standardizing the unitage of therapeutic products. The International Standard for Interleukin-2 and a number of international standards for murine and human interferon α, β, and γ were established at the Committee's thirty-eighth meeting (WHO Technical Report Series, No. 771, 1988). In addition, the Committee had recognized that international standards for interleukin-1 were required (WHO Technical Report Series, No. 745, 1987, p. 26) and was informed at its present meeting of the need for an international reference material for interleukin-6. The Committee agreed that this group of compounds should be considered under the heading of "Cytokines"; interleukin-1 and interleukin-6 are therefore dealt with in this way in the present report.

Standardization of allergenic extracts

The Committee discussed the problems associated with the standardization of allergenic extracts and recognized the importance to world health of allergic diseases, for the diagnosis and treatment of which such extracts have been used clinically for over 70 years. They are complex mixtures of a number of active components, however, and the Committee therefore reaffirmed the need for their standardization. It considered a document prepared by the Allergen Standardization Subcommittee of the International Union of Immunological Societies (BS 88.1599) following the decisions contained in the Committee's thirty-eighth report (WHO Technical Report Series, No. 771, 1988, pp. 20–21). In this report, concern was expressed about the results of preliminary stability studies on two proposed reference materials, Alternaria alternata extract and Bermuda grass (Cynodon dactylon) pollen extract, and of the results of accelerated degradation studies made on the International Standard for Dog (Canis domesticus) Hair and Dander Extract, and the Committee had requested the WHO Secretariat to arrange for further stability studies.

The Committee agreed that, in the case of such complex mixtures, the assumption on which the accelerated degradation test is based,
namely that degradation at elevated temperature is a single-order reaction, may not hold good. In some instances, these tests have overestimated the instability of the preparation; in such cases, real-time stability data are critically important. The Committee emphasized the importance of using several assays of activity with different specificities when studying the stability of allergenic extracts. As knowledge increases, reference materials for specific components may be needed.

Snake venoms and antivenoms

The Committee recognized the importance in terms of world health of the preparation of international reference materials for snake venoms and antivenoms; many thousands of deaths from snake-bite occur annually in certain areas of Africa, South-East Asia, the Indian subcontinent, and Central and South America. There is an urgent need for international reference materials for snake antivenoms and defined methodologies whereby preparations of such antivenoms can be tested in these areas. The Committee therefore asked the WHO Secretariat to investigate the possible need for requirements for the manufacture and control of snake antivenoms.

The Committee noted the progress made in the preparation and characterization of reference materials for snake venoms and antivenoms and recommended that this work, which is being coordinated at the WHO Collaborating Centre for the Control of Antivenoms, Liverpool, England, should be continued, be expanded to include other laboratories, and receive further support. In future, the selection of venoms for study should reflect the scale of the problem in different regions; for example, *Crotalus durissus* and *Bothrops jararaca* from South America, *Vipera russelli* and *Bungarus caeruleus* from Sri Lanka and India, and *Bitis arietans* and *Naja nigricollis* from Africa are not currently being studied although they are significant causes of death and morbidity. The Committee also recommended that venoms from scorpions of medical importance and their related antivenoms should be considered for inclusion in the programme.
SUBSTANCES

Antibiotics

1. Teicoplanin

The Committee noted that the preparation of teicoplanin referred to in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 17) had been shown to have adequate stability when assayed microbiologically and tested by high-performance liquid chromatography (BS/88.1597). The Committee also noted that the National Institute for Biological Standards and Control, Potters Bar, was arranging a collaborative study of this antibiotic.

Antibodies

2. Cytomegalovirus immunoglobulin

The Committee was informed that, in accordance with the request made in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 18), a candidate international reference material from Australia had been offered to the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, and that a collaborative study would be arranged.

3. Tetanus immunoglobulin

The Committee was informed that, in accordance with the request made in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 18), some 5000 ampoules of candidate reference material of tetanus immunoglobulin had been obtained and that an international collaborative study was being organized by the National Institute for Biological Standards and Control, Potters Bar.

4. Varicella zoster immunoglobulin

The Committee was informed that the stability studies referred to in the thirty-eighth report (WHO Technical Report Series, No. 771,
1988, p. 19) had continued for over 12 months at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam. These studies had confirmed the stability of the International Standard for Varicella Zoster Immunoglobulin.

5. Anti-toxoplasma IgM serum

The Committee noted that, in accordance with the request made in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, pp. 19–20), the State Serum Institute, Copenhagen, had conducted further stability studies on the proposed international standard of anti-toxoplasma IgM serum (BS/88.1588). The Committee noted that the results were still inconclusive and that studies were continuing, with the involvement of a second laboratory.

6. Anti-rabies serum, equine, and rabies immunoglobulin, human

The Committee noted the report of the international collaborative study in eight laboratories, referred to in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 20), on two methods used to assay the potency of rabies antibody preparations (BS/88.1587 Rev. 1). This study had been arranged by the State Serum Institute, Copenhagen, and the Center for Biologics Evaluation and Research, Bethesda, following the report in 1986 that some laboratories had obtained different estimates of relative potencies when the mouse neutralization test (MNT) and the neutralization test in cell culture (rapid fluorescent focus-inhibition test (RFFIT)) were used. The Committee noted that the conclusions of this study were as follows:

1. The differences between the two methods reported earlier were not generally confirmed. In three of the eight laboratories, the MNT/RFFIT potency ratios were significantly greater than unity, but were not the same. In contrast, the ratios obtained in the other five laboratories were neither significantly different from each other nor significantly different from unity. It appeared that the differences in the MNT/RFFIT ratios could be explained by the MNT results, since the RFFIT potencies obtained were the same in all eight laboratories.

2. The slopes of the dose–response curves were different for the RFFIT and MNT results, and differences were also found
between laboratories. It is noteworthy, however, that in none of the eight laboratories were slope differences between the equine and human preparations observed.

(3) In the three divergent laboratories, MNT/RFFIT ratios were reduced when a human immunoglobulin preparation was used as reference instead of the equine standard, but ratios higher than unity were still observed in two of the three laboratories. No explanation can be given for these observations.

(4) The potency estimates obtained for the International Standard for Rabies Immunoglobulin were consistent with the potency of 59 International Units of rabies antibodies per ampoule assigned to this preparation at the thirty-fifth meeting of the Committee (WHO Technical Report Series, No. 725, 1985, p. 17). This applies to the RFFIT results obtained in all eight laboratories, but to the MNT results only if the potency estimates are based solely on data from the five laboratories that obtained comparable results.

The Committee was informed that the problems were being investigated by the three divergent laboratories, since in the other five it was possible to perform the MNT in such a way that species differences between reference and test preparations did not influence the outcome.

7. Sera containing antibodies to human immunodeficiency virus type 1 (HIV-1)

The Committee noted a report (BS/88.1602) on two preparations of human serum, one reactive against HIV-1 and the other non-reactive, that had been tested in the international collaborative study referred to in its thirty-seventh report (WHO Technical Report Series, No. 760, 1987, pp. 30–31). The Committee noted that no data were presented on the precision of the ampoule-filling process or the stability of the materials. The Committee therefore noted the existence of the materials, which could serve as negative and positive controls in HIV-1 antibody tests, but requested the WHO Secretariat to obtain further information on the adequacy of the viral inactivation procedure used for the materials, their stability, and possible mechanisms for distributing them.
8. Botulinus antitoxins

The Committee noted that stocks of the International Standard for Clostridium botulinum Type C Antitoxin, Equine were so low that distribution had ceased (BS/88.1589). The Committee also noted that toxins of types A, B, and E are those for which therapeutic antisera are used in humans, so that continued supply of the international standards of antitoxins to these types is important. Antitoxins to types C, D, and F are mainly of interest for typing purposes. The Committee agreed that the international standards for these antitoxins should be retained, since they are used to define the relevant international units, but that the International Standard for Clostridium botulinum Type C Antitoxin need not be replaced.

Antigens

9. Measles vaccine (live)

The Committee noted a report on the stability studies, referred to in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 22), of the International Reference Reagent for the Assay of Measles Vaccine (Live), which is freeze-dried in rubber-stoppered vials (BS/88.1603). Interim results obtained at the National Institute for Biological Standards and Control, Potters Bar, showed no loss in potency after four years at \(-20^\circ\text{C}\). Further accelerated degradation studies are in progress to establish a predicted loss on long-term storage at \(-20^\circ\text{C}\).

10. Rabies vaccine

The Committee was informed that the 4000 ampoules of the proposed replacement for the International Standard for Rabies Vaccine referred to in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 22) had been obtained and that the stability studies requested were in progress. The Committee was also informed that small supplies of two additional vaccines produced from different virus strains and on different cell substrates had been offered and that, if the stability studies were successful, all four vaccines would be included in an international collaborative study arranged by the State Serum Institute, Copenhagen, which would include both \textit{in vivo} and \textit{in vitro} methods. The Committee was
further informed that the International Standard for Rabies Vaccine and the proposed replacement preparation would both be included in a collaborative study arranged by another organization, which would use an in vivo assay method different from the one generally used for the potency assay of rabies vaccines for human use (the so-called NIH method). The Committee requested the WHO Secretariat to continue monitoring methodological developments in the area of rabies vaccine testing.

11. Acellular pertussis vaccines

The Committee noted the report (BS/88.1586) on the international collaborative study of candidate reference materials for the assay of acellular pertussis vaccines arranged by the National Institute for Public Health and Environmental Protection, Bilthoven. Three freeze-dried preparations (JNIH-3, acellular pertussis vaccine; JNIH-4, filamentous haemagglutinin, FHA; and JNIH-5, leukocytosis-promoting factor, LPF) had been studied in ten laboratories. The products appeared to be of appropriate purity, immunogenicity, and stability to serve as reference materials but additional statistical evaluation of the immunogenicity data was required. The Committee also noted that the three candidate reference materials had been used for the characterization of adsorbed JNIH-6 vaccine; this had been produced from the same bulk material as JNIH-3 and had recently been investigated for efficacy in a controlled field trial in Sweden.

The Committee considered that further validation studies of the use of JNIH-3, JNIH-4, and JNIH-5 as reference reagents for vaccines from other manufacturers were necessary.

The Committee was also informed about three freeze-dried antibody preparations (JNIH-10, human anti-pertussis FHA and anti-pertussis LPF immunoglobulin; JNIH-11, mouse anti-pertussis FHA serum; and JNIH-12, mouse anti-pertussis LPF serum) that appeared to be stable in a study performed in one laboratory.

The Committee considered all these products to be potentially valuable reagents for the characterization of acellular pertussis vaccines and noted that the reagents were available from the State Serum Institute, Copenhagen, though they were not yet established as international reference reagents.
12. Diphtheria toxoid and tetanus toxoid for flocculation tests

The Committee noted the results (BS/88.1590) of an international collaborative study arranged by the State Serum Institute, Copenhagen, of proposed reference reagents of diphtheria toxoid and tetanus toxoid for flocculation tests, which estimate antigen content in “limit of flocculation” or Lf units. The Committee noted that the results from the 14 participating laboratories were reproducible and showed the materials to be stable; it therefore established the materials studied as the International Reference Reagent of Diphtheria Toxoid for Flocculation Tests, with a unitage of 900 Lf units of diphtheria toxoid per ampoule, and the International Reference Reagent of Tetanus Toxoid for Flocculation Tests, with a unitage of 1000 Lf units of tetanus toxoid per ampoule.

Blood products

13. Apolipoproteins A-I and B

The Committee noted the results of a collaborative study in 28 laboratories arranged under the auspices of the International Union of Immunological Societies on a candidate reference material of apolipoprotein A-I and apolipoprotein B, measurements of which are increasingly used in screening for cardiovascular disease (BS/88.1600). The Committee noted that the material consisted of aliquots of human sera freeze-dried in vials, rather than ampoules, and that no stability data had been presented; furthermore, material in a freeze-dried form might not be suitable for assays based on nephelometry. The Committee therefore requested the WHO Secretariat to consider the matter further.

14. Factor VIII and von Willebrand factor in plasma

The Committee noted the results of the collaborative study performed in 25 laboratories in 13 countries of the proposed replacement for the International Reference Preparation of Factor VIII-Related Activities in Plasma established in 1982 (WHO Technical Report Series, No. 687, 1983, pp. 23–24), stocks of which were depleted (BS/88.1598). The Committee noted that the candidate replacement material had been assayed against the
International Reference Preparation in four different assay systems and that it had been found to be of adequate stability. Taking into account the new nomenclature recommended by the International Committee on Thrombosis and Haemostasis, the Committee established the material in ampoules coded 87/718 as the second International Standard for Factor VIII and von Willebrand Factor in Plasma and assigned activities in terms of the four assay systems: factor VIII clotting activity, 0.60 International Unit per ampoule; factor VIII antigen, 0.91 International Unit per ampoule; von Willebrand factor antigen, 0.91 International Unit per ampoule; von Willebrand factor ristocetin cofactor activity, 0.84 International Unit per ampoule.

**Endocrinological and related substances**

15. Erythropoietin

The Committee was informed that, in accordance with the request made in its thirty-sixth report (WHO Technical Report Series, No. 745, 1987, p. 23), candidate reference materials for human erythropoietin prepared by recombinant DNA techniques had been obtained and ampouled at the National Institute for Biological Standards and Control, Potters Bar, and that, after initial stability studies had been carried out, an international collaborative study would be arranged.

16. Luteinizing hormone, pituitary

The Committee noted the results of the collaborative study referred to in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 28) of a candidate material to replace the International Reference Preparation of Human Pituitary Luteinizing Hormone (LH) for Immunoassay, stocks of which were almost exhausted (BS/88.1604). The Committee also noted that the collaborative study had been carried out in 18 laboratories in 11 countries using in vivo and in vitro bioassays, a receptor binding assay, and a variety of different immunoassays. The Committee noted further that the potency of the proposed replacement (in ampoules coded 80/552) was consistent with that of the International Reference Preparation in all the systems studied and
that it was of adequate stability. The Committee therefore established the material studied as the second International Standard for Pituitary Luteinizing Hormone, and assigned an activity of 35 International Units of pituitary luteinizing hormone to the contents of each ampoule.

17. Somatropin

The Committee had noted in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 27) that there might be a need for an international reference material for human growth hormone of relative molecular mass 22,000 prepared by recombinant DNA techniques (somatropin). The Committee noted that a batch of somatropin had been obtained by the National Institute for Biological Standards and Control, Potters Bar; it had been distributed into ampoules coded 88/624 and a collaborative study, including the International Standard for Human Growth Hormone (of pituitary origin), was being arranged.

18. Inhibin

The Committee was informed that the proposed reference material of porcine inhibin described in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 27) was being examined in a collaborative study organized by the National Institute for Biological Standards and Control, Potters Bar, in which laboratories in 15 countries were participating, using a variety of in vivo and in vitro bioassays and immunoassays.

19. Matrix standard for assays of thyroid-stimulating hormone

The Committee noted a progress report (BS/88.1605) on the collaborative study being carried out by WHO and the International Federation of Clinical Chemistry on the use of a common matrix standard for assays of thyroid-stimulating hormone, as referred to in its thirty-sixth report (WHO Technical Report Series, No. 745, 1987, p. 11). The nine participants in the study were manufacturers of assay kits for thyroid-stimulating hormone based on the ultrasensitive two-site immunometric method. The Committee noted that data from these participants were undergoing statistical analysis at the National Institute for Biological Standards and Control, Potters Bar, and that a report would be prepared.
20. Insulin-like growth factor I (somatomedin C)

The Committee noted that the collaborative study of candidate reference materials of insulin-like growth factor I (formerly known as somatomedin C) referred to in its thirty-seventh report (WHO Technical Report Series, No. 760, 1987, p. 28) had been completed (BS/88.1606). The Committee also noted that the material was of adequate stability when tested in immunoassay systems, but that the data obtained from bioassays were inconclusive.

On the basis of the study, and with the agreement of eight of the nine participants, the Committee established one of the preparations, in ampoules coded 87/518, as the first International Reference Reagent of Insulin-like Growth Factor I, for Immunoassay, with 3.1 μg of insulin-like growth factor I per ampoule.

21. Thyroid-stimulating antibodies

The Committee was informed that, in accordance with the request made in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 26), the National Institute for Biological Standards and Control, Potters Bar, had obtained a candidate reference material for thyroid-stimulating antibodies, consisting of 4 litres of plasma from a patient with high levels of these antibodies. The Committee was also informed that an immunoglobulin fraction (prepared from this pool by ammonium sulfate precipitation and diethylaminoethyl cellulose chromatography) was highly active in a radioreceptor assay, and that an international collaborative study was being arranged.

22. Follicle stimulating hormone and luteinizing hormone, urinary

The Committee noted that stocks of the first International Standard for Human Urinary Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) for Bioassay, established in 1974 (WHO Technical Report Series, No. 565, 1975, p. 6), were nearly exhausted and that material in ampoules coded 71/223 could serve as a replacement (BS/88.1595). The Committee noted that these ampoules had been prepared from the same batch of material as the first International Standard and had been included in the same international collaborative study. The Committee also noted that a recent supplementary study had confirmed the stability of the
proposed replacement material, and therefore established the material in ampoules coded 71/223 as the second International Standard of Urinary Follicle Stimulating Hormone and Luteinizing Hormone. It assigned an activity of 54 International Units of urinary follicle stimulating hormone activity and 46 International Units of urinary luteinizing hormone activity per ampoule.

23. Prolactin

The Committee was informed that stocks of the second International Standard for Human Prolactin established in 1986 (WHO Technical Report Series, No. 760, 1987, p. 28) were depleted and that a replacement was needed. The Committee noted that two other preparations (coded 84/500 and 83/573) had been examined in an earlier study and found to have similar characteristics to the second International Standard, but had at that time been predicted to have higher rates of degradation on the basis of short-term accelerated degradation studies (BS/86.1520). The Committee noted that further stability studies had been carried out which showed the predicted rates of loss to be less than 0.1% per year (BS/88.1596). The Committee therefore established the preparation in ampoules coded 84/500 as the third International Standard for Prolactin, and assigned an activity of 0.053 International Units of prolactin to the contents of each ampoule.

24. Calcitonin-gene-related peptide

The Committee was informed that there was a need for an international reference material of calcitonin-gene-related peptide, a 37-amino-acid peptide that is a potent vasodilator and is under investigation in clinical trials. The Committee therefore requested the National Institute for Biological Standards and Control, Potters Bar, to obtain suitable material and arrange a collaborative study.

25. Salmon calcitonin

The Committee was informed that, following the report at its thirty-seventh meeting (WHO Technical Report Series, No. 760, 1987, pp. 23–24) of stability problems when the proposed replacement international standard for salmon calcitonin was freeze-dried with trehalose as a carrier, a further batch of material,
ampouled with mannitol, had been tested and appeared to be of adequate stability. It was to be investigated in an international collaborative study involving laboratories in six countries and including the candidate reference material for eel calcitonin.

26. Eel calcitonin

The Committee was informed that there was a need for an international reference material for eel calcitonin. Pilot studies of candidate material at the National Institute for Biological Standards and Control, Potters Bar, with mannitol as a carrier, had shown the material to be of adequate stability. A definitive fill would be made and the material included in the collaborative study of salmon calcitonin.

27. Thyroxine-binding globulin

The Committee was informed that there was a need for an international reference material for the quantification of thyroxine-binding globulin. Assays of this globulin are widely used in the diagnosis and management of thyroid diseases but have been hindered by the lack of an international standard. The Committee therefore requested the National Institute for Biological Standards and Control, Potters Bar, to obtain suitable material and arrange a collaborative study.

28. Thyroglobulin

The Committee was informed that there was a need for an international reference material for thyroglobulin, a protein present in the colloid of the thyroid gland; the assay of this substance is used in the management of thyroid cancer. The Committee therefore requested the National Institute for Biological Standards and Control, Potters Bar, to obtain suitable material and arrange a collaborative study.

Cytokines

29. Interleukin-1

The Committee was informed that the international collaborative study of candidate reference materials of human alpha and beta

30. Interleukin-6

The Committee was informed that there was a need for an international reference material for interleukin-6, a cytokine of relative molecular mass 21 000 of potential therapeutic importance as a stimulant of the immune system. Some interleukin-6 material of recombinant DNA origin had been offered to WHO, and the Committee requested the National Institute for Biological Standards and Control, Potters Bar, to obtain the material and arrange a collaborative study.

31. Other cytokines

The Committee was informed that there was a need for international reference materials for a number of other cytokines, including: interleukin-3, interleukin-4 (B-cell stimulatory growth factor), granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, tumour necrosis factor, and transforming growth factor beta. The Committee requested the National Institute for Biological Standards and Control, Potters Bar, to obtain suitable materials and arrange collaborative studies.

Allergens

32. Alternaria (Alternaria alternata) extract

The Committee was informed that the further studies on the stability of the Alternaria (Alternaria alternata) extract requested in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, pp. 20–21) had confirmed its high moisture content and instability and thus its unsuitability to serve as an international reference material.

33. Bermuda grass (Cynodon dactylon) pollen extract

The Committee was informed that the results of the studies on the Bermuda grass (Cynodon dactylon) pollen extract requested in its
thirty-eighth report (WHO Technical Report Series, No. 771, 1988, pp. 20–21) had shown that it had adequate stability, as measured by the radioallergosorbent (RAST) inhibition test. The Committee therefore established the preparation as the International Reference Reagent of Bermuda Grass (Cynodon dactylon) Pollen Extract and assigned 100 000 RAST inhibition units to the contents of each ampoule.

34. Dog (Canis domesticus) hair and dander extract

The Committee was informed about the results of the stability studies of the International Standard of Dog (Canis domesticus) Hair and Dander Extract requested in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 21). It agreed that, although the results of the RAST inhibition test were inconclusive, the rocket immunoelectrophoresis assays for three specific antigens (Ag3, Ag13, and albumin) showed these to be of adequate stability.

35. Other allergens

The Committee was informed that there was a need for international reference materials for the following respiratory allergens: pollen from white oak trees (Quercus alba), olive trees (Olea europaea), mugwort (Artemisia vulgaris), and Parietaria judaica vines, the house dust mite Dermatophagoides farinae, and the mould Cladosporium herbarum (Hornodendrum). It noted that the International Union of Immunological Societies was planning to obtain candidate materials and organize collaborative studies.

Miscellaneous

36. Snake venoms and antivenoms

The Committee noted the progress made in the standardization of snake venoms and antivenoms since its thirty-sixth meeting (WHO Technical Report Series, No. 745, 1987, p. 16) (BS/88.1585). It noted that eight proposed international reference venoms—Echis carinatus (Islamic Republic of Iran and Mali), Vipera russelli (Thailand), Naja naja kaouthia (Thailand), Bothrops atrox asper (Costa Rica), Trimeresurus flavoviridis (Japan), Notechis scutatus (Australia), and
Crotalus atrox (Mexico)—had been characterized using biological tests (lethality and haemorrhagic, necrotizing, procoagulant, and defibrinogenating activities) and that stability tests on the ampouled venoms were in progress. The Committee also noted that tests for neuromuscular paralytic and systemic myotoxic activities were under development. It noted further that antivenoms had been prepared against six of the venoms and that a protocol for the assessment of their neutralizing activities was being prepared by the WHO Collaborating Centre for the Control of Antivenoms, Liverpool. The test for neutralization of lethality is currently the most useful general assay. More specific tests are: for viperid antivenoms, neutralization of haemorrhagic and procoagulant activities; for elapid antivenoms, neutralization of neuromuscular paralytic activity; for sea snake and certain viperid and elapid antivenoms, neutralization of systemic myotoxic activity.1

37. Digitalis

The Committee was informed that, in accordance with the request made in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 32), the WHO Secretariat had made inquiries but had failed to identify a continuing need for an International Standard for Digitalis; the Committee therefore recommended that the third International Standard for Digitalis be disestablished.

REQUIREMENTS FOR BIOLOGICAL SUBSTANCES

38. Requirements for hepatitis B vaccines made by recombinant DNA techniques

The Committee noted that, in accordance with the request made in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 34), the WHO Secretariat had arranged for the preparation of a single set of proposed requirements for hepatitis B vaccines made by recombinant DNA techniques using either yeast, for which requirements were adopted in 1986 (Requirements for Biological Substances No. 39; WHO Technical Report Series, No. 760, 1987,

Annex 6), or mammalian cells (BS/88.1582). The Committee also noted that the proposed revised requirements incorporated a reference to the appropriate sections of the Requirements for Continuous Cell Lines Used for Biologicals Production (Requirements for Biological Substances No. 37; WHO Technical Report Series, No. 745, 1987, Annex 3). After making certain modifications to the draft, the Committee adopted the revised Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques and agreed that they should be annexed to this report (Annex 2).

39. Requirements for human interferons prepared from lymphoblastoid cells

The Committee was informed that, in accordance with the request made in its thirty-eighth report (WHO Technical Report Series, No. 771, 1988, p. 33), the WHO Secretariat had arranged for further review of the draft requirements for human interferons prepared from lymphoblastoid cells (BS/87.1546). The Committee decided that wherever possible the format of the requirements should correspond to that of the Requirements for Human Interferons Made by Recombinant DNA Techniques (Requirements for Biological Substances No. 41; WHO Technical Report Series, No. 771, 1988, Annex 7). After making a number of modifications, including the introduction of references to the International Standards for Interferons established at its thirty-eighth meeting (WHO Technical Report Series, No. 771, 1988, pp. 29–31), the Committee adopted the Requirements for Human Interferons Prepared from Lymphoblastoid Cells and agreed that they should be annexed to this report (Annex 3).

40. Requirements for the collection, processing, and quality control of blood, blood components, and plasma derivatives (revised 1988)

The Committee studied the proposed requirements for the collection, processing, and quality control of blood, blood components, and plasma derivatives (BS/88.1584 Rev. 1) that had been prepared by the WHO Secretariat in collaboration with a number of experts to supersede the Requirements for the Collection, Processing and Quality Control of Human Blood and Blood
Products (Requirements for Biological Substances No. 27). The earlier requirements were adopted in 1977 (WHO Technical Report Series, No. 626, 1978, Annex 1) and were later included in The collection, fractionation, quality control and uses of blood and blood products (Geneva, World Health Organization, 1981). After making a number of modifications, the Committee adopted the revised Requirements for the Collection, Processing, and Quality Control of Blood, Blood Products, and Plasma Derivatives and agreed that they should be annexed to this report (Annex 4).

41. Requirements for poliomyelitis vaccine (oral) prepared in continuous cell lines

The Committee noted that a revision of the draft requirements for poliomyelitis vaccine (oral) prepared in continuous cell lines considered at its thirty-eighth meeting (WHO Technical Report Series, No. 771, 1988, p. 36) had been distributed for comments (BS/87.1580 Rev. 1), and reviewed the revised draft and the comments received. It expressed concern regarding the safety of such vaccines and was informed that studies were continuing and that the results would be reported. After making some modifications, the Committee agreed that the modified draft requirements should be combined with the existing requirements for poliomyelitis vaccine (oral) (Requirements for Biological Substances No. 7, revised 1982 and addendum 1987; WHO Technical Report Series, No. 687, 1983, Annex 4, and No. 771, 1988, Annex 4). It therefore requested the WHO Secretariat to prepare unified draft requirements for discussion at the next meeting of the Consultative Group on Poliomyelitis in 1989.

42. Requirements for diphtheria toxoid, pertussis vaccine, tetanus toxoid, and combined vaccines

The Committee was informed that a scientific consultation had been held in 1988 to review the Requirements for Diphtheria Toxoid, Pertussis Vaccine, Tetanus Toxoid, and Combined Vaccines (Requirements for Biological Substances Nos. 8 and 10) in the light of changes in the production and control methods for diphtheria, pertussis, and tetanus vaccines that had occurred since the last revision in 1978 (WHO Technical Report Series, No. 638, 1979, Annex 1). The proposed revision (BS/88.1593) is currently open for
comments and suggestions, after which it will be proposed for adoption by the Committee. The Committee was also informed that a retrospective survey conducted by one laboratory of results obtained by 11 laboratories had not revealed any evidence of instability of the International Standard for Pertussis Vaccine. It therefore requested the WHO Secretariat to obtain further information on stability.
Annex 1

PROCEDURE FOR EVALUATING THE ACCEPTABILITY IN PRINCIPLE OF VACCINES PROPOSED TO UNITED NATIONS AGENCIES FOR USE IN IMMUNIZATION PROGRAMMES

(Revised 1988)

The WHO Biologics Unit acts as adviser to UNICEF on matters related to the quality of vaccines offered for use in immunization programmes. Since the publication in 1987 (f) and 1988 (2) of the first WHO procedures for evaluating the acceptability of vaccines for use in such programmes, experience has been gained which justifies several changes in the procedures. This annex deals with the six commonly used vaccines, including BCG, and supersedes the procedures published in 1987 and 1988. The procedure used by WHO is described below, and the order in which the various steps should be taken is indicated in Appendix 1.

1. Preliminary assessment of acceptability

WHO will make an initial evaluation of the acceptability of a vaccine by reviewing the following items, four copies of which must be submitted by the manufacturer in English or French:

(a) the standard operating procedure, i.e., a complete description of the method(s) used for producing and controlling the vaccine(s) that is (are) offered;

(b) a history of the seed strain of virus or bacterium and, for virus vaccines, the substrates used;

(c) a copy of the licence issued by the national control authority of the country in which the vaccine is manufactured;

(d) a copy of the licence(s) issued by the national control authorities of other countries in which the vaccine is sold or distributed (if no national control authority exists in a country in which the vaccine is sold or distributed, this should be indicated);
(e) a copy of all the correspondence from the national control authorities concerning the reservations expressed, if any, about an application for a licence for the product;

(f) a copy of a recent manufacturing protocol;

(g) a plan of the relevant production and testing facilities, and a list of professional and senior technicians, together with their qualifications, experience, and duties;

(h) a summary of the experience with the vaccine, namely:
   —the total number of batches produced, tested, and rejected during the previous three years by the manufacturer and tested and/or rejected by the national control authority, with the reasons for such rejection;
   —the total number of doses distributed since licensing;
   —the total number of doses distributed since the previous year;
   —the number and nature of the adverse reactions following vaccination reported over the previous five years;
   —the number of final containers offered per year to UNICEF and the number of single human doses in each container (to qualify for entry to the WHO procedure, the manufacturer should offer at least 10% of the total number of doses of the particular vaccine that UNICEF intends to acquire in the same year);
   —the results of any clinical studies, and especially of comparative studies in which efficacy and safety were assessed, including information on the dose and immunization schedule. (As a general principle, candidate vaccines should be shown to be, in the intended target populations and when the immunization schedules of the countries concerned are used, as immunogenic as similar vaccines already approved for use in those countries, and no more likely to produce adverse reactions. Candidate BCG vaccines should be tested in humans, in consultation with WHO, in comparison with an ad hoc BCG reference vaccine; the aim is to determine the tuberculin sensitivity conversion and the extent of local lesions and other complications. The test BCG vaccine used in the trial must be similar to the one offered for sale as regards absence of contamination and variables such as optical density, heat stability, number of culturable particles per single human dose, and microscopic pattern after reconstitution.)
2. Consistency of production

The most important information concerning the safety of a biological substance is that relating to consistency of production. Tests for such consistency are complementary to those on the final material in containers. In routine production, failure of a single batch to meet the safety requirements may be considered a breakdown in production, and consistency of production may need to be re-established.

WHO will evaluate the consistency of production of acceptable lots of vaccine by:

(a) Reviewing the summary protocol of production for each of five lots selected by WHO and produced from five different bulks.

(b) Reviewing the evidence that the product meets WHO requirements, including the minimum potency requirements at the expiry date.

(c) Reviewing a copy of the certification and release document issued by the national control authority for each of five consecutive lots selected by WHO, including the results of any tests performed by the national control laboratory. The certification and release documents (Appendix 2) should state that the batches meet WHO as well as national requirements.

(d) For all vaccines except BCG vaccine, having tests performed by one or more WHO collaborating laboratories on samples from five lots selected by WHO. For this purpose five batches will be selected by WHO from all those certified and released by the national control authority over the preceding 12 months and a sample of 20 final containers from each of the batches will be shipped to WHO by or under the responsibility of that authority. If required, appropriate amounts of corresponding bulks will be similarly sampled and shipped. Particular emphasis will be placed by WHO on tests for potency and, when applicable, for thermostability.

(e) For BCG vaccines, having tests performed by the WHO Collaborating Laboratory for BCG Vaccine. A minimum of 20 randomly selected batches of vaccine produced by the manufacturer over a period not less than the preceding two years must have been tested by the WHO Collaborating Laboratory with satisfactory results before acquisition by UNICEF may be considered. If unsatisfactory results are obtained, the vaccine will be rejected or further batches will be
tested; when satisfactory results are obtained and a vaccine proves acceptable in principle, every batch supplied to UNICEF during a period of one year will also be tested by the WHO Collaborating Laboratory. Testing of further batches after that time will be more limited and be performed on a random selection of batches. The main tests that will be performed are those for absence of contamination, optical density, number of culturable particles, thermostability, and microscopic pattern after reconstitution. The cost of such testing will be chargeable in advance to the manufacturer.

3. Inspection

The overall acceptability of the vaccine is based on a satisfactory outcome of the procedures described in sections 1 and 2, and a satisfactory inspection. The inspection will focus primarily on the manufacturing and control facilities and on the records of the producer, as well as on those of the national control laboratory. The inspection will be performed by two or three WHO consultants familiar with vaccine production and/or quality control, together with the person responsible for national control in the country of production, or his or her representative. One or two members of WHO staff will accompany the consultants. The extent to which, and the manner by which, the national control laboratory tests individual batches will be reviewed; this is an important factor in assessing acceptability. The cost of the inspection will usually be borne by the manufacturer, who will be required to provide round-trip transportation to the manufacturing and controlling sites for each consultant and WHO representative plus a subsistence allowance.

REFERENCES

SEQUENCE OF STEPS

The appropriate sequence of steps to be followed by an applicant vaccine supplier is as follows:

1. A letter should be sent to the Director, UNICEF Procurement and Assembly Centre (UNIPAC), UNICEF Plads, Freeport, 2100 Copenhagen, Denmark, with a copy to Chief, Biologicals, WHO, 1211 Geneva 27, Switzerland, to ask for the procedure to be started. The nature of the proposed vaccine and the number of doses offered per year should be indicated and the following included:

   (a) a certificate from the national control authority certifying that, as a matter of principle, the vaccine meets the requirements published by WHO as well as (if applicable) national requirements (Appendix 2);

   (b) concise technical information in the form of a summary protocol on production and testing, in accordance with the models published by WHO in the relevant requirements;

   (c) a certificate from the national control authority listing all the batch numbers of similar vaccines produced by the applicant that have been certified and released over the previous 12-month period, together with the number of containers in each batch and the number of single human doses in each container.

2. When requested by WHO, all the other information mentioned in this Annex should be sent.

When all of the above information and the results of independent testing of the products have been reviewed and found satisfactory, WHO will organize the inspection of the control and/or production facilities of both the manufacturer and the national control laboratory.
MODEL CERTIFICATE FOR THE RELEASE
OF VACCINES
ACQUIRED BY UNITED NATIONS AGENCIES
(Revised 1988)

(to be completed by the national control authority of the country where the vaccines have
been manufactured, and to be sent by the vaccine manufacturer to UNICEF)

The following lots of ...................... ¹ vaccine produced by ...................... ²
in .......................... ³ whose numbers appear on the labels of the final containers, meet all
national requirements, ⁴ Part A ⁵ of Requirements for Biological Substances No.
.......................... ⁶ (Requirements for ...................... ⁷ published in 19..... [if
applicable, revised 19......, addendum 19......]) and Requirements for Biological
Substances No. 1 (General Requirements for Manufacturing Establishments and
Control Laboratories, published in 1959; revised 19......). ⁷

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As a minimum, this certificate is based on examination of the manufacturing
protocol.

The Director of the National Control Laboratory (or Authority as appropriate) ⁸
Name (typed).................................................................
Signature...........................................................................
Date....................................................................................

¹ Indicate type of vaccine (measles, oral poliomyelitis, tetanus, diphtheria–
² Name of manufacturer.
³ Country.
⁴ If any national requirements are not met, specify which one(s) and indicate why
release of the lot(s) has nevertheless been authorized by the national control authority.
⁵ With the exception of the provisions on shipping, which the national control
authority may not be in a position to control.
⁶ Indicate the reference number of the relevant Requirements for Biological
Substances published by WHO.
⁷ These requirements were revised in 1965; a further revision is in preparation for
consideration by the WHO Expert Committee on Biological Standardization in 1989.
⁸ Or his or her representative.
Annex 2

REQUIREMENTS FOR HEPATITIS B VACCINES MADE BY RECOMBINANT DNA TECHNIQUES

(Requirements for Biological Substances No. 45)

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GENERAL CONSIDERATIONS

Advances in molecular genetics and nucleic acid chemistry have made it possible to identify genes coding for biologically active substances, to analyse them in detail, to transfer them within and
between organisms, and to obtain gene expression under controlled conditions with efficient synthesis of the encoded product. A gene that codes for a specific product can be isolated and propagated by insertion into a suitable vector with the aid of highly specific restriction endonuclease enzymes (which cleave the vector DNA at predetermined sites) and ligases (which join the gene insert to the vector). The vector can then be introduced into host organisms, and individual clones that carry the desired gene can be selected and propagated in mass culture.

A gene is characterized by a specific nucleotide sequence in one strand of the double-stranded DNA molecule. When the strands are separated, each forms a template for the synthesis of a complementary copy, thus providing a mechanism for the faithful reproduction of genes with conservation of the linear sequence of the four types of mononucleotides. The process of decoding the genetic information and of synthesizing the gene product occurs in two phases, of which the first is transcription of the DNA coding strand in the form of messenger RNA, and the second translation of the information carried by the messenger RNA molecule into an amino acid sequence. The factors affecting the expression of foreign genes introduced into prokaryotic and eukaryotic cells are complex; the efficient and controlled expression of stable, cloned DNA sequences is an important field of current research.

Hepatitis B virus has several characteristics that distinguish it from the other families of DNA viruses. It has an outer coat (more substantial than a membrane or envelope) consisting of protein, lipid, and carbohydrate and bearing a unique antigen complex, hepatitis B surface antigen (HBsAg). Its nucleic acid consists of a circular DNA genome of relative molecular mass about 2 million, part of which is double-stranded and part single-stranded—an unusual feature among viruses. Virus recovered from the plasma of a hepatitis B carrier has been used to prepare viral DNA; that DNA has been cloned in Escherichia coli and the gene coding for HBsAg has been isolated.

This gene has been inserted into yeast and mammalian cells by means of appropriate expression vectors. Purified antigens obtained from transfected cultures containing it have been shown to induce antibodies in mice and guinea-pigs and have been formulated into vaccines. Electron microscopy has revealed that the purified HBsAg used for these vaccines exists as particles 15–30 nm in diameter, with the morphological characteristics of free surface antigen in plasma
and of the purified antigen now used in plasma-derived hepatitis B vaccines. Some of the vaccine formulations containing these materials have already been shown to be immunogenic in mice, chimpanzees and other monkeys, and human beings, with antigenic potencies similar to those of vaccine made from plasma-derived antigen.¹

The requirements that follow apply to the control and testing of hepatitis B vaccine made by recombinant DNA methods, and have been formulated to take account of the scale-up required for commercial production. Particular emphasis is placed on introducing “in-process” control, which has been highly effective for other bacterial and viral vaccines, rather than relying entirely on tests on the end-products. General requirements, such as tests for potency, purity, toxicity, pyrogenicity, and sterility, will apply as much to hepatitis B vaccines made by recombinant DNA methods as to those derived from human plasma. Certain tests will be required on every production batch of vaccine, whereas others will be required only to establish the validity, acceptability, and consistency of a given manufacturing process.

A detailed description of how the product is made should be given. Evidence should be presented to show that any HBsAg made by recombinant DNA techniques possesses the characteristics of an immunogen that protects against hepatitis B virus.

Rigorous identification and characterization of recombinant DNA-derived vaccines will be required. The ways in which these products differ chemically, structurally, biologically, or immunologically from the naturally occurring antigen must be fully documented. Such differences could arise during processing at the genetic or post-translational level, or during purification. Differences between batches of the product may result from genetic instability during serial cultivation. Microbial contamination during fermentation may occur, and tests for contaminants must be thorough.

Special attention should be given to purity because:

—unwanted gene products may be co-expressed unexpectedly with the HBsAg, for example if transcription is initiated at several sites, or if changes occur during culture that affect transcription,

initiation, or termination processes or favour the expression of other genes in the vector or the host cell;
— biologically active extraneous components, such as DNA, proteins, and endogenous retroviruses derived from the host-cell system may be found in the final product;
— agents used in the purification process (column matrices, antibodies) may give rise to specific contaminants in the final product.

The product arising from the recombinant system must be shown to elicit specific antibody responses to HBsAg in laboratory animals, including, where possible, a non-human primate species. HBsAg of diverse subtypes should be used to characterize the specificity of the response fully.

One approach to evaluating the protective potential of recombinant DNA-derived vaccines in human beings is through immunization and challenge studies in chimpanzees. The requirement for a test of vaccine efficacy in chimpanzees should be at the discretion of the national control authority but, if possible, on at least one occasion, chimpanzees given the recommended human immunization schedule should be shown to be protected against challenge with hepatitis B virus. In some studies, more than 1000 chimpanzee infectious doses of hepatitis B virus of homotypic and heterotypic subtypes, given intravenously, have been used as a challenge.

The vaccine should reliably induce antibody responses to HBsAg in human recipients. The frequency and titre of the antibody responses should be at least equivalent to those induced by plasma-derived vaccines that fulfil WHO requirements. The aims of immunogenicity studies in human subjects should be to define the quantity of antigen and number of doses required to elicit reliably antibody responses to HBsAg. The titre, duration, and quality of the responses should be clearly defined. In vitro tests of antigenicity may be difficult to standardize, and it is therefore proposed that the content of pure HBsAg in the product should be used as the basis for comparing immunogenicity in mice and responses in human subjects. The relationship between the antigenicity of the product in in vitro tests, in mouse immunogenicity tests, and in human beings should be established. Studies in human subjects should be designed to provide information on the frequency and severity of any local and systemic adverse reactions to vaccination. Vaccine recipients in
such studies should be representative of the intended target group for immunization in terms of age and risk.

Each of the following sections constitutes a recommendation. The parts of each section that are printed in large type have been written in the form of requirements, so that, if a health administration so desires, these parts as they appear may be included in definitive national requirements. The parts of each section that are printed in small type are comments and recommendations for guidance.

Should individual countries wish to adopt these requirements as the basis of their national regulations concerning hepatitis B vaccine, it is recommended that a clause should be included permitting modifications of the manufacturing requirements on the condition that it can be demonstrated, to the satisfaction of the national control authority, that such modifications ensure a degree of safety and potency of the vaccine at least equal to those provided by the requirements formulated below. The World Health Organization should then be informed of the action taken.

The terms “national control authority” and “national control laboratory”, as used in these requirements, always refer to the country in which the vaccine is manufactured and/or used.

PART A. MANUFACTURING REQUIREMENTS

1. Definitions

1.1 International name and proper name

The international name shall be *Vaccinum hepatitidis B recombinatum*. The proper name shall be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

1.2 Descriptive definition

*Vaccinum hepatitidis B recombinatum* is a preparation of purified hepatitis B surface antigen (HBsAg) that has been produced by recombinant DNA techniques. The preparation shall satisfy all the requirements formulated below.

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1.3 International reference materials

International standards and reference reagents are needed for the control of HBsAg or to determine antigenic content and potency.

The reference materials mentioned below are available but are not specified for use in tests in these requirements.

For the assay of antigenic content by techniques such as radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), single radial immunodiffusion, and rocket electrophoresis, an international reference material consisting of an aqueous preparation without an adjuvant is required (see Part A, section 7.3). Such a preparation was established in 1985 as the first International Standard for Hepatitis B Surface Antigen ad Subtype (100 IU per ampoule).

The International Reference Reagent of Plasma-Derived Hepatitis B Vaccine for Immunogenicity Studies was established in 1986. It is a liquid preparation containing approximately 20 µg/ml of plasma-derived hepatitis B surface antigen and contains aluminium hydroxide as an adjuvant.

Both the above-mentioned reference materials are available on request from the National Institute for Biological Standards and Control, Potters Bar, England.

For the calibration of techniques used to measure antibody responses to hepatitis B vaccines, an international reference preparation is available. This International Reference Preparation of Hepatitis B Immunoglobulin (established in 1977) is dispensed in ampoules containing 50 IU of hepatitis B immunoglobulin (from fractionated human plasma, and freeze-dried). This preparation is in the custody of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands.

1.4 Terminology

*Hepatitis B virus*: a 42-nm double-shelled virus particle, originally known as the Dane particle, which contains the DNA genome of the virus.

*HBsAg*: hepatitis B surface antigen, comprising a complex of antigens associated with the virus envelope and subviral forms (22-nm spherical and tubular particles). Native HBsAg is encoded by envelope gene sequences (S plus pre-S) in the viral DNA. Recombinant DNA-derived hepatitis B vaccines may contain the S gene product or products of the S/pre-S combination.

*Cell seed lot*: a quantity of cells stored frozen at −70 °C or below in aliquots of uniform composition, one or more of which would be used for the production of a manufacturer’s working cell bank.
Manufacturer's working cell bank (MWCB): a quantity of cells, derived from one or more ampoules of the cell seed, stored frozen at 
−70°C or below in aliquots of uniform composition.

In normal practice, a cell seed is expanded by serial subculture
up to a passage number (or population doubling, as
appropriate) selected by the manufacturer, at which point the
cells are combined to give a single pool and preserved
cryogenically to form the MWCB.

Production cell culture: a collection of cell cultures being used for
biological production that have been derived from one or more
ampoules of the MWCB.

Single harvest: the biological material prepared from a single
production run.

Final aqueous bulk: the final bulk before the addition of an
adjuvant.

Final bulk: the finished biological material prepared from one or
more batches of purified HBsAg, and present in the container from
which the final containers are filled.

Final lot: a collection of sealed final containers that are
homogeneous with respect to the risk of contamination during filling
and during the preparation of the finished vaccine. A final lot must
therefore consist of finished material distributed into containers in
one working session from a single final bulk.

2. General manufacturing requirements

The general manufacturing requirements contained in the revised
Requirements for Biological Substances No. 1 (General Require-
ments for Manufacturing Establishments and Control Laboratories)
(1, p. 11) shall apply to establishments manufacturing hepatitis
B vaccine, with the addition of the following directives:

(a) Production areas shall be decontaminated before they are used
for the manufacture of hepatitis B vaccine.

(b) Hepatitis B vaccine shall be produced by staff who have not
handled animals or infectious microorganisms in the same
working day. The staff shall consist of persons who have been
examined medically and have been found to be healthy and not
to be carriers of hepatitis B.

(c) No cultures of microorganisms or eukaryotic cells other than
those approved by the national control authority shall be
introduced into or handled in the production area at any time during manufacture of the vaccine.

(d) Persons not directly concerned with the production processes, other than official representatives of the national control authority, shall not be permitted to enter the production area.

Particular attention is drawn to the recommendations contained in Part A, section 1 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (I, p. 13) regarding the training and experience of persons in charge of production and testing and of those assigned to various positions of responsibility in the manufacturing establishment, and to the registration of such personnel with the national control authority.

The preparation of hepatitis B vaccine shall be based strictly on the cell seed lot system. A description of the system used should be provided, including the number of vials of seed available and details of their storage. Particular attention should be paid to the stability of the expression vector and to the plasmid copy number in the seed stock under conditions of storage and recovery.

Full details of the cell culture process used in manufacture should be provided to the national control authority with particular reference to tests to monitor microbial contamination in the cell culture vessels. Information on the sensitivity of methods of detecting such contamination and the frequency of testing should be provided, together with criteria for the rejection of contaminated materials.

The yield of HBsAg shall be monitored during the course of individual production runs. Criteria, based on yield, for the acceptance of culture harvests for further processing into vaccine shall be defined, and consistency of production shall be established by testing at least five consecutive lots prepared by the same procedures.

3. Validation and control of manufacturing procedures

The general production precautions formulated in Part A, section 3 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (I, p 15) shall apply to the manufacture of hepatitis B vaccine.
3.1 Strategy for cloning and expressing the gene

A full description of the biological characteristics of the host cell and expression vectors used in production should be given. This should include details of: (a) potential retrovirus-like particles in and genetic markers of the host cell; (b) the construction, genetics, and structure of the expression vector; and (c) the origin and identification of the gene that is being cloned.

The physiological measures used to promote and control the expression of the cloned gene in the host cell should be described in detail.

Data that demonstrate the stability of the expression system during storage of the MWCB and beyond the passage level used for production should be provided. Any instability of the expression system occurring in the seed culture or after a production-scale run, for example involving rearrangements, deletions, or insertions of nucleotides, must be documented. Unstable preparations must not be used until approval to continue use has been obtained from the national control authority.

3.2 Biochemical characterization of recombinant vector

The nucleotide sequence of the gene insert and of adjacent segments of the vector and restriction-enzyme mapping of the vector containing the gene insert shall be provided as required by the national control authority.

3.3 Purification procedures

The methods used to purify the HBsAg from culture harvests should be fully described. The capacity of each step of the purification procedure to remove and/or inactivate substances other than HBsAg derived from the host cell or culture medium, including, in particular, virus particles, proteins, and nucleic acids, shall be evaluated. If individual contaminants are difficult to monitor, the results of pilot-scale studies to follow the removal of individual, deliberately added contaminants at appropriate stages of purification will provide valuable information.

If antibodies are used in the purification procedures, their origins and characteristics should be fully described. The degree of purity of monoclonal antibodies produced from hybridoma cell lines and the criteria for freedom from cell-derived DNA and murine viruses
should conform to the regulations laid down by the national control authority.

3.4 Characterization of gene products (HBsAg)

3.4.1 Particle characterization

The morphological characteristics of the HBsAg particles and degree of aggregation should be established by electron microscopy and by physicochemical methods, for example by gradient centrifugation. In addition, the protein, lipid, nucleic acid, and carbohydrate content should be measured.

3.4.2 Determination of protein content

The protein content (composition) in comparison with a reference reagent should be established by quantitative amino acid analysis or by another accurate method.

3.4.3 Protein characterization

An ultraviolet absorption spectrum should be recorded. The protein composition should be established by techniques such as sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions. The bands should be identified by sensitive staining techniques and, where possible, by specific antibodies to confirm the presence of the expected products of the hepatitis B virus envelope gene. The identity of the protein should be established by partial N-terminal and C-terminal sequence analysis.

3.4.4 Antibody responses

The recombinant DNA-derived vaccine should be shown to induce antibody responses in humans comparable to those elicited by plasma-derived vaccines that have proved effective in the field. The antibodies induced by the vaccine in human beings should be titrated and characterized with respect to their activities against relevant determinants of the hepatitis B virus envelope, for example group and subtype determinants.
3.4.5 Consistency of yield

Data on the consistency of yield between runs and during individual production runs shall be provided, and the national control authority shall approve the criteria for an acceptable production run.

4. Manufacturer's working cell bank (MWCB)

4.1 Origin of cell banks

Only cells approved by and registered with the national control authority shall be used to produce HBsAg. The national control authority shall have responsibility for approving the cell seed. A short history of the cell banks shall be provided.

4.2 Characteristics of cell seed lot

The characteristics of the cell seed (host cell in combination with the expression vector system) shall be fully described, and information given on the absence of extraneous agents and on genetic homogeneity. The nucleotide sequence of the HBsAg gene insert and its flanking regions shall be specified where relevant. A peptide map and/or terminal amino acid sequence of the gene products shall be obtained.

4.3 Phenotypic indicators of purity and genetic consistency of recombinant cultures

Cells must be maintained in a frozen state that allows recovery of viable cells without alteration of genotype. The cells should be recovered from the frozen state, if necessary, in selective media such that the genotype and phenotype consistent with the unmodified host and unmodified recombinant DNA vector are maintained and are clearly identifiable. The cell seed must be identified by means of appropriate tests.

4.4 Sterility tests

Tests for bacteria, fungi, and mycoplasmas shall be performed in accordance with the requirements of Part A, section 5 of the revised
Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (2, p. 49).

4.5 Additional tests for MWCB of mammalian cells

The cells shall have been characterized as specified under the Requirements for Biological Substances No. 37 (Requirements for Continuous Cell Lines Used for Biologials Production) (3).

The national control authority shall have the responsibility for approving the cell seed.

5. Production precautions

5.1 Production cell cultures

Only cell cultures derived from the MWCB shall be used for production. All processing of cells shall be done in an area in which no cells or organisms are handled other than those directly required for the process.

5.2 Culture conditions for production cell cultures

Production cell cultures shall be grown under conditions agreed with the national control authority. These conditions shall include details of the culture system used, the cell doubling time, the number of subcultures or the duration of the period of subcultivation permitted, and the incubation temperature.

Cell cultures shall be monitored for freedom from microbial contamination as required by the national control authority.

5.3 Cell culture medium

If serum is used for the propagation of cells for vaccine production, it shall be tested by methods approved by the national control authority, to demonstrate freedom from bacteria, fungi, viruses, and mycoplasmas, according to the requirements given in Part A, sections 5.2 and 5.3 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (2, p. 49) as well as freedom from pathogens of the species of origin of the serum.
In some countries, sera are examined for freedom from phages.

Antibiotics of the β-lactam type shall not be used at any stage in the production.

Minimal concentrations of other suitable antibiotics may be used if approved by the national control authority. Nontoxic pH indicators may be added, e.g., phenol red in a concentration of 20 mg/l.

6. Single harvests

6.1 Sterility

Microbial contamination in the cell culture vessels shall be monitored during and at the end of the production runs by methods approved by the national control authority.

6.2 Consistency of yield

The yield of HBsAg from each single harvest shall be shown to be within the limits approved by the national control authority (see Part A, section 3.4.5).

6.3 Plasmid retention

A sample of cells that are representative of each harvest must be tested to confirm that the recombinant phenotype has been retained. The method used shall be approved by the national control authority.

7. Purification

The purification procedure can be applied to a single harvest, a part of a single harvest, or a pool of single harvests. The maximum number of single harvests that may be pooled shall be fixed by the national control authority. The antigen must be purified before adsorption on to an adjuvant. Adequate purification may require several purification steps based on different principles. This will minimize the possibility of co-purification of extraneous cellular materials. The methods used for the purification of the HBsAg
should be appropriately validated (see Part A, section 3.3) and approved by the national control authority.

The experience of several manufacturers has shown that it is possible to produce batches in which HBsAg accounts for at least 95% of the total protein. One suitable method of analysing the proportion of potential contaminant proteins in the total protein of the product is separation of the proteins by polyacrylamide gel electrophoresis under both non-reducing and reducing denaturing conditions. Individual gels should be stained by Coomassie Blue and by silver stain, or a single gel may be successively stained by both methods. The silver stain is considerably more sensitive for the detection of very small quantities of proteins and is also useful for identifying any non-protein materials, such as nucleic acids, carbohydrates, and lipids, which may be present. The protein in each band can be quantified by densitometric analysis. The staining procedure should be sensitive enough to reveal a potential contaminant protein present at a level of 1% of total protein.

High-performance liquid chromatography is another very useful method for determining the purity of a protein or peptide and for investigating its molecular configuration. A widely used method is reverse-phase high-performance liquid chromatography. A protein or peptide that elutes as a single symmetrical peak in two markedly different systems, including an ion-pair system, is generally of high purity.

7.1 Protein and other components of the vaccine

The total protein content of the vaccine should be determined (see also Part A, section 3.4.2).

The total concentration of protein may be determined by measuring absorbance at 280 nm by the micro-Kjeldahl method, the Lowry technique, or another suitable method.

Lipid and carbohydrate contents may also provide useful information.

If serum is used in the medium for the production cell cultures or at any stage in the purification process, for example as a reagent in immunoabsorption chromatography, tests shall be made for residual serum in the purified vaccine.

The concentration of animal serum in the vaccine shall be not more than 1 µl per litre of vaccine.

A concentration of 1 µl of animal serum per litre of vaccine is approximately equivalent to 50 ng of albumin per ml. Methods such as enzyme-linked immunosorbent assay (ELISA) and
immunoprecipitation can detect as little as 1 ng of albumin per ml.

7.2 Tests for agents used during purification or other phases of manufacture

A test shall be made for the presence of any potentially hazardous agent used in manufacture. The method used and the permitted concentration shall be approved by the national control authority.

7.2.1 Monoclonal antibodies

Where a monoclonal antibody is used in vaccine preparation, for example for immunological affinity chromatography to purify HBsAg, the product should be tested for residual antibody. The methods used and the permitted concentrations of antibody shall be approved by the national control authority.

Several national control authorities have drafted guidelines for the control of monoclonal antibody preparations used for the manufacture of biological products for human use.

7.3 Determination of HBsAg content

The HBsAg content of the purified preparation shall be determined by an appropriate method.

Tests that have been found suitable include radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), single radial immunodiffusion, and rocket electrophoresis in which the purified preparation is compared with a known standard. Analysis of the results by the parallel-line method has been found suitable for most of these techniques.

7.4 Test for antigenic identity (molecular and immunochromical identity)

A test shall be made for the molecular characteristics of the HBsAg gene product by polyacrylamide gel electrophoresis under reducing denaturing conditions.

In one country, the detection of pre-S antigen by SDS-PAGE is required.

The gene products shall be shown to possess antigenic determinants characteristic of HBsAg by means of tests with
monoclonal antibodies or other antibodies of defined specificity directed against epitopes of HBsAg known to be relevant to the protective efficacy of the vaccine. Such tests shall be approved by the national control authority.

7.5 Test for sterility of purified surface antigen

Each batch shall be tested for 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) (2, p. 48).

7.6 Test for inactivating agents

If the HBsAg has been treated with formaldehyde and/or other inactivating agents, then the material shall be tested for the presence of free formaldehyde and/or the other agents. The method used and the permitted concentration shall be approved by the national control authority.

8. Final bulk before addition of adjuvant (aqueous bulk)

The final aqueous bulk consists of one or more purified HBsAg batches. Only batches that have satisfied the requirements of sections 2–7 above shall be included in the final bulk. The national control authority shall determine the maximum number of batches that may be pooled.

8.1 Test for sterility

The final aqueous bulk shall be tested for bacterial and mycotic sterility according to the requirements given in Part A, section 5.2 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (2, p. 49).

8.2 Test for HBsAg

The quantity of HBsAg as a proportion of the total protein in the final bulk shall be determined by an appropriate quantitative procedure. The protein load should be specified so as to ensure that
the assay is of reasonable sensitivity. The lower limit of the ratio of HBsAg to total protein shall be approved by the national control authority. The final aqueous bulk should contain at least 90% of the total protein as HBsAg.

In one country, the amount of pre-S antigen is determined by means of a suitable quantitative assay.

8.3 Test for DNA

The amount of residual cell or plasmid DNA in each batch of vaccine should be determined by sensitive methods which must be validated and approved by the national control authority. Suitable tests for the homologous vector used in the production of the HBsAg should be included. Maximum acceptable levels of DNA per vaccine dose shall be approved by the national control authority.

The WHO Study Group on Biologicals concluded that the probability of risk associated with heterogeneous contaminating DNA in a product derived from a continuous cell line is negligible when the amount of such DNA is 100 pg or less in a single dose given parenterally (4).

9. Final bulk

9.1 Addition of adjuvant

The adjuvant and the concentration used shall be approved by the national control authority.

A preservative may need to be added at this stage.

9.2 Tests on final bulk

9.2.1 Tests for sterility

The final bulk shall be tested for bacterial and mycotic sterility according to the requirements given in Part A, section 5.2 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (2, p. 49).

9.2.2 Tests for preservative

The final bulk may be tested for the presence of preservative. The method used and the permitted concentration should be approved by the national control authority.
9.2.3 Assay for adjuvant

Each final lot may be assayed for the content of adjuvant. The method used and the permitted concentration should be approved by the national control authority. Where aluminium compounds are used, the concentration of aluminium should not be greater than 1.25 mg per single human dose.

10. Filling and containers

The requirements concerning filling and containers given in Part A, section 4 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (I, p. 16) shall apply.

Care should be taken to ensure that the material of which the container is made does not adversely affect the HBsAg under the recommended storage conditions. Adjuvanted HBsAg vaccine must be stored at 5–8°C.

11. Control of final lot

Samples shall be taken from each final lot for the tests described in the following sections.

11.1 Sterility test

Each final lot shall be tested for 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) (6).

11.2 Innocuity tests

Each final lot shall be tested for innocuity by appropriate tests in mice and guinea-pigs, using parenteral injections. The tests shall be approved by the national control authority.

11.3 Test for pyrogenic substances

Each final lot shall be tested for pyrogenic substances. The test shall be approved by the national control authority.
11.4 Test for preservative

Each final lot shall be tested for the presence of preservative. The test used and the permitted concentration shall be approved by the national control authority.

11.5 Assay for adjuvant

Each final lot shall be assayed for the content of adjuvant. The method used and the permitted concentration shall be approved by the national control authority. Where aluminium compounds are used, the concentration of aluminium shall not be greater than 1.25 mg per single human dose.

11.6 Potency and identity tests

The vaccine shall be identified as envelope antigens of hepatitis B virus by appropriate methods. An appropriate quantitative test for antigen content and an immunogenicity assay shall be performed on samples representative of the final filling lots. The vaccine potency shall be compared with that of a reference preparation, and the national control authority shall determine the lower limit of potency.

A suitable quantitative extinction test in mice is as follows. Several groups of at least 20 suitable mice, five weeks of age, are tested. Each mouse is vaccinated intraperitoneally with the adjuvanted hepatitis B vaccine diluted in the adjuvant used in the vaccine; a different graded dose is used for each group of mice. Similar groups of mice are inoculated with the adjuvanted reference preparation. The reactivity of positive sera towards important epitopes on HBsAg should be verified by means of appropriate tests. Blood samples are taken from the mice after sufficient time has elapsed to permit the development of an adequate antibody response, and individual sera are assayed for antibodies to products of the envelope gene of hepatitis B virus by means of sensitive quantitative tests such as radioimmunoassay. The concentrations of vaccine tested should be selected to permit the calculation of 50% seroconversion to antibodies against HBsAg. The strain of mice used for this test must give a suitable dose–response curve with the reference and test antigens. In any test, a calibrated reference preparation should be included and the potency of the vaccine expressed in terms of it.
12. Records

The requirements given in Part A, section 6 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (I, p. 17) shall apply.

13. Samples

The requirements given in Part A, section 7 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (I, p. 18) shall apply.

14. Labelling

The requirements given in Part A, section 8 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (I, p. 18) shall apply, with the addition of the following directive.

The leaflet accompanying the package shall include the following information:

— the nature of the cells used to produce the antigen;
— the nature and amount of any preservative, adjuvant, or stabilizer present in the vaccine;
— the volume of one recommended human dose, the immunization schedules, and the recommended routes of administration (this information shall be given for newborn babies, children, adults, and immunosuppressed individuals, and shall be the same for a given vaccine for all regions of the world);
— the amount of total protein contained in one recommended human dose; and
— the amount of HBsAg protein contained in one recommended human dose.

15. Distribution and shipping

The requirements given in Part A, section 9 of the revised Requirements for Biological Substances No. 1 (General Require-
ments for Manufacturing Establishments and Control Laboratories (1, p. 18) shall apply.

In addition, the conditions of shipping shall be such as to ensure that the adjuvanted vaccine does not freeze.

Temperature indicators should be packaged with each vaccine shipment to show whether freezing occurs. If freezing has occurred, the vaccine should not be used.

16. Storage and expiry date

The requirements given in Part A, section 10 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (1, p. 19) shall apply.

16.1 Storage conditions and stability

Before being distributed by the manufacturing establishment, or before being issued from a depot for the maintenance of reserves of vaccines, all vaccine in bulk form or in final containers shall be kept at 5–8°C. The vaccine shall also be stored at this temperature after distribution or issue. The vaccine shall have been shown to maintain its potency for a period equal to that between the date of issue and the expiry date. During storage the vaccine shall not be frozen.

16.2 Expiry date

The expiry date shall be fixed with the approval of the national control authority and shall relate to the date of the last satisfactory potency test, i.e., the date on which the animals were inoculated with the vaccine.

PART B. NATIONAL CONTROL REQUIREMENTS

1. General

The general requirements for control laboratories contained in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (1, p. 19) shall apply.
The national control authority shall:

— approve the methods for producing HBsAg by recombinant DNA techniques;
— approve the tests for HBsAg concentration and define its minimum value;
— approve the methods used for purification;
— approve the purity of the final product;
— approve the tests for extraneous substances and total protein;
— approve the tests for preservative and the agents used for purification and in other aspects of manufacture;
— approve the tests used to determine whether the final product is free from abnormal toxicity;
— approve the adjuvant assay and define the permitted concentration of adjuvant in the final product;
— approve the types of animals used in the assay of potency; and
— approve the data showing that the vaccine produces an adequate antibody response (titre, duration, and quality) in human beings.

The national control authority shall also provide national reference preparations for defining the activity of HBsAg in a given quantity of protein.

Before releasing a vaccine, the national control authority shall be satisfied that the results of all tests, including those on individual batches during the process of manufacture, are satisfactory and that consistency has been established.

2. Release and certification

A hepatitis B vaccine shall be released only if it satisfies Part A of these requirements.

A statement signed by the appropriate official of the national control laboratory shall be provided at the request of the manufacturing establishment and shall certify whether or not the final lot of vaccine in question meets all national requirements as well as Part A of these requirements. The certificate shall state the date of the last satisfactory HBsAg potency test, the lot number, the number under which the lot was 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 the certificate is to facilitate the exchange of hepatitis B vaccine between countries.

AUTHORS AND ACKNOWLEDGEMENTS

These Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques are based on four previous drafts; for the authors of the first three of these drafts and corresponding acknowledgements, please see WHO Technical Report Series, No. 760, 1987, Annex 6. For the fourth draft, acknowledgements are also due to Dr G.R.E. Swaniker, University of Papua New Guinea, Boroko, Papua New Guinea. The fourth draft was prepared in January 1988 by Dr V. Grachev, Scientist, and Dr D. Magrath, Chief, Biologicals, WHO, Geneva, Switzerland.

REFERENCES

SUMMARY PROTOCOL FOR PRODUCTION AND TESTING OF HEPATITIS B VACCINES MADE BY RECOMBINANT DNA TECHNIQUES

Based on Requirements for Biological Substances No. 45
(Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques)

Identification of final lot

Name and address of manufacturer

International name and proprietary name of vaccine

Lot number of final product

Date of manufacture of final lot

Date of filling containers

Number and nature of containers (ampoules or vials)

Date of last potency test

Number of doses in each container

Volume of single dose

Expiration date

3.1 Validation and control of manufacturing procedures

3.1 Strategy for cloning and expressing the gene

Details of:
(a) potential retrovirus-like particles in and genetic markers of the host cell
(b) construction, genetics, and structure of the expression vector
(c) origin and identity of the gene that was cloned

1 Numbers refer to the corresponding numbered sections in the text of the Requirements.
2 Section 3.1 does not necessarily need to accompany each batch of vaccine. If necessary, it should be typed on a separate sheet.
Description, in detail, of the measures taken to promote and control the expression of the cloned gene in the host cells.

Information on the stability of the expression system during storage and cell propagation to the production level.

3.2 Biochemical characterization of recombinant vector

Nucleotide sequence of the surface-antigen gene insert

Restriction-endonuclease mapping of the recombinant vector, where relevant

3.3 Purification procedures

Methods used to purify the HBsAg

Results

Origins and characteristics of antibodies, if used

3.4 Characterization of gene products (HBsAg)

3.4.1 Particle characterization

Morphological characteristics of the particles

Results of examination by electron microscopy

Degree of aggregation

Method

Results

Quantity of protein

Method

Results

Quantity of lipid

Method

Results

Quantity of nucleic acid

Method

Results

Quantity of carbohydrate

Method

Results
3.4.2 Determination of protein content
   Method
   Results

3.4.3 Protein characterization
   Ultraviolet absorption spectrum
   Protein composition
      Method
      Results
   Identity of the protein by partial
   N-terminal and C-terminal analysis
      Method
      Results

3.4.4 Antibodies induced by the vaccine in human beings
   Titres
   Characteristics

3.4.5 Consistency of yield
   Between runs
   During individual runs

4. Manufacturer's working cell bank (MWCB)

4.1 Origin of cell banks
   Date of establishment of cell banks
   Quantity of cells stored
   Passage level of the MWCB
   Storage conditions

4.2 Characteristics of cell seed lot
   Purity and homogeneity of the cell seed lot
      Method
      Results
   Genetic characteristics of the cell seed lot
      Method
      Results
   Purity of recombinant DNA vector
      Method
      Results
Genetic characteristics of recombinant DNA vector
Methods
Results
Nucleotide sequence of HBsAg gene insert
Peptide map of gene product (HBsAg)
Terminal amino acid sequence of gene product

4.3 Phenotypic indicators of purity and genetic consistency of recombinant cultures
Tests on cells after recovery from preserved state
Methods
Results
Identity of cell seed
Method
Results

4.4 Sterility tests
Results

4.5 Additional tests for MWCB of mammalian cells
Tests for adventitious agents
Tests in suckling mice
No. and weight of animals
Quantity injected (intramuscular)
Observation period
Results of injection (pathological changes, no. survived, etc.)

Tests in adult mice
No. and weight of animals
Quantity injected (intramuscular)
Observation period
Results of injection (pathological changes, no. survived, etc.)

Tests in guinea-pigs
No. and weight of animals
Quantity injected (intramuscular)
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Tests in rabbits

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Tests in embryonated chicken eggs

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Tests in adult mice

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\(^1\) Only if immunoabsorption was used in the purification process.
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Method
Results

**Internal certification**

*Certification by person taking overall responsibility for production of the vaccine*

I certify that lot no. ................ of the vaccine satisfies Part A of the Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques published by WHO.

Signature
Name (typed)
Date

The protocol must be accompanied by a sample of the label and a copy of the leaflet.

**Release certification by the national control authority**

Whenever hepatitis B vaccines produced by recombinant DNA techniques are to be exported, they should be accompanied by a release certificate from the national control authority.

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Sample release certificate

I hereby certify that batch no. .................. of hepatitis B vaccine produced by (name of producer) by recombinant DNA techniques meets all national requirements as well as Part A of the Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques published by WHO.

The date of the last satisfactory potency test carried out by the national control laboratory is ..................

The final lot has been released by us under no. ..................

The number appearing on the label of the containers is ..................

Signature

Name (typed)

Date
Annex 3

REQUIREMENTS FOR HUMAN INTERFERONS PREPARED FROM LYMPHOBLASTOID CELLS

(Requirements for Biological Substances No. 42)

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Acknowledgements

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GENERAL CONSIDERATIONS

The interferons (IFNs) are a family of proteins initially thought to be of interest as inhibitors of virus growth. They are now known
to have many other biological effects \textit{in vivo}, including effects on cell
growth and on many functions of the immune system. These
probably reflect metabolic changes produced after an interferon is
bound to a specific receptor on the cell surface.

Interferons are formed by cells in response to stimuli of various
types, collectively known as inducers, which may be viruses,
chemical substances, or immunological interactions. They are
classified on the basis of antigenicity into three types: IFN-\(\alpha\), IFN-\(\beta\),
and IFN-\(\gamma\). In humans, many individual subtypes of IFN-\(\alpha\) are
formed, and at least 23 are now known. These are termed HuIFN-
\(\alpha_1\), -\(\alpha_2\), -\(\alpha_3\), etc. (HuIFN-\(\alpha_1\) was previously known as HuIFN-\(\alpha_D\)
and HuIFN-\(\alpha_2\) as HuIFN-\(\alpha_A\)). They are potent antiviral agents
and in pure form have specific activities generally of the order of
\(2 \times 10^5\) units/mg of protein. Most of these subtypes of HuIFN-\(\alpha\)
contain 166 amino acids, of which about 65\% are the same in all the
subtypes, but there are reports that some shorter forms have been
isolated from human leukocyte interferon preparations. Their
chemical composition suggests that their relative molecular masses
are in the region 18 000–20 000, but when analysed by gel filtration,
some appear to have higher relative molecular masses of about
26 000–28 000. Few if any of the HuIFN-\(\alpha\) subtypes contain
carbohydrate in an N-glycosylated form, but some may be O-
glycosylated. Individual subtypes differ not only in their chemical
composition, but also in physical and antigenic characteristics.
Furthermore, when tested \textit{in vitro}, each has a unique spectrum of
biological effects; differences in \textit{in vivo} effects have also been seen in
very limited clinical studies with subtypes \(\alpha_1\) and \(\alpha_2\).

One major species of human IFN-\(\beta\) has been identified, composed
of 166 amino acids; it shows 34\% sequence similarity to HuIFN-\(\alpha_2\).
It is also a potent antiviral agent with a specific activity in the range
of 1–5 \(\times 10^6\) units/mg of protein. HuIFN-\(\gamma\) has little or no sequence
similarity to HuIFN-\(\alpha\) or HuIFN-\(\beta\) and is a more potent modulator
of the immune response. The mature gene codes for a protein of 143
amino acids. Natural HuIFN-\(\beta\) and HuIFN-\(\gamma\) are glycosylated.

In general, an interferon derived from the cells of one animal
species is most active when it is used to treat cells from the same
species, but being a protein, it may be immunogenic when
administered to an animal of another species. Therefore, although
human interferons have been used in a number of animal studies,
these have for the most part little relevance to their use in humans.
The antiviral and antitumour activity of some human interferon preparations in human subjects is now well established.

Preparations of HuIFN-α for clinical use can be obtained in several quite different ways. Originally, so-called "leukocyte" interferon was obtained from human peripheral blood cells, with Sendai virus as the inducer. However, only small amounts of this interferon are obtained from each blood donation so that a very large number of donors are needed to produce it in amounts sufficient for large-scale clinical use. For this reason HuIFN-α preparations are now generally obtained by two other routes. One involves recombinant DNA procedures; in particular, HuIFN-α2 has been expressed by cultures of *Escherichia coli* which have been transformed by a vector carrying the cloned DNA sequence specifying the mature protein. In the second approach, cultured human lymphoblastoid cells are treated with an inducer.

Cells of lymphoblastoid lines have the characteristics of transformed cells. For the most part, they grow readily *in vitro* and can be subcultured indefinitely. They are usually aneuploid and have chromosomal markers which may be characteristic for the particular line. Some lines, particularly those derived from Burkitt's lymphoma tissue, are tumorigenic.

The use of a transformed cell line, particularly one of neoplastic origin, as the substrate for the manufacture of a human pharmaceutical product raises questions of safety, which were first posed in connection with the production of alpha-interferons from cells of the Namalwa human lymphoblastoid line, which originated from Burkitt's lymphoma tissue. Over a period of some ten years, these questions were debated at a number of international and national scientific meetings. There is now a consensus that continuous cell lines can be used for pharmaceutical manufacture, provided that the final product is adequately purified, and subject to certain safeguards (1).

The requirements which follow take account of these points and should accordingly be met in the production control and testing of any human lymphoblastoid interferon preparation. These requirements have been formulated in relation to large-scale production, with particular reference to IFN-α but, with appropriate changes, are likely to be applicable to preparations of IFN-β or IFN-γ similarly derived from lymphoblastoid cells. They apply to the cells used as the substrate, the final purified product, and the method used for purification, which must be shown to inactivate or eliminate any
actual or potential noxious contaminants present in the crude product. Particular emphasis is placed on “in-process control” and consistency of the manufacturing process, a concept which has been highly effective in the control of other biological products, rather than reliance entirely on tests on the final product. General requirements, such as tests for potency, identity, purity, toxicity, pyrogenicity, and sterility, will apply as much to interferon prepared from lymphoblastoid cells as to those made from other cell substrates or by recombinant DNA techniques. Certain tests will be required on every production batch of interferon. Others will be required only to establish the validity and acceptability of a particular part of the proposed manufacturing process. Any such tests must form part of or be in addition to those so far applied during the manufacture of conventional biologicals, such as vaccines, derived from cell cultures.

Special attention should be given to the potential presence in the final product of contaminants such as:

(a) biologically active extraneous components (e.g., DNA, proteins, adventitious infectious agents, and endogenous retroviruses derived from the host cells), which may be present in the crude product but should be excluded from the final product;

(b) materials derived from the culture medium, substances used to enhance interferon production, the interferon inducer used, and chemical substances derived from those used during purification (e.g., column matrices and antibodies).

The methods used for purification and to identify and characterize the product must therefore be described.

The manufacturer should give a value for the specific activity of the purified human lymphoblastoid interferon in each lot of the final product, i.e., the biological potency (in International Units, whenever possible) per milligram of total protein (before the addition of any proteinaceous stabilizer) and its confidence limits. A minimum acceptable specific activity for each lot of final product should be proposed by the manufacturer. Tests that merely measure interferon protein, whether radioimmunometric or other, should not be used to control the potency of the final product, since evidence is required that the protein is biologically active.

Each of the following sections constitutes a recommendation. The parts printed in large type have been written in the form of requirements so that, if a health administration so desires, they may
be included in definitive national requirement as they stand. The parts printed in small type are comments or recommendations for guidance.

Should individual countries wish to adopt these requirements as the basis of their national regulations on human lymphoblastoid interferon, it is recommended that a clause should be included permitting modifications of manufacturing requirements on the condition that it can be demonstrated, to the satisfaction of the national control authority, that such modified requirements ensure that the degree of safety and the potency of the product are at least equal to those provided by the requirements formulated below. The World Health Organization should then be informed of the action taken.

The terms “national control authority” and “national control laboratory”, as used in these requirements, always refer to the country in which the interferon is manufactured and/or used.

PART A. MANUFACTURING REQUIREMENTS

1. Definitions

1.1 International name and proper name

The international name shall be *Interferonum lymphoblastoidis humanum, alpha or beta or gamma*, followed in parenthesis by the name or designation of the cell line used as the source, e.g., “(Namalwa)”. The proper name shall be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to preparations that satisfy the requirements given below.

1.2 Descriptive definition

Human lymphoblastoid alpha-interferon is a preparation containing a number of individual alpha-interferon subtypes produced by the induction of a human lymphoblastoid cell line; these interferons are harvested from the supernatant fluids, purified, and prepared in a form suitable for injection and that satisfies all the requirements formulated below.
1.3 International reference materials

The International Standard for Human Lymphoblastoid Interferon (Namalwa) (HuIFN-α (Ly)) was established in 1984. Each ampoule contains, by definition, 25 000 IU. The interferon included in the standard preparation was derived from Namalwa cells and induced with Sendai virus. It will be necessary to establish that this International Standard is appropriate for the standardization of other preparations of lymphoblastoid interferon derived from other lines or lymphoblastoid cells or with the use of inducers other than Sendai virus.

A number of other standard preparations for human interferons are available.

These include: the Second International Standard for Interferon, Human, Fibroblast, β, containing 15 000 IU/ampoule; the International Standard for Interferon, Human, Recombinant, α₁(αD), containing 8000 IU/ampoule; the International Standard for Interferon, Human, Recombinant, α₂(αN), containing 17 000 IU/ampoule; and the International Standard for Interferon, Human, Recombinant, β-α₁β₁7, containing 6000 IU/ampoule.

In addition, three standard preparations for murine interferons are available: the International Standard for Interferon, Murine, α, containing 16 000 IU/ampoule; the International Standard for Interferon, Murine, β, containing 15 000 IU/ampoule; and the International Standard for Interferon, Murine, γ, containing 1000 IU/ampoule.

Interferon standards and reference reagents are held and distributed by the National Institutes of Health, Bethesda, MD, USA, and the National Institute for Biological Standards and Control, Potters Bar, England.

1.4 Terminology

The following definitions are given for the purposes of these Requirements only.

Cell seed: a quantity of cells stored frozen at −70 °C or below in aliquots of uniform composition, one or more of which is used for the production of a manufacturer’s working cell bank.

Several national control authorities have drafted documents relating to cell substrates used in the manufacture of biologicals for human use.

Manufacturer’s working cell bank (MWCB): a single uniform suspension of lymphoblastoid cells which have been dispensed in a
single working session into a number of containers which are stored at -70°C or below. Cells revived from one or more of these containers are used as a source of production cell cultures.

In normal practice a cell seed is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer, at which point the cells are combined to give a single pool and preserved cryogenically to form the MWCB.

Production cell culture: a lymphoblastoid cell culture derived from one or more containers of MWCB that, in the production process, is induced to form interferon.

Inducer: a substance added to a culture of lymphoblastoid cells which leads to or stimulates the production of interferon.

Enhancer: any substance added to cultures of lymphoblastoid cells during the process of manufacture in order to increase the production of interferon when the cells are treated with an inducer.

Single harvest: the cell-free fluid containing unpurified interferon harvested from an individual production culture of induced lymphoblastoid cells.

Purified interferon solution: lymphoblastoid interferon, from a single harvest, that has been subjected to a designated purification process.

Purified interferon bulk solution: the result of blending two or more batches of purified interferon solution.

Stabilized interferon: a purified interferon bulk solution to which a stabilizer has been added.

Final bulk: the finished biological material prepared from purified interferon bulk solution and present in the container from which the final containers are filled.

Final lot: a collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling or preparation of the finished product. A final lot must therefore consist of finished material dispensed into containers in one working session from a single final bulk.

Manufacturer's reference material: a large number of samples from one or more final lots of material that has been shown to be active in clinical use (or from lots directly related to such material) shall be fully characterized in ways to be specified by the national control authority and be suitably stored to serve as the manufacturer's reference material. For certain tests, a sample of such reference material shall be included in parallel with each lot of
production material, which must match the specification of the reference batch within limits to be agreed by the national control authority.

2. General manufacturing requirements

The general manufacturing requirements contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, pp. 11–22) shall apply to establishments manufacturing human interferon, with the addition of the following directives.

Production areas shall be decontaminated before they are used for the manufacture of human interferon.

Human interferon shall be produced by staff who have not handled animals or infectious microorganisms, other than those microorganisms directly required in the process, in the same working day. The staff shall consist of persons whose state of health does not compromise the quality of the product.

No culture of any microorganisms or eukaryotic cells, other than those required for the process and approved by the national control authority, shall be introduced or handled at any time during the manufacture of the human interferon in the areas used for cell culture and product purification.

Persons not directly concerned with the production processes, other than official representatives of the national control authority, shall not be permitted to enter the production area without valid reason and specific authorization.

Particular attention shall be given to the recommendations contained in Part A, section 1 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, p. 13) regarding the training and experience of persons in charge of production and testing and of those assigned to various areas of responsibility in the manufacturing establishment, as well as to the registration of such personnel with the national control authority.

There shall be strict adherence to the use of a seed lot system. A description of the system used should be provided, including the number of vials of seed available and details of their storage. It must be possible to trace every culture back to the MWCB.
Full details of the production and control procedures used in manufacture shall be provided to the national control authority. Information on the sensitivity of test methods and the frequency of the tests shall be provided, together with criteria for the rejection of material. All test methods shall be validated to the satisfaction of the national control authority.

The yield of human lymphoblastoid interferon shall be monitored during the course of manufacture. Criteria for the acceptance of single harvests and intermediate products for further processing shall be defined. Consistency of production shall be established by testing a number of consecutive lots prepared by the same procedure, which shall be determined by the national control authority.

3. Validation and control of manufacturing procedures

The general production precautions formulated in Part A, section 3 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, pp. 15–16) shall apply to the manufacture of human lymphoblastoid interferon.

3.1 Production strategy

A full description shall be given of the biological characteristics of the lymphoblastoid cells and any additives, for example the inducer and any enhancer or other substances (e.g., antibiotics), used in production. The information should include:

(a) documentation concerning the origin of the cell line, and the nature of any known relevant condition in the donor, such as Burkitt’s lymphoma, active infectious mononucleosis, or infection with any other virus;

(b) data that can be used to establish the identity of the cell line, for example the karyology and, in particular, any constant chromosomal markers which may be used to characterize the cells;

(c) the growth characteristics of the cell line; and

(d) data that document the stability of the cell line under the culture conditions used, if cells are to be subcultured for an extended or indefinite period (if stability is checked only at intervals, the lymphoblastoid interferon made between such checks must not
be approved for issue until the appropriate analysis has been made).

3.2 Purification procedures

The methods used to purify the lymphoblastoid interferon from the culture harvests should be fully described. The capacity of the purification procedure to remove and/or inactivate substances derived from the host cells or culture medium other than interferon, including, in particular, viruses, proteins, and nucleic acids, must be evaluated.

The results of pilot-scale studies which monitor the removal of individual marker substances deliberately added at appropriate stages in the purification procedure will provide valuable information in this respect.

If any substance is added or used during purification, it must be shown to the satisfaction of the national control authority that its concentration is reduced to an insignificant level during further purification or that it does not adversely affect the safety and efficacy of the final product.

If antibodies are used in the purification procedures, their origins and characteristics should be fully described. The degree of purity and freedom from viruses or cell-derived DNA of any monoclonal antibodies produced from hybridoma cell lines should conform to the regulations laid down by the national control authority.

3.3 Characterization of lymphoblastoid interferon

3.3.1 Biological and antigenic characterization

Tests shall be performed on at least two successive filling lots to show that the product has the biological characteristics expected for the relevant type of interferon.

These tests should be of at least two quite different types. One test might be to determine whether the preparation reduces the growth of at least two unrelated viruses in human cells (for HuIFN-a, appropriate animal cells such as bovine or porcine cells may be more convenient). Other tests might determine the inhibitory effect of the preparation on the growth of sensitive cells, for example the Daudi line of human lymphoblastoid cells, or measure the induction of histocompatibility antigens or the formation of the enzyme 2',5'-oligoadenylate synthetase in treated cells.
The antigenic type of interferon present in the preparation shall be established by neutralization of the antiviral activity of the preparation by an appropriate reference antiserum (for details of the reference antisera available, see WHO Technical Report Series, No. 687, 1983, p. 39).

3.3.2 Chemical characterization

Interferons should be characterized by means of techniques approved by the national control authority, which will specify those characterization tests to be applied only to one or more reference batches of purified interferon bulk solution and those to be applied to each bulk solution.

The protein composition should be analysed by techniques such as sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) performed under reducing and non-reducing conditions, or high-performance liquid chromatography. With SDS–PAGE, the stained gels should be analysed by an appropriate method, such as scanning densitometry, to determine the percentage purity of the interferon preparation. If bands other than the interferon monomers are observed on the gel, Western blot analysis using antibodies should be used to identify which species are the interferon products (e.g., oligomers, fragments) and which are non-interferon contaminants.

The following tests have also been useful in characterizing the protein product: isoelectric focusing, size exclusion chromatography, amino acid analysis, ultraviolet spectroscopy, affinity chromatography, circular dichroism, and neutralization by anti-interferon antibody.

3.3.3 Consistency of production

Data to show the consistency of the production process shall be provided in terms of the specific activity of the interferon (i.e., the number of units of biological activity per mg of total protein present) at different stages in the production process, and the purity (percentage content of extraneous protein) of each lot of purified interferon bulk solution. The national control authority shall approve the criteria for an acceptable production run.
4. Manufacturer's working cell bank (MWCB)

Only cells approved by the national control authority shall be used to produce human interferon. The continuous cell lines used shall have been characterized as specified in the Requirements for Biological Substances No. 37 (Requirements for Continuous Cell Lines) (3). The national control authority shall have responsibility for approving the cell seed.

The manufacturer shall show to the satisfaction of the national control authority that the MWCB satisfies the requirements outlined in this section for freedom from bacteria, fungi, and adventitious viral agents.

Preserved cell cultures must be maintained in a state that allows recovery of viable cells without alteration of genotype. The identity of the revived cells must be determined by appropriate tests.

In one country, cells recovered from the preserved state are checked to confirm that they have retained the karyological characteristics of the lymphoblastoid cell line concerned.

5. Controls for mammalian cell cultures

If serum is included in the medium for the production cell cultures, it shall be tested to determine whether it is free from bacteria, fungi, viruses, and mycoplasmas, according to the requirements in Part A, sections 5.2 and 5.3 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (4, pp. 49–52) and by methods approved by the national control authority.

Alternatively, the serum may be processed in various ways, e.g., by filtration together with irradiation or chemical treatment(s), which eliminate or inactivate any bacteria, fungi, viruses, or mycoplasmas that might be present in the untreated serum. The supporting data shall be presented to the national control authority and, if accepted, it may be agreed by that authority that tests on each batch of serum used for production are not needed.

Antibiotics of the β-lactam type shall not be used at any stage in the production process.

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6. Controls for additives

Any additives, including any inducers and enhancers, shall be defined and approved by the national control authority. If the inducer is a virus, it shall be obtained from a seed lot system under approved manufacturing conditions.

Additives shall be used at a concentration within a range previously shown to yield a satisfactory product from production cell cultures incubated at a given temperature for a specified period. These details shall be agreed with the national control authority. The manufacturer shall demonstrate to the satisfaction of the national control authority that the presence of inducers or enhancers in a single harvest does not adversely affect the stability of the product.

7. Production precautions

7.1 Production cell cultures

Only cell cultures derived from the MWCB shall be used for production. All cells shall be processed in an area in which no other cells or organisms are handled other than those directly required for the process.

7.2 Culture conditions for production cell cultures

Production cell cultures shall be grown under conditions agreed with the national control authority. Information to be provided should include the culture system used, the cell doubling time, the number of subcultures or the duration of the period of subcultivation permitted, and the incubation temperature.

Cell cultures shall be monitored for freedom from microbial contamination as required by the national control authority.

8. Single harvests

Each single harvest shall be processed to remove cells and cellular debris.
8.1 Assays for pyrogens and interferon activity

These shall be carried out by methods approved by the national control authority.

8.2 Sterility

The degree and nature of any microbial contamination shall be monitored during and at the end of production by methods approved by the national control authority. The sensitivity of the test methods and criteria for the rejection of harvests shall be defined.

8.3 Consistency of yield

The yield of human interferon in a single harvest shall be shown to be within the limits approved by the national control authority.

9. Purification

9.1 The purification procedure

The purification procedure to be applied at any stage in the manufacturing process shall be approved by the national control authority.

Several purification steps are likely to be required, yielding progressively purer intermediate products. Details of any control procedures to be applied shall be agreed with the national control authority.

For manufacturing convenience, the intermediate products derived from two or more single harvests that have passed any applicable control tests may be combined. If required, the bulk may be subjected to further processing. The product after all purification procedures have been applied is a purified interferon solution or purified interferon bulk solution, which may be stabilized by the addition of protein or other substances of a nature and at a concentration approved by the national control authority. A stabilizing substance of human origin must have been manufactured in such a way as to ensure its freedom from contamination with adventitious agents. Any substances added must not impair the safety and efficacy of the product. Before any proteinaceous
stabilizer is added, all the samples required for certain tests, including those for protein content, purity, content of specific extraneous proteins, e.g., bovine proteins derived from the medium, chemical characterization, and DNA content, must be taken. All such tests, as well as tests for materials of animal origin (e.g., serum protein) or plant origin (e.g., lectins) used at any stage in production or purification, shall be carried out using methods approved by the national control authority.

9.2 Control of purified interferon solutions or bulk solutions

9.2.1 Assay for protein content

The total protein content of the purified solution shall be determined.

9.2.2 Assay for interferon activity

The interferon content of the purified solution shall be determined by a method which measures antiviral activity or some other biological activity, as agreed with the national control authority.

Additional tests which have been found useful include SDS-PAGE, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), and single radial immunodiffusion in comparison with a known standard. Analysis of the data by the parallel-line method has been found suitable for most of these techniques.

9.2.3 Test for additives used during purification or other phases of manufacture

A test shall be made for the presence of any potentially hazardous agent, including monoclonal antibodies, antibiotics, and other additives used at any stage in manufacture. The method used in the test and the permitted residual concentration in the final product shall be approved by the national control authority.

9.2.4 Test for identity

The human interferon product shall be identified by means of SDS-PAGE and/or by neutralization of biological activity, as required by the national control authority.
9.2.5 Protein purity

Before any stabilizing substance is added, the purity of each purified interferon solution or bulk solution shall be established, as approved by the national control authority.

One manufacturer has used for this purpose SDS–PAGE of a sample containing at least 10 μg of total protein. The protein peaks obtained are scanned in a densitometer and stained with specific antisera to interferon protein and to potential protein contaminants derived from the cells, the medium, or the inducer used in the production process. Identification of the protein bands and their relative amounts enables the purity of the preparation to be assessed.

A purity of at least 95% has been achieved by one manufacturer.

9.2.6 Test for serum proteins

If serum is used in the medium for the production cell cultures, or at any stage in the purification process, for example as a reagent in immunoabsorption chromatography, tests shall be made to determine whether serum remains in the purified interferon solution or bulk solution.

Animal serum at 1 μl per litre of interferon solution is approximately equivalent to 50 ng/ml albumin. Methods such as ELISA and immunoprecipitation can detect as little as 1 ng of albumin.

9.2.7 Test for DNA

The amount of residual DNA in each batch of product should be determined by means of sensitive methods, which must be validated and approved by the national control authority. The maximum acceptable level of DNA per human dose shall be approved by the national control authority.

The WHO Study Group on Biologics concluded that the probability of risk associated with heterogeneous contaminating DNA in a product derived from a continuous cell line is negligible when the amount of such DNA is 100 μg or less in a single dose given parenterally (3).
10. Final bulk

Any substances such as diluent, preservatives, and stabilizers added to the purified interferon bulk solution shall be approved by the national control authority.

10.1 Tests for sterility

The final bulk shall be tested for bacterial and mycotic sterility in accordance with 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) (4, pp. 48–53).

10.2 Tests for pyrogenic substances

The pyrogen content of the final bulk shall be determined by a method agreed with the national control authority.

11. Filling and containers

The requirements covering filling and containers given in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, pp. 16–17) shall apply.

Care should be taken to ensure that the materials of which the container and closure are made do not react with the interferon.

12. Final lot

Samples shall be taken from each final lot for the tests described below.

12.1 Sterility test

Each final lot shall be tested for sterility according to the requirements in Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (4, pp. 48–53).
12.2 Identity test

Samples from each final lot shall be identified as human interferon by a method approved by the national control authority.

12.3 Potency test

Potency should be determined by means of a test measuring a biological activity. Statistical analysis of the data should show that the mean potency value obtained has confidence limits within a range accepted by the national control authority.

The detailed procedures for carrying out this test shall be approved by the national control authority and an appropriate interferon standard preparation homologous with the product should be tested in parallel. For interferon preparations made from Namalwa cells induced with Sendai virus, the International Standard for Human Lymphoblastoid Interferon (Namalwa) (HuIF-N-a (Ly)) is appropriate.

12.4 Innocuity test

The innocuity of the final product shall be tested parenterally in guinea-pigs and mice by a method approved by the national control authority.

12.5 Test for pyrogenic substances

Each final lot shall be tested for pyrogenic substances by a method approved by the national control authority.

12.6 Test for preservative

Each final lot shall be tested for the presence of any preservative added. The test used and the permitted concentration shall be approved by the national control authority.

12.7 Determination of moisture content

For lyophilized products, the moisture content shall not exceed a level approved by the national control authority.
12.8 Determination of hydrogen ion concentration and degree of clarity

The pH and degree of clarity of the final reconstituted interferon solution shall be within the limits approved by the national control authority.

13. Records

The requirements given in Part A, section 6 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, p. 17) shall apply.

14. Samples

The requirements given in Part A, section 7 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, p. 18) shall apply.

15. Labelling

The requirements given in Part A, section 8 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, p. 18) shall apply, with the addition of the following directive.

The leaflet accompanying the package should include the following information, together with any other items required by the national control authority:

—description of the product,
—clinical pharmacology,
—indications and usage,
—contraindications,
—warnings,
—precautions,
—use during pregnancy,
---adverse reactions,
---dosage and route(s) of administration,
---directions for use,
---how supplied,
---storage conditions, and
---references.

16. Distribution and shipping

The requirements given in Part A, section 9 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, pp. 18–19) shall apply.

17. Storage and expiry date

The requirements given in Part A, section 10 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, p. 19) shall apply.

17.1 Storage conditions

Filled containers of human lymphoblastoid interferon intended for clinical use shall be stored under conditions such that the product conforms with the specification agreed with the national control authority during the claimed shelf-life.

17.2 Expiry date

The expiry date for each preparation of human lymphoblastoid interferon shall be that agreed by the national control authority on the basis of evidence for stability supplied by the manufacturer.

PART B. NATIONAL CONTROL REQUIREMENTS

1. General

The general requirements for control laboratories contained in Part B of the revised Requirements for Biological Substances No. 1
(General Requirements for Manufacturing Establishments and Control Laboratories) (2, pp. 19–22) shall apply.

The national control authority shall:

— approve the methods for producing human interferon from lymphoblastoid cells;
— approve the tests for interferon concentration and specify the statistical precision required for a claimed value;
— approve the purity of the final product;
— approve the tests for extraneous substances and total protein;
— approve the tests for preservatives and the agents used for purification and in other aspects of manufacture;
— approve the tests used for freedom from abnormal toxicity in the final product;
— approve the tests used in the assay of potency; and
— approve the data from which it has been concluded that therapeutic responses are adequate and that the product is safe in humans.

The national control authority shall be satisfied that the results of all tests, including those done on individual batches during the process of manufacture, are satisfactory and that consistency has been established.

2. Release and certification

Interferon shall be released only if it fulfils the above requirements.

A statement signed by the appropriate official of the national control authority shall certify whether or not the final lot of interferon in question meets all national requirements as well as the above requirements. The certificate shall state the date of the last satisfactory potency test, the lot number, the number under which the lot was 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 the certificate is to facilitate the exchange of human interferon between countries.
AUTHORS

The first draft of the proposed Requirements for Human Interferons Prepared from Lymphoblastoid Cells was prepared by Dr N.B. Finter, Wellcome Biotech, Beckenham, Kent, England.

The second draft of these Requirements was formulated in March 1987, contributions being made by Dr V. Grachev, Scientist, and Dr J.C. Petricciani, Chief, Biologicals, World Health Organization, Geneva, Switzerland.

The third draft of these Requirements was formulated at the WHO Informal Consultation on the Standardization of Interferons, Geneva, 23-25 March 1987; for a list of the participants, please see WHO Technical Report Series, No. 771, 1988, Annex 1.

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REFERENCES

Annex 4

REQUIREMENTS FOR THE COLLECTION, PROCESSING, AND QUALITY CONTROL OF BLOOD, BLOOD COMPONENTS, AND PLASMA DERIVATIVES

(Requirements for Biological Substances No. 27) (Revised 1988)

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INTRODUCTION

In 1976, a WHO Working Group on the Standardization of Human Blood Products and Related Substances (1, pp. 24–51) 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).

A number of other WHO publications have dealt with whole blood and its components, among them guidelines intended mainly for blood transfusion services (3).

This Annex contains a revised version of the Requirements for the Collection, Processing and Quality Control of Human Blood and Blood Products; the Requirements have been updated and the recent
WHO recommendations concerning testing for antibodies to the human immunodeficiency virus (anti-HIV) noted (4).

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. The collection and distribution of whole blood, the separation of whole blood into components, and the fractionation of plasma is a logical developmental sequence in a comprehensive organization. It is not always possible to be specific about the details of the procedures employed, the in-process controls, or the tests applied at each stage of production. This is particularly true with regard to whole blood and component cells. Although the general principle of fractionation of plasma is well established, there are in practice numerous variations in the details of the various production steps. Any country wishing to begin the collection and fractionation of blood and blood components should therefore 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 in considering whether fractionation of plasma should be started is whether the country has a suitable donor population of sufficient size to guarantee an adequate supply of source material. It is not possible to set a lower limit for the quantity of source material that would be necessary to make such an operation economic because too many factors are involved. In order to ensure that production standards are met at all times and to avoid certain contamination risks, it is important to have sufficient source material to keep the fractionation plant in continual 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 would 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 hepatitis and AIDS, and of parasitic diseases, differs so markedly from one geographical region to another that each national authority must decide for itself whether the testing of each blood donation by means of the most sensitive test available is cost-effective and whether it is feasible to collect suitable source material. In any case, a brief protocol should be drawn up for the collection of source material (see Appendix 1). Great emphasis should be placed on the production of blood fractions by means of 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 reputation of being free from contamination with HIV and hepatitis B virus, as have albumin products prepared by the same method and heated for 10 hours at 60°C (3). Nevertheless, ensuring that these products are free from infectious viruses requires extreme care in manufacture and it cannot be assumed that new fractionation methods will be equally effective.

The protection of the health of the staff calls for special care, and appropriate protective measures approved by the national control authority must be taken.

The transport of source materials from blood collecting centres and hospitals to the 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 and spillage or leakage from containers.

In these requirements, the word “human” has been omitted from the names of the products derived from human blood. Blood products of animal origin are immunogenic, and their administration to humans should be avoided wherever 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 the following five parts:

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

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

Although these requirements are concerned primarily with the collection of plasma by plasmapheresis for further processing to produce injectable drugs or diagnostic reagents, they may be adapted by means of minor alterations to apply also to the following blood components for use in transfusion:

(a) plasma,
(b) fresh-frozen plasma,
(c) platelet-rich plasma,
(d) platelets, and
(e) leukocytes.

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

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.

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

**International 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 in the last 38 years. A number of materials are currently under investigation for use in the preparation of new standards.

The activity of blood and 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 1986) under the title *Biological substances: international standards and reference reagents*.

The international standards are in the custody of the State Serum Institute, Copenhagen, Denmark, the National Institute for Biological Standards and Control, Potters Bar, England, the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands, the National Institute of Public Health, Bilthoven, Netherlands, and the Centers for Disease Control, Atlanta, USA, and samples are distributed on request to national control laboratories. The international standards are intended for the calibration of national standards for use in the manufacture and laboratory control of human blood and blood products.

**DEFINITIONS**

The following definitions are intended for use in this document and are not necessarily valid for other purposes.
Centres for the collection of source material

Blood donor centre: an establishment in which blood and/or blood components are obtained from donors.
Placenta collecting centre: an establishment in which placentas are received and stored.

Activities of collection centres

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

Donors

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, plasma-reduced (sometimes referred to as “plasma-reduced blood”): whole blood in which the erythrocyte volume fraction (“packed cell volume”) has been elevated to approximately 0.6 by the removal of part of the plasma.
Whole blood, modified: whole blood from which plasma has been separated for the purpose of obtaining cryoprecipitated factor VIII, platelets, or leukocytes and to which the modified plasma has then been returned.
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, cryoprecipitate-depleted: a plasma from which cryoprecipitate has been removed.
Plasma, frozen: a plasma separated more than 6 h after the collection of the blood and stored below −20°C.

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

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

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

Plasma, specific immune: a plasma that can be used for the manufacture of specific immunoglobulins and which may also sometimes be used for passive immunization.

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

Plasma, recovered: a plasma that does not meet the requirements of “plasma, fresh-frozen” or “plasma, frozen” and is intended for further processing rather than transfusion.

Source plasma: a plasma collected by apheresis and intended only for use in further manufacture.

Cryoprecipitated factor VIII: a crude preparation that contains factor VIII obtained from single units (or 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.

Serum, specific immune: a serum that can be used for the manufacture of specific immunoglobulins and may also be used for passive immunization.

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

Red cells suspended in preservative solution: red cells to which a preservative solution, for example containing adenine, glucose, and mannitol, is added so as to enable them to be stored 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 remaining plasma has been removed by one or more stages of washing with an isotonic solution.

Red cells, leukocyte-poor: a red-cell preparation containing at least 80% of the red cells and less than 25% of the leukocytes of the original whole blood.
Red cells, leukocyte-free: a red-cell preparation containing at least 80% of the red cells and less than 2% of the leukocytes of the original whole blood.

Red cells, frozen: frozen red cells to which a cryoprotective agent such as glycerol has been added prior to 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 or by apheresis and suspended in a small volume of autologous plasma.

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

Postpartum source materials

Placental blood: the placental blood from a single delivery.

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 revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (5, p. 13) and in addition provide adequate space, lighting, and ventilation for the following activities, where applicable:

1) 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 exclusion of unsuitable potential donors.

2) The withdrawal of blood from donors and, where applicable, the re-infusion of blood components with minimum risk of contamination and errors.

3) The care of donors, including the treatment of those who suffer adverse reactions.
(4) The storage of whole blood and blood components in quarantine pending completion of processing and testing.
(5) The laboratory testing of blood and blood components.
(6) The processing and distribution of whole blood and blood components in a manner that prevents contamination and loss of potency.
(7) The performance of all the steps in apheresis procedures, if applicable.
(8) The performance of labelling, packaging, and other finishing operations in a manner that prevents errors.
(9) The storage of equipment.
(10) The storage of finished products prior to distribution.
(11) The documentation and recording 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 tested and validated before initial use, and shall be kept clean and maintained and checked regularly. The revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (5, p. 13) 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. 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/cm² or 15 lbf/in²) or by a temperature of 170°C maintained for 2 h with dry heat.
Tests for sterility are given in the WHO General Requirements for the Sterility of Biological Substances (6, pp. 40–61). The disposal of contaminated material should comply with the relevant local by-laws.

3. Personnel

An organization for the collection of blood or blood components 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 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 employees.

The persons responsible for the collection of 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. Donor selection

4.1 Donors

Source materials for further processing are obtained from donations of blood or its components. The medical criteria for the
acceptance of donors—relating to the safety, purity, potency, and

efficacy of the final products—must be the same for donors of whole

blood and of cellular components or blood components collected by

haemapheresis.

The physical fitness of a donor shall be determined by a licensed

physician or a person under the direct supervision of a licensed

physician. Donors shall be healthy persons of either sex between the

ages of 18 and 65 years.

The recruitment of volunteer (non-remunerated) donors should be

the aim of any national blood programme.

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 donation include: (1) healthy persons;
(2) persons with antibody levels that have been increased, either
naturally or by immunization; (3) persons with markedly increased
or markedly depressed levels of specific plasma proteins, whose
plasma is essential for diagnostic purposes; and (4) persons whose
blood may be used in the preparation of certain vaccines, such as
hepatitis B vaccine.

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

To minimize the possibility of transmitting retrovirus infections,
all potential donors should be advised of factors in their history or
behaviour which may increase their risk of being infected. The
national control authority must determine the appropriate exclusion
criteria for the country concerned.
It is recommended that persons in the following categories be excluded from acting as donors:

— those with clinical or laboratory evidence of infection with human retroviruses;
— past or present intravenous drug abusers;
— those with haemophilia and other clotting-factor defects who have received clotting-factor preparations;
— sexual partners of any of the above.

The following should be excluded from acting as donors for a minimum period of two years:

— men who have had sexual intercourse with another man one or more times;
— men and women who have engaged in prostitution;
— sexual partners of any of the above;
— persons who have received blood transfusions.

Donors should be made aware before donating blood that it will be tested for the presence of serological markers of virus infection. It is advisable that provision for the right to test donations should be made in appropriate legislation.

4.2 Donation frequency

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 2 litres.

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 blood donors, and those who donate at a maximum of twice a week. The first group shall be accepted on the basis of the criteria for donors of whole blood.
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. In 1967, the Council of Europe Subcommittee of Specialists on Blood Problems recommended that not more than 8 single units of plasma (each of approximately 300 ml) should be removed in one month, and not more than 50 single units in one year. However, different limits are imposed in certain countries, e.g., in the USSR and France the limit is 10–12 litres per year, and in the USA 50 or 60 litres per year depending on whether donors weigh less than or more than 80 kg.

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 for 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, such as malignant disease, diabetes or other endocrine imbalance (e.g., thyrotoxicosis), epilepsy, hypertension, or renal disease or infection.

A donor who appears to be suffering from an acute or chronic disease or who is receiving oral or parenteral medication, with the exception of vitamins, postmenopausal hormone therapy, or oral contraceptives, may not be accepted unless approved by a physician.

In one country, donors are excluded for one month after having received the vitamin A analogue retinoic acid.

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 which places them at increased risk of transmitting infection shall not donate blood or plasma for
at least two years following the last known incident of risk. A donor shall be permanently excluded if a previous blood donation was believed to be responsible for transmitting disease.

In most countries questions concerning the signs and symptoms of AIDS will be part of the routine assessment of medical history and appropriate monitoring for possible indications of AIDS, as defined by the national control authorities, will be included. Such monitoring may include assessment of weight loss, visual examination of the oral mucosa and of the skin of all the extremities, and palpation of the cervical lymph-nodes. As a result of this assessment, a donor may be disqualified.

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

Acupuncture within the previous two years 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) are permanently excluded. In others, such donors are accepted providing that recovery occurred more than one year previously and that the reaction for HBsAg 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 known to be capable of transmitting hepatitis or HIV should be avoided if the donor population shows a prevalence of acute or chronic hepatitis B, non-A non-B hepatitis, or HIV infection higher than that found in the general population. Specific approval may be given by the 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.

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

Blood and plasma shall be tested for the presence of \textit{HBsAg} and anti-HIV by the methods described in Part B, section 3; the tests used should be approved by the national control authority.

Anyone whose blood has repeatedly been shown, in screening tests, to contain anti-HIV should be permanently excluded as a donor unless sufficient data are available to determine with certainty that the test results were nonspecific.

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

In countries where malaria is not endemic, donors of cellular 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 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 which will be fractionated.

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

Anyone who has received pituitary growth hormone of human origin should be permanently excluded as a donor because of possible infection with the agent causing \textit{Creutzfeldt-Jakob disease}. Although transmission of this agent through blood products has not been proved, it is believed to be possible.
4.3.3 Minor surgery

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

4.3.4 Pregnancy

Pregnant women shall be excluded from blood donation. In general, mothers shall also be excluded for the duration of the period of 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 following an abortion during the first trimester.

In some countries, donors are accepted when pregnant or during the period of lactation if their blood contains rare 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. There is no deferral period for recipients of prophylactic therapy or therapy associated with a bite from an animal subsequently proven to be non-rabid.
— 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 should be disqualified for at least six months after the last such exposure. If hepatitis B immune globulin has been administered, the period of deferral should be increased to at least 12 months because disease onset may be delayed.
4.4 Physical examination

Donors shall generally have a weight, blood pressure, pulse rate, and temperature within the established normal limits. If, when these are measured, the results lie outside the limits for weight, blood pressure, and pulse rate, the donor concerned may be accepted only if approved by the responsible licensed physician.

The following recommendations may be useful for guidance:

(1) Blood pressure: systolic blood pressure between 12 and 24 kPa (90 and 180 mmHg); diastolic blood pressure between 6.67 and 13.3 kPa (50 and 100 mmHg).
(2) Pulse: between 50 and 100 beats per minute and regular. Lower values may be accepted in healthy athletes with endurance training.
(3) Temperature: oral temperature not exceeding 37.5°C. In some countries, the body temperature does not have to be measured but decisions to this effect should be made by the national control authority.
(4) Weight: donors weighing less than 50 kg may donate a volume of blood proportionately 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 Haemoglobin or erythrocyte volume fraction

The haemoglobin or erythrocyte volume fraction of the donor’s blood shall be within normal limits, as defined by the national control authority; in many countries, minimum values for haemoglobin of 125 g/l for females and 135 g/l for males are specified.

4.6 Additional requirements applicable to donors for haemapheresis

All phases of apheresis, including explaining to donors what is involved in the process and obtaining their informed consent, shall be performed under the direct supervision of a licensed physician. In the absence of such a physician, use may be made by trained personnel of a manual of standard operating procedures.
4.6.1 *First-time plasma donors*

Usually, when donors present themselves to a centre for the first time, they are received by a receptionist, who explains the procedure of plasmapheresis. Only after the donor has given his or her consent does the initial screening begin.

The following information shall be permanently recorded:

1. **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.

2. **A preliminary medical history as required for blood donors, covering infectious diseases and the general state of health.**

If there are no contraindications to plasmapheresis, laboratory tests shall be carried out, namely reading of the erythrocyte volume fraction, determination of total serum proteins, and screening for protein and sugar in the urine. If normal values are obtained in the laboratory tests (see section 4.6.3), 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 prior to 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, a serological test for syphilis, tests for HBsAg and anti-HIV, 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.

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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), the tests for HBsAg and anti-HIV, and those for the quantification of plasma proteins. The results of these tests should be reviewed by the physician before subsequent plasmapheresis procedures.

4.6.2 Donors who have undergone plasmapheresis previously in the same programme

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

1. The receptionist shall note the date and time of the last donation (at least 48 h must have elapsed since that time). No more than two donations shall be permitted within a seven-day period.

2. The medical history and weight of the donor shall be recorded; blood pressure, temperature, pulse rate, and haemoglobin shall be measured by trained personnel.

On the day of each donation, in addition to meeting the requirements for donors (section 4.1), plasma donors shall be shown to have a total serum protein 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 authorities, and tests carried out as specified in section 4.6.3. The interval between laboratory examinations shall not exceed four months, unless the national control authorities have established other limits.

Whenever the result of a laboratory test is found to be outside the established normal limits or a donor exhibits any important abnormalities either 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 the national health authorities to define normal ranges of test results on the basis of data from a sufficiently large sample of healthy individuals not undergoing plasmapheresis.

In the case of non-A non-B hepatitis, 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.

4.6.3 *Tests for plasma donors*

The following tests shall be performed at each visit:

1. Measurement of haemoglobin or erythrocyte volume fraction.
2. Determination of total serum protein: this shall show a minimum value of 60 g/l. If plasma is used for this assay, the appropriate correction for fibrinogen should be made. Acceptable methods include the use of a Goldberg refractometer or the biuret reaction read with a photometer.
3. Determination of serum alanine aminotransferase: this shall have a value no more than twice the upper limit of the normal range in a photometric determination using approved reagents.
4. Test for HBsAg: unless the plasma is to be used for hepatitis B vaccine production, it shall be negative by a sensitive method such as: (a) radioimmunoassay; or (b) enzyme-linked immunoassay.
5. Test for anti-HIV antibodies or HIV antigen: this shall be negative.

The following tests shall be performed initially and every four months or after every ten donations:

1. Urine analysis (test for glucose and protein): this should be negative. A paper test strip method is suitable.
2. If required by the national health authorities, a serological test for syphilis: this shall be negative.
3. Serum protein electrophoresis: this should 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. The serum proteins should show the following values: albumin: minimum 35 g/l; IgM: minimum 0.5 g/l; IgG: between 5 and 20 g/l.

Specific exceptions might be considered appropriate by the national control authorities based on the therapeutic benefit and safety of the product.

4.7 *Donors for platelet and leukocyte apheresis*

In general, platelet and leukocyte donors shall meet the criteria for whole blood and plasma donors (sections 4.1–4.6). In addition,
platelet donors should not have taken aspirin or other platelet-active drugs for at least 72 hours before donation.

The requirements to be satisfied in the performance of platelethpheresis 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 platelethpheresis should have an absolute platelet number concentration ("count") of not less than $200 \times 10^9/\text{l}$ and donors for leukapheresis should have an absolute granulocyte number concentration of not less than $3 \times 10^9/\text{l}$. Both types of donor should have a normal leukocyte count ("differential count") and haemoglobin level.

Although circulating platelet and leukocyte levels recover promptly in donors, data are not at present available from which the maximum numbers of platelets and leukocytes that can safely be 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 in order 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.

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

4.8 Donor immunization practices and plasma for special purposes

4.8.1 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 registered 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 such medically useful plasma may be identified by screening voluntary 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 some examples of medically useful plasma:

1. Antibody-rich, convalescent, or post-vaccination plasma for the production of therapeutic or prophylactic immunoglobulins; plasma or serum for use against such infections as hepatitis A, hepatitis B, measles, tetanus, rabies, varicella zoster, poliomyelitis, and tick-borne encephalitis, as well as against diseases caused by such uncommon agents as Lassa, Ebola and Marburg viruses.

Although anti-D (anti-RhD) was at one time obtained by therapeutic plasmapheresis, the effectiveness of this treatment is not well established and the benefit-risk ratio of other types of therapy should be considered.

The effectiveness of anti-HIV therapy has not yet been proved.

Although there is still no clinical evidence of efficacy, it would seem useful for human diphtheria immunoglobulins to be available.

2. 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; collection procedures should be carried out in appropriately isolated premises when products are being prepared that are known to be associated with an increased risk of transmitting infection.

3. Antibodies to human cellular and serum antigens for diagnostic use, such as HLA typing reagents, erythrocyte typing reagents, and immunoglobulin allotyping reagents.

4. Plasma containing reagents useful for diagnostic tests, such as reagin, rheumatoid factors, heterophile antibody, and C-reactive protein.

5. Factor-deficient plasma for specific assays, such as factor-VIII-deficient plasma. Donors who have been recipients of factor VIII are at increased risk of transmitting hepatitis B, non-A, non-B hepatitis, and HIV; collection procedures should therefore be carried out in appropriately isolated premises.

6. Plasma containing HBsAg for vaccine production. Special precautions should be taken with donors of HBsAg-positive plasma to monitor the status of their chronic liver disease, as well as for the protection of other donors and of the personnel handling the blood. The premises in which HBsAg-positive plasma is collected must be strictly separated from those for normal plasma. Ideally, collection should be restricted to special centres for vaccine production (7). In this case, a minimum level of monitoring would include baseline and periodic measurement of levels of serum alanine aminotransferase.
4.8.2 Precautions to be taken when handling blood or blood products containing infectious agents

All blood or 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 in the apheresis of HBsAg-positive donors and other donors who may be associated with an increased risk of transmission of infection include:

(a) 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;
(b) disinfection of all work surfaces and equipment with a disinfectant of known efficacy, such as freshly prepared 0.25% sodium hypochlorite solution;
(c) protection of staff by means of adequate training, avoidance of aerosols, and use of gloves, gowns, masks, and eye protection;
(d) application of the labelling, shipping, and waste-disposal requirements appropriate to the etiological agents in question.

4.8.3 Immunization of donors

There is a scientifically valid need for specific immunoglobulins, serum, 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 of the requirements listed in the previous sections.

Immunization of donors with antigens shall be carried out only when sufficient supplies of material of suitable quality cannot be obtained by selection of appropriate donors or from donations selected by screening. Donors must be fully informed of the risk of any proposed immunization procedure, and pressure shall not be brought to bear on a donor to agree to immunization. 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 may reveal hypersensitivity to a proposed antigen. (See also Part B, section 1.)

A schedule of immunization for each antigen shall be submitted to the national control authority. Every effort should be made to use the minimum dose and number of antigen 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.

When immunization is considered necessary, potential donors should be:

(1) 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 initially by informing small groups of potential donors);

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

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

(4) required to indicate agreement by signing an informed-consent form; and

(5) informed that they are free to withdraw consent at any time.

All vaccines used for the immunization of donors shall be registered or recognized by the national health authority, and 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.

4.8.4 Immunization with human erythrocytes

Erythrocytes

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.

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

The cells from a single donor shall be used to immunize no more than three persons (preferably who have not previously had a blood transfusion) in an initial six-month period, during which monthly determinations of anti-HIV, antibody to hepatitis B core antigen (anti-HBc), HBsAg, and serum alanine aminotransferase should be made in both the donor and the recipients. If, after six 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 the immunization of not more than ten recipients. The group of ten should be followed for at least one year and the absence of disease markers documented before the cell donor is declared “safe” for unrestricted use. As small a number of donors of erythrocytes should be used as possible. Ideally, erythrocytes obtained for immunization purposes should be frozen for at least six months prior to use and the donor should be recalled and retested for anti-HIV, anti-HBc, and HBsAg before the stored cells are used for immunization.

Programmes for producing anti-A and anti-B should use soluble blood-group substances rather than whole erythrocytes.

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

Laboratory tests. Each donation should undergo: (1) a serological test for syphilis, which should be negative; (2) an HBsAg test, which should be negative; (3) a test for serum alanine aminotransferase, which should be within normal limits as established by the national
health authority; (4) a test for anti-HIV, which should be negative; and (5) a test for anti-HBc, which should be negative.

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

Preparation of erythrocyte antigens for immunization

Collection. Antigens should be collected from donors 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 environment in which aliquots are prepared should be monitored to ensure that it is free from bacterial and fungal contamination.

Storage. Antigens should be stored at 5°C ± 3°C or frozen. The length of the frozen storage period permissible will depend in part on the temperature; if the antigens are stored at 5°C ± 3°C, the storage period should not exceed 45 days. The method selected should give more than 70% cell recovery in vivo. If erythrocytes are frozen, they should be washed after storage to remove the cryoprotective agent. If washed before use, they should be used within 2 h of entry into the container for washing; after washing they should be stored at 5°C ± 3°C.

Dating period. Adequate sterility data to support the requested dating period should be submitted by the manufacturer. A test for bacterial and fungal contamination should be made on all blood dispensed in aliquots in an open system (6). The test should also be performed on at least one single-dose vial from each bleed lot that has been stored unfrozen for more than seven days. One sterility test should be made on the eighth day after collection on at least one single-dose vial from each lot of antigen, and an additional test should be made on the last day of the dating period. Cultures for the sterility test should be maintained for at least 14 days, with subculturing on days 3, 4, or 5.

Washing of erythrocytes. Washing is desirable but not required for erythrocytes stored in suspension at 5°C ± 3°C. If the cells are washed, however, sterile pyrogen-free sodium chloride solution should be used and the cells must be used within 2 h of entry into the container for washing. If they are not washed, a single-dose vial of erythrocytes should be stored upside down so that only sedimented
cells are aspirated for immunization and the leukocyte content is reduced. The removal of leukocytes reduces the risk of contamination with HLA antigens.

Record keeping

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

Additional testing of erythrocyte recipients

The following additional testing of erythrocyte recipients is necessary:

1. The recipient should be phenotyped for ABO, Rh, Kell, and Duffy prior to 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(a), S, and s phenotypes is also desirable.

2. Screening for unexpected antibodies by methods that demonstrate coating and haemolytic antibodies should include the antiglobulin method or a procedure of equivalent sensitivity.

Whenever antibody screening tests demonstrate the presence of erythrocyte antibodies other than those deliberately stimulated through immunization by the plasmapheresis centre, the prospective erythrocyte recipients should be asked whether they have ever been pregnant or had a transfusion, tissue graft, or injection of erythrocytes for any reason. This history should form part of the permanent record and should identify the cause of the earlier immunization as clearly as possible. The recipient 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.

Recommended immunization schedules

Because of the risk of infection, it is important that an immunization schedule should be adopted that requires the smallest number of doses of erythrocytes to obtain the maximum response.

Primary immunization. Two injections of erythrocytes, each of the order of 1–2 ml and given at an interval of three months, have been shown to elicit antibody formation within three months of the second injection in approximately 50% of subjects; the result is not
improved by injecting larger amounts or giving more frequent injections.

**Secondary immunization.** It is advantageous to choose as donors of anti-D (anti-Rhₐ) subjects who are already immunized, since useful levels of anti-D are usually attained within a few weeks of reimmunization. In some subjects, 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 5–8 weeks. Eventually, about 70% of immunized subjects produce antibody levels well in excess of 100 IU/ml. Once attained, such levels can be maintained by injections of 0.1–0.5 ml of erythrocytes, given at intervals of 2–9 months, as required. If injections of erythrocytes are discontinued, antibody levels usually fall appreciably within 6–12 months.

The equilibrium constant of the antibody produced increases during continuing immunization.

**Antibody response.** The antibody baseline titre should be established and the antibody response, including both the type and titre, should be monitored monthly.

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

**Risks to recipients**

Recipient of erythrocytes for immunization purposes may run the risk of:

1. Viral hepatitis (B and non-A, non-B) and HIV infection;
2. Other infectious diseases;
3. HLA immunization;
4. The production of unwanted erythrocyte antibodies which may complicate future blood transfusion;
5. A febrile reaction if the antigen dose is too great;
6. The production of antibodies that may interfere with future organ transplantation if it is needed.

**5. Collection of blood**

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

The skin of the donor at the site of venepuncture shall be prepared by a method that has been shown to give reasonable assurance that the blood collected will be sterile. 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 (see Part B, section 3.1).

With apheresis procedures, care shall be taken to ensure that the maximum volume of the donors' erythrocytes is returned to them by intravenous infusion. If the red cells cannot be returned to the donor, no further collection shall be made for eight weeks. Several checks shall be made to ensure that donors receive their own erythrocytes. Donors shall identify their own erythrocytes before reinfusion.

5.1.1 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 involved in plasmapheresis procedures. If any serious adverse reaction occurs, a physician should be called.

5.1.2 Types of adverse reaction

The following types of adverse reaction may occur.

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

Haemolytic transfusion reactions. Such reactions should not occur, 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 in all donors at the centre concerned should be discontinued until reidentification procedures have been completed. Automated plasmapheresis is preferred in some circumstances because it avoids the risk of errors that result in incompatibility.

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. In order to avoid the transmission of infectious organisms to the donor, care should be taken at all stages of plasmapheresis.

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 with a dating period of 24 h or less. Containers shall be uncoloured and transparent and the labelling shall be placed in such a position as to allow visual inspection of the contents. They shall be hermetically sealed by means of suitable closures so that contamination of the contents is prevented. The container material shall not interact with the contents under the prescribed conditions of storage and use, because such interaction may have an adverse effect on the safety or efficacy of the products.

The specifications for containers should be approved by the national control authority.1 Wherever possible, it is desirable to use sterilized integrally attached satellite containers to prepare components in a closed

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system in order to minimize the likelihood of microbial contamination.

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-glucone, citrate-phosphate-glucone, and citrate-phosphate-glucone-adipine (Appendix 2); various amounts of adenine are used in different countries. Solutions of adenine, glucose, and mannitol used for red-cell preservation may be added after removal of the plasma; these solutions require several containers.

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

5.4 Volume of blood

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

5.5 Pilot samples

Pilot samples are blood samples provided with each unit of whole blood or of red blood cells. They shall be collected at the time of donation by the person who collects the whole blood. The containers for pilot samples shall be marked at the collection site before the samples are collected, and the marking used must be identical with that of the 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.

Laboratory samples for use in testing donated blood may be collected in addition to the pilot samples. They should also meet the above requirements.
5.6 Identification of samples

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

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

An example of a protocol that may be useful for such purposes is given in Appendix 1.

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

1. 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 those such as 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, that can be used 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 make good deficits in patients. Although not dealt with further in these requirements, these uses include: (1) stimulation of plasma donors with red blood cells in order to raise antibody levels for the preparation of anti-D (anti-Rh) immunoglobulin (§) and special blood-grouping reagents; and (2) preparation of in vivo diagnostic products, such as radiolabelled fibrinogen for the diagnosis of deep vein thrombosis. It is of the utmost importance that the donors of cells and plasma for such purposes be carefully studied both initially as well as on a continuing basis to minimize the likelihood of the
transmission of infectious agents to recipients. The use of red cells, stored frozen, which have been shown to be free from infectious agents by retesting of the donor six 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 4.8.

2. Summary of minimum general requirements for apheresis

The minimum general requirements for apheresis can be summarized as follows:

1. **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 surveyable and interruptable, and provided with suitable automatic alarms.

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

3. **Disposables:** these must be pyrogen-free, sterile, and bio-compatible (e.g., there must be no activation of enzyme systems).

3. Testing of whole blood

3.1 Sterility

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

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

Each donation of whole blood shall be inspected visually immediately before issue. It shall not be issued if there is any evidence of leakage or suspicion of microbial contamination, such as unusual turbidity, haemolysis, or change of colour.

3.2 Laboratory tests

Laboratory tests shall be made on laboratory samples taken 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, and particularly those used for blood grouping and for the detection of anti-HIV and HBsAg, must be approved by the national control authorities.

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

3.3 Tests for infectious agents

3.3.1 Test for syphilis

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

3.3.2 Test for viral hepatitis

Each unit of blood or plasma collected shall be tested for HBsAg by an approved method and only those giving a negative result shall be used (9). Units giving a positive result shall be so marked, segregated, and disposed of by a method approved by the national control authorities, unless designated for the production of a reagent or experimental vaccine in an area designed and segregated for such production.

The test used should preferably be a highly sensitive one, such as radioimmunoassay or enzyme-linked immunosassay.
With respect to plasma intended for pooling, all donations entering the pool should be tested and found non-reactive for HBsAg. In some countries, small subpools and the final products are tested by highly sensitive methods, but this is considered to be inferior to the testing of single donations.

There may be geographical areas of such high levels of hepatitis B endemicity in donors and immunity in recipients that national control authorities may wish to modify this testing requirement to meet their special needs and conditions.

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

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

3.3.3 Test for anti-HIV

Blood for transfusion and for use in the preparation of components must be tested by a method approved by the national control authority for antibodies to HIV (anti-HIV) when the benefits of such testing outweigh other important factors in providing blood. WHO has published recommendations concerning testing for anti-HIV (4).

All blood from which plasma derivatives are manufactured must be shown by a method approved by the national control authority to be free of serological markers of HIV. Specific exceptions might be considered appropriate by national control authorities, based on the therapeutic benefit and safety of the product.

Human retroviruses other than HIV have been described (e.g., HTLV-I) and more may be identified in the future. For this reason, it is important for the national control authority to reassess testing requirements from time to time in the light of current knowledge and the prevalence of these viruses in different populations, together with the availability of tests for serological markers of infection.

3.4 Red blood cell 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 A1) 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 (Rhₐ) antigen is present, each unit of blood shall be classified according to Rh blood type based on the results of testing for the D (Rhₐ) red cell antigen. The D (Rhₐ) type shall be determined with anti-D (anti-Rhₐ) typing serum.

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

4. Separation of red cells

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 used 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.

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 3.

The following may be prepared for therapeutic purposes:

1. whole blood, plasma-reduced;
2. red cells;
3. red cells suspended in preservative solution;
4. modified red cells:
   a. red cells, leukocyte-poor and red cells, leukocyte-free;
   b. red cells, washed;
   c. red cells, frozen and red cells, deglycerolized.

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 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 containers and the blood container is broached 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 plasma-reduced blood and red cells (but not necessarily for 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 the removal of any component, such samples shall be reattached to the container of plasma-reduced blood or 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. When the final container is filled and if it 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 6 h of the blood donation.

When platelets and coagulation factors are to be prepared, it is 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 whole blood unit, the blood shall be kept at a temperature as close as possible to 20–24 °C for up to 6 h until the platelet-rich plasma has been separated from the red blood cells.

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

Sedimentation is the least expensive method for 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 at the same time preventing haemolysis.

4.2 Plasma-reduced whole blood and red cells

Plasma-reduced whole blood is obtained when sufficient plasma has been withdrawn to yield a product with an erythrocyte volume fraction ("packed cell volume") of approximately 0.6. Red cells are obtained when more plasma is removed, yielding a product with an erythrocyte volume fraction of approximately 0.7–0.9.

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

4.3 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.

When red cells are prepared with very high erythrocyte volume fractions, an expiry date of 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 exceeds 21 days.

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.

The shelf-life of stored blood has been extended to 35 days in some countries 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, or to 42 days by adding a preservative solution, for example 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.

Provided that sterility is maintained, the expiry date of red cells is not influenced by the method of separation used. However, if an open system is used which does not maintain sterility, the expiry date is 24 h after separation and the cells should be used as soon as possible. Red cells should be stored at 5°C ± 3°C and transported with wet ice in insulated boxes at 5°C ± 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 such time as they are used.

4.4 Modified red cells

4.4.1 Red cells, leukocyte-poor and red cells, leukocyte-free

Leukocyte-poor red cells is the name given to a red-cell preparation containing at least 80% of the red cells and less than 25% of the leukocytes of the original whole blood. A preparation of leukocyte-free red cells contains at least 80% of the red cells and less than 2% of the leukocytes of the original whole blood. The number
and type of the residual leukocytes vary with the method of preparation.

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

Leukocyte-poor blood can be prepared by means of filtration, freezing and washing, or by washing alone. It should be prepared as soon as possible after the blood has been collected. Filtration is an efficient method for the elimination of the leukocytes if used within 48 h of blood collection, but the filtration of heparinized blood should take place within 1 h. The red cells should be frozen as described below for frozen red cells.

4.4.2 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°C ± 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, unless the national control authority has specified a longer expiry date. They should be stored at all times at 5°C ± 3°C.

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

4.4.3 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 (−30°C or lower and preferably −65°C or lower) in the presence of a cryoprotective agent. The red cells must be washed to remove the cryoprotective agent prior to use for transfusion. The methods of preparation, storage, thawing, and washing used should be such as to ensure that a 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 storage period of frozen cells is at least three years below −65 °C 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 on the glycerol concentration used. Deglycerolized red cells should be stored at a temperature of 1–6 °C and shipped at 5 °C ± 3 °C.

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

5. Other single-donor or small-pool components

5.1 Plasma

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 give rise to prekallikrein activation. If the plasma thaws and is not intended for transfusion, it is regarded as recovered plasma.

5.1.1 Plasma, fresh-frozen

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

Plasma may be frozen at or below −40 °C or with a combination of solid carbon dioxide and an organic solvent such as ethanol. If the latter 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. Prior to use for infusion, the 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.

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

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.

5.1.2 Plasma, frozen

Frozen plasma is, by definition, separated from whole blood 6 h or more 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. In addition, 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 such 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 the use of closed systems.

In some countries, when frozen plasma is stored at or below -20°C (preferably below -30°C), it is given an expiry date of five years from the date of collection.

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

Plasma may be pooled at any time after collection. To avoid microbial growth in contaminated plasma, recovered plasma should preferably be stored and transported in the frozen state. A preservative should not be added.

5.1.3 Plasma, freeze-dried

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

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

The purposes for which freeze-dried plasma can be used and the expiry date will depend on the source material, storage conditions, and residual moisture of the product.

5.1.4 Plasma, recovered

Recovery 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 container system is not closed, the plasma shall be stored and transported frozen.

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

5.1.5 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 6 h of the collection of the whole blood, and the temperature and time of processing as well as storage shall be consistent with the maintenance of platelet survival and function.

Platelet-rich plasma shall be transfused as soon as possible after collection (and not later than 72 h afterwards, unless stored in containers approved for a longer storage period) in order to achieve the desired haemostatic effect.

5.2 Platelets

Platelets can be separated from whole blood or from buffy coat or platelet-rich plasma, or by means of automated cell separators. Aspirin ingestion less than three days previously precludes a donor from serving as the sole source of platelets.

The whole blood from which platelets or platelet-rich plasma are derived shall be maintained at 22°C ± 2°C until the platelets have
been separated. The separation shall be performed preferably within 6 h after the collection of 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 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.

A pH of 6.5 or higher shall be maintained throughout storage of platelets. The volume of original plasma to be used for resuspension of the platelets will depend on the storage temperature. Platelets stored at room temperature shall be resuspended in not less than 50 ml of plasma.

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

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

Platelet products with high platelet counts and stored at 22 ± 2°C may require up to 70 ml or more of plasma in order to maintain the pH above 6.5 throughout the storage period. This period may be as long as seven days with containers made of certain special plastics, but it is prudent to restrict platelet storage to five days because of the risk of proliferation of potential bacterial contaminants.

The product should be ABO typed and, in countries where D (Rh) is polymorphic, D (Rh) typed; it may also be desirable to determine 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 the labelling of the final container are the same as those given in section 7. In addition to the customary data, the label shall bear the following: (1) the recommended storage temperature; (2) the statement that, when stored at 22 ± 2°C, the platelets should be gently agitated throughout storage to obtain maximum haemostatic effectiveness; and (3) a statement to the effect that the contents shall be used as soon as possible, and preferably less than 4 h after the containers have been broached for pooling.
5.2.1 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 authority for a longer period.

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

Single-donor platelet concentrates may be pooled under aseptic conditions prior to issue. Such small pools should be used as soon as possible, and not later than 4 h after preparation if stored at room temperature.

5.3 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.

Methods of processing leukocytes shall comply with the requirements and recommendations given in section 4.1 for the separation of red cells.

The label of the final container shall bear, in addition to the 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 broached for pooling. The temperature of storage and transport shall be 5°C ± 3°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 of high relative molecular mass.

The product should be ABO typed and, in countries where D (RhD) is polymorphic, D (RhD) typed; it may also be desirable to determine the HLA type.
The large number of red cells present in products prepared by some methods makes compatibility testing before transfusion necessary.

5.3.1 Expiry date

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

5.4 Cryoprecipitated factor VIII

Cryoprecipitated factor VIII is a preparation of factor VIII obtained from single units or small pools of plasma from whole blood 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.

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

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 6.5).

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

1. In order to prevent coagulation, venepuncture should be performed in such a way that tissue damage is minimal. The blood should flow freely without interruption, and be mixed thoroughly with anticoagulant initially and during collection.

2. Microbial contamination should be avoided during the separation of the plasma. This is accomplished by using multiple-plastic-bag closed systems, or laminar-flow cabinets when an open procedure is used.

3. The recovery of factor VIII depends on the interval between venepuncture and freezing of the plasma, and on the temperature at which the plasma is held as well as 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.

4. The plasma may be frozen in a freezer at –20°C or preferably at a lower temperature, or in a mixture of solid carbon dioxide and an organic solvent such as ethanol. Contamination of the plasma...
by the solvent or leaching of substances from the container into
the plasma should be avoided. Rapid freezing by the use of ultra-
low temperatures has been shown to increase the yield of factor
VIII.

(5) 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 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.

5.4.1 Expiry date

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

6. Control of single-donor and small-pool products

6.1 General

Single-donor and small-pool products shall comply with any
specifications established by the national control authority. Cellular
blood components and some plasma components may deteriorate
during 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 assure 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 in liquid
storage.
The methods employed for component separation should be checked before they are used and thereafter at regular intervals so as to ensure that the final product is of the required quality. The characteristics assessed should include yield, purity, in vivo recovery, biological half-life, functional behaviour, and sterility. The intervals at which such checks are to be carried out should be determined by the national control authorities.

Immediately before issue for transfusion or for other purposes, the 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.

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. Blood 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.

6.2 Red cells, single-donor plasma, and leukocytes

When red cells and leukocytes are obtained from units of whole blood, such units shall comply with the requirements of Part A, section 5 and Part B, section 3. Single-donor plasma shall be obtained by plasmapheresis or from units of whole blood that comply with the requirements in the above-mentioned sections.

6.3 Platelets

Platelets shall be obtained by cytopheresis or from units of whole blood that comply with the requirements of Part A, section 5 and Part B, section 3.

Randomly selected units at the end of their shelf-life shall be tested on a regular basis. They shall be shown to have: (1) plasma volumes appropriate to the storage temperature (see section 5); and (2) a pH between 6.0 and 7.4.

The number of units to be tested and the platelet number required shall be specified by the national control authorities.
6.4 Leukocytes

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

6.5 Cryoprecipitated factor VIII

Cryoprecipitated factor VIII 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 3.

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 authorities. 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 preparation may be tested by measuring its ability to correct the prolonged activated partial thromboplastin time of haemophilia A plasma or by another method and by comparing the activity of the cryoprecipitate with that of an appropriate standard.

When cryoprecipitated factor VIII is produced from fresh-frozen plasma (frozen within 6 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, an average yield of 400 International Units of factor VIII per litre of starting plasma is obtained. The average yield of freeze-dried cryoprecipitate is then at least 300 International Units of factor VIII per litre of starting plasma. Whether this yield can be obtained will depend on local technical possibilities. In some countries, the yields will be much lower, and the national control authority must decide as to the yield that is acceptable.

7. 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:

(1) the proper name of the product;
(2) the unique number or symbol identifying the donor(s);
(3) the expiry date;
(4) any special storage conditions or handling precautions that are necessary;
(5) a reference to a package insert containing instructions for use, warnings, and precautions;
(6) the name and address of the blood donor centre and, where applicable, the manufacturer and distributor.

The results of red blood cell grouping shall be stated on the label of whole blood, red cells, plasma products, platelets, and leukocytes but not necessarily on that of cryoprecipitated factor VIII.

8. Placental source material

Placental blood may serve as the source material for certain plasma fractions, but should be used only for products such as pasteurized products and immunoglobulins prepared by the Cohn fractionation process that have not been associated with the transmission of infectious agents. If another fractionation method is used, the manufacturer must provide data validating the ability of the method to remove or inactivate infectious agents.

Where it is impracticable to test individual source material for the presence of disease markers, the national control authority may authorize the use of such source material after the manufacturer has demonstrated the efficacy of the viral inactivation processes.

It is important that national control authorities assume the responsibility for the method of testing, the production method, and the use of the products obtained from this source material. In countries where placentas are harvested, the incentives for the collection of placental material should be such as to ensure that newborn infants are not adversely affected.

PART C. REQUIREMENTS FOR THE MANUFACTURE OF BLOOD PRODUCTS

1. Buildings

The buildings used for the fractionation of plasma shall be of such size, construction, and location as to facilitate their proper
operation, cleaning, and maintenance in accordance with general rules of hygiene. They shall comply with the revised Requirements for Biological Substances No.1 (General Requirements for Manufacturing Establishments and Control Laboratories) (5, p. 13) and in addition provide adequate space, lighting, and ventilation for the activities listed below.

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

1.1 Storage of whole blood and its components

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

1.2 Separation of cells and fractionation of components

Cells shall be separated and components fractionated in a building isolated from those in which the manufacture or processing of non-human proteins or microbiological materials, such as vaccines, is carried out and separate from the animal house.

In some countries, cell constituents are separated in an area separate from that in which components are fractionated.

1.3 Supply and recovery of ancillary materials

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

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

1.4 Freeze-drying and filling

Final containers shall be filled under sterile conditions in a separate area.

A separate area and separate equipment should be used for the freeze-drying of bulk product and of final product.
1.5 Packaging, labelling, and storage

Separate facilities shall be used for the 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 prior to dispatch.

1.6 Keeping of records

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

1.7 Quality control

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

Those parts of the quality-control laboratories that are hazardous to production should be separated from the production area.

1.8 Disposal of infective material

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

The disposal of these materials should comply with local legislation.

2. Equipment

Equipment used for the collection, processing, storage, and distribution of source materials and plasma fractions shall comply with the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (5, p. 14).

Particular attention shall be paid to the following:
(1) The validation of the reliability, maintenance, monitoring, and recording of the operation of continuously operating equipment and the provision of stand-by equipment.

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

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

(3) 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.

It should preferably be possible to inspect visually all surfaces with which plasma and solvents come into contact and to dismantle stainless steel tubing.

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.

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

3. Provision of support services

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

3.1 Water supply

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

The two most commonly used types of water are pyrogen-free distilled water (10, p. 50) and pyrogen-free deionized water, each of which should be maintained at 80 °C. Water preparation and delivery systems should be tested at regular intervals for pyrogenicity and conductance. The water system should be a continuously circulating one and should have no dead ends. Water for injection is generally used for the preparation of final products (10, p. 50).
3.2 Steam supply

An adequate supply of steam shall be provided for the cleaning of the equipment and for the operation of the apparatus used to sterilize the equipment and containers. The steam shall be clean and shall be derived from water of the quality of water for injection.

3.3 Other support facilities

The other support facilities required are as follows:

(1) A supply of electrical and thermal energy.
(2) A means of refrigeration for the following purposes:
   (a) the storage of various source materials and fractions;
   (b) keeping the various fractionation areas at the correct temperature;
   (c) keeping the process equipment at the correct temperature;
   (d) the storage of final products under test;
   (e) the storage of final products awaiting dispatch.

(3) A system of ventilation providing the following two grades of filtered air:
   (a) air filtered to remove particles 5 μm or greater in diameter, which shall be supplied to the entire work area; and
   (b) 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 (11).

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 serviced by engineering staff skilled in the maintenance and repair of such equipment should be available.

4. Personnel

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

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

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

Personnel known to be carriers of specific pathogenic organisms (such as those of salmonellosis, tuberculosis, and viral hepatitis) shall be excluded from the production area.

Personnel should be medically examined at regular intervals. Vaccination against hepatitis B is recommended for employees routinely exposed to blood or blood products.

5. Fractionation of source materials

The general conditions for carrying out the fractionation procedure for preparing prophylactic or therapeutic plasma protein fractions from source material shall comply with good manufacturing practices (II) 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.

Only those fractionation procedures shall be used that give a good yield of products meeting the quality requirements of international or national authorities. They shall be carried out in such a manner that the risk of microbiological contamination 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 fractionation procedures used shall not significantly denature the proteins.

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 such procedures to the extent that they are unacceptable for clinical use.

When possible, methods shall be used that exclude or inactivate pathogenic organisms, and particularly hepatitis viruses and human retroviruses, from the final products intended for clinical use. All manufacturers shall validate the ability of their manufacturing processes to inactivate and/or remove contaminating viruses such as hepatitis B virus and human retroviruses.

There appears to be increasing evidence that certain manufacturing procedures, coupled with strict control to ensure that the final product complies with precise specifications, may result in a product free from infectivity in relation to HIV, hepatitis B, and non-A non-B hepatitis. Nevertheless, additional studies and clinical experience will be required to establish optimal inactivation procedures.

For coagulation products, viral inactivation methods, such as dry heat treatment, wet heat treatment, the use of steam under pressure or of organic solvents, or solvent-detergent treatment, have been successful in reducing or eliminating the risk of HIV transmission. Under certain conditions, some of the procedures appear to reduce or eliminate hepatitis B or non-A non-B hepatitis infectivity.

Recent advances in donor selection and in the viral inactivation procedures used in manufacturing plasma coagulation concentrates have significantly improved the safety of these products. However, seroconversion for HIV antibody has still occasionally been reported.
Fibrinogen prepared from plasma pools continues to carry a risk of infection unless it is treated to remove or inactivate viruses. For this reason, 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 cryo-precipitate from one unit of plasma (200 ml) frozen within 6 h of collection from the donor.

Placenta-derived source materials must be handled in separate equipment in rooms separate from those in which other fractionation processes are carried out until the stage in processing when the infectious risk associated with the product is not greater than that associated with a plasma-derived product.

The operating manual for the fractionation procedure shall specify the times of sampling of 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, pyrogenicity, and toxicity in accordance with The International Pharmacopoeia (10) or national pharmacopoeia.

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

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

6. Storage and heat treatment of blood products

At all stages of the manufacturing process, the source materials and resulting fractions shall be stored at temperatures and under conditions shown to prevent further contamination and the growth of microorganisms, protect the identity and the integrity of the proteins, and 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.
6.1 Heat treatment of albumin and plasma protein fraction

Albumin and plasma protein fraction in solution shall be heated in the final container to 60°C ± 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.

7. In-process control

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

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.

7.1 Quality 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 testing before release for prekallikrein 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 desirable for highly purified albumin products (purity greater than 95%).
Since albumin solutions are often administered to patients with cardiovascular disorders, a careful watch should be kept for hypotensive reactions associated with vasoactive substances. This is particularly important for products prepared from placental source material and for plasma protein fraction.

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, the possibility has not been eliminated 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 control should be capable of detecting contamination with bacteria and moulds. Similarly, care should be taken to ensure that all equipment and reagents used in the manufacturing process are scrupulously clean and free from toxic materials. The process used for this purpose shall be validated.

7.2 Stability of albumin solutions

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

(1) the quality of the starting plasma;
(2) the quality of the fractionation, particularly with respect to the degree of purity achieved and the number of reprecipitation and reheating procedures involved; and
(3) the storage conditions with respect not only to the 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:

(1) The addition of stabilizing chemicals is necessary. Commonly used products are sodium octanoate and sodium acetyltryptophanate.
(2) Albumin prepared from aged liquid or dried plasma is less stable than albumin made from fresh-frozen plasma.

(3) Reprocessing steps, such as reprecipitation and reheating, may reduce the stability of albumin solutions.

(4) Albumin solutions, on long-term storage, are more stable at 5°C ± 3°C than at 32–35°C. Storage above 30°C should be avoided.

These findings have been taken into consideration by national control authorities in determining the expiry dates.

8. Record keeping

Records shall be kept of the performance of all steps in the manufacture, quality control, and distribution of blood products and related substances (II).

These records shall:

—be original (not a transcription), indelible, legible, and dated;
—be made at the same time as each operation and test is 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 interrelationships 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 dating periods and legal requirements;
—indicate that processing and testing were carried out in accordance with procedures established and approved by the designated responsible authority;
—if necessary, allow a prompt and complete recall of any particular lot; and
—show the lot numbers of the materials used for specified lots of products.
PART D. REQUIREMENTS FOR THE CONTROL OF PLASMA FRACTIONS

1. Introduction

A number of requirements common to albumin, plasma protein fraction, immunoglobulin G, and coagulation-factor concentrates are considered in sections 3–11. However, for clarity, it has proved convenient to bring together certain specific requirements for coagulation-factor concentrates into a separate section (section 12).

2. Terminology

Bulk purified material: powder or liquid material prepared by the fractionation of pooled source material.
Final bulk: a sterile solution prepared from bulk purified material and used to fill the final containers. It must be given the corresponding batch number.

In some countries, the final bulk is distributed into containers through a sterilizing filter.

Filling lot (final lot): a collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling and (where appropriate) drying or other further processing such as heat treatment. A filling lot must therefore have been filled and (where appropriate) dried in one working session. If the total final bulk is not distributed into containers in one session, it must bear a sub-batch number.

3. Control of bulk purified material

3.1 Storage

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

3.2 Tests on bulk purified material

Tests on the purified bulk powder or solution shall be made if the manufacturer sends the material to another institution for further
processing. Samples for these tests shall be taken under conditions that do not impair the quality of the bulk purified material. Tests shall be carried out on a specially dissolved sample processed to a stage equivalent to the final product, after sterile filtration. The tests shall be those listed in section 4.3 to 4.7 inclusive.

4. Control of final bulk solution

4.1 Preparation

The final bulk solution shall be prepared from bulk purified powder or by the dilution of concentrates by a method approved by the national control authority. The final bulk solution shall meet all of the requirements described in sections 4.2–4.5 inclusive. In addition, the final bulk solution of normal immunoglobulin shall be made from material from a large number (at least 1000) of donors.

In the case of normal immunoglobulins, a large number of donors is necessary in order to obtain adequate amounts of the various desired antibodies in the final product.
In the case of specific immunoglobulins, whether intended for intravenous or intramuscular injection, the number of donors represented is less important because the particular antibody present will be tested.

4.2 Preservatives and stabilizers

No preservative shall be added to the albumin, plasma protein fraction, intravenous immunoglobulin (normal or specific), or coagulation-factor concentrates either during fractionation or at the stage of the final bulk solution.

To prevent protein denaturation, stabilizers shall be added. Such substances shall have been shown to the satisfaction of the national control authority not to have any deleterious effect on the final product in the amounts present and to cause no untoward reactions in humans. Antibiotics shall not be used as preservatives or for any other purpose in the fractionation of plasma.

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

The albumin concentration in the final bulk albumin solutions shall be between 35 and 265 g/l. Not less than 95% of the proteins present shall be albumin as determined by a suitable electrophoretic method. The test shall be carried out on a sample after heating (see Part C, section 6.1).

The protein concentration in the final bulk solution 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 fractions shall be gamma globulin.

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

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

4.4 Hydrogen ion concentration

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

In some countries a different range of pH-values is permitted.

4.5 Sterility

Where feasible, the final bulk material shall be tested for sterility. Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (6, p. 48) shall apply.
4.6 Sodium content

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

4.7 Potassium content

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

5. Filling and containers

The requirements concerning filling and containers given in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (5, p. 16) 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 within 24 h of the start of filling to a temperature of 60°C ± 0.5°C and shall be maintained at that temperature for 10 h in order to inactivate any infectious agents that may be present. In order to prevent protein denaturation, a stabilizer shall be added to the albumin solution prior to heating (see section 4.2).

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

6. Control tests on final product

The tests given below shall be performed on representative samples of the product in the final container from every final filling of each lot of product. If the product is processed further after filling, e.g., by freeze-drying or heating, the tests shall be performed on samples from each filling of each drying chamber or heating vessel.

6.1 Identity test

An identity test shall be performed on at least one labelled container from each filling lot to verify that the preparation is of human origin. The test shall be one approved by the national control authority.
For immunoglobulin, albumin, and plasma protein fraction, additional tests shall be made to determine that the protein is predominantly immunoglobulin G or albumin as appropriate. The tests mentioned in section 4.3 shall be used.

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

6.2 Determination of protein concentration and purity

The protein concentration and purity of each filling lot shall be within the limits prescribed in section 4.3.

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.

6.3 Tests on immunoglobulins

Each lot of immunoglobulin (whether normal or specific) shall have a minimum concentration of antibody to HBsAg of 1 IU/g of immunoglobulin.

Tests shall be conducted on each 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 G 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 G 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 G monomer and dimer, most will consist of monomer. If a minimum level of monomer per se is to be established, however, the methods shall specify the time and temperature at which samples must be incubated before analysis.

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:
A. A test for hypotensive activity.

An appropriate test for hypotensive activity is that for prekallikrein activator content.

B. A test for anticomplementary activity.

Several methods are available for the determination of anticomplementary activity, and the test method used as well as the maximum level permitted shall be approved by the national control authority.

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

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

6.4 Potency tests for normal immunoglobulins

A 16% solution of normal immunoglobulin shall be prepared from pooled material by a method that has been shown to be capable of concentrating by a factor of ten from source material at least two different antibodies, one viral and one bacterial, for which an international standard or reference preparation is available\(^1\) (e.g., antibodies against poliomyelitis virus, measles virus, streptolysin O, diphtheria toxin, tetanus toxin, and staphylococcal \(\alpha\) toxin).

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

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

Since preparations of normal immunoglobulins produced in different countries can be expected to differ in their content of various antibodies, depending on the antigenic stimulation to which the general population has been subjected (either by natural infection or by 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

\(^1\) WHO publishes a list of such preparations (revised from time to time and most recently in 1986) under the title Biological substances, international standards and reference reagents.
at least the minimum antibody levels required by the national control authority.

6.5 Potency tests for specific immunoglobulins

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

For varicella zoster immunoglobulin, at least 100 IU/ml of varicella zoster antibody, as measured by a comparative enzyme-linked immunosorbent assay or by a method shown to be equivalent.

For anti-D (anti-RhD) 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 stated potency.

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

6.6 Tests for sterility

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) (6, p. 48) shall apply. For albumin and plasma protein fraction, samples for sterility testing shall be taken from final containers selected at random after heating at 60 °C for 10 h.

In one country, the sterility test is carried out at least ten 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.

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6.7 Tests for freedom from abnormal toxicity

Each filling lot shall be tested for freedom from abnormal toxicity 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. For coagulation-factor concentrates except fibrinogen, the test dose should not exceed 500 IU of the coagulation factor per kg of body weight of the test animal.

6.8 Tests for freedom from pyrogenicity

Each filling lot shall be tested for pyrogenicity by the intravenous injection of the test dose into three or more rabbits that have not previously received blood products. In general, the dose shall be at least equivalent proportionately, on a rabbit body-weight basis, to the maximum single human dose recommended, but not more than 10 ml/kg 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, for albumin at concentrations of 35 g/l and 50 g/l and for plasma protein fraction, 10 ml/kg of body weight, and for immunoglobulins, 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.

6.9 Determination of 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: (1) drying over phosphorus pentoxide for at least 24 h at a pressure not exceeding 2.7 Pa (0.02 mm Hg); and (2) the Karl Fischer method.
The acceptable moisture content shall be determined by the national control authority.

6.10 Determination of hydrogen ion concentration

The final product, reconstituted if necessary, diluted to give a concentration of 10 g/l protein with 0.15 mol/l sodium chloride, shall, when measured at a temperature of 20°C ± 2°C, have a pH of 6.9 ± 0.5 for albumin and immunoglobulin and 7.0 ± 0.3 for plasma protein fraction.

In some countries, a different range of pH-values is permitted.

6.11 Tests for stability

For immunoglobulin solutions, the test shall be performed by heating an adequate sample at 37°C for four weeks. No gelation or flocculation shall occur.

Alternatively (or in addition), molecular size distribution and/or enzyme assays may be used when shown to predict stability reliably, and when approved by the national control authority.

For solutions of albumin and plasma protein fraction which have been heated for 10 h at 60°C, the test shall be performed by heating an adequate sample at 57°C for 50 h. The solutions shall remain visually unchanged when compared with a control sample that has been heated for only 10 h at 60°C.

6.12 Determination of 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 405 nm.

6.13 Inspection of filled containers

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

The normal colour of albumin solutions may range from colourless to yellow or green to brown. When turbidity indicates the possibility of microbial contamination, tests should be done to isolate and identify the microorganisms.

7. Records

The requirements given in Part A, section 6, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (5, p. 17) shall apply.

8. Samples

The requirements given in Part A, section 7, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (5, p. 18) shall apply.

9. Labelling

The requirements for labelling given in Part A, section 8, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (5, p. 18) shall apply, except that the recommended human dose need not be specified on the label on the container, and with the addition of the information given below.

For albumin and plasma protein fraction, the label on the container shall 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”;
— a warning that the contents must be used within 4 h of breaking the seal;

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— the sodium and potassium concentrations.

For immunoglobulins, the label on the container shall state:

— the type of source material;
— the protein concentration;
— the concentration of preservative, if any (in the case of specific immunoglobulin, the content of specific antibody expressed in international units or equivalent national units);
— "For intramuscular use only" (if the immunoglobulins are not specially prepared for intravenous use).

For freeze-dried preparations, the label on the container shall, in addition, show the name and volume of the reconstituting liquid to be added.

In addition, the label on the package or the package insert shall state:

— the approximate concentration of electrolytes and excipients and the approximate osmolality;
— the buffering capacity when the pH of the diluted product is lower than that indicated in section 4.4;
— the concentration of preservative;
— the recommended dose for each particular disease or condition;
— the fact that the preparation satisfies the requirements of this document, including those for donor selection and for the testing of the source material for disease markers or, if derived from placental source material, that the national control authority has approved the efficacy of the fractionation, purification, and viral inactivation processes.

10. Distribution and shipping

The requirements given in Part A, section 9, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (5, p. 18) shall apply.

11. Storage and expiry date

The requirements given in Part A, section 10, of the revised Requirements for Biological Substances No. 1 (General Require-
ments for Manufacturing Establishments and Control Laboratories) (5, p. 19) shall apply.

Final containers of albumin solution shall have a shelf-life of not more than three years provided that they are stored at a temperature not higher than 30°C, and of not more than five years provided that they are stored at 5°C ± 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 shelf-life of not more than three years provided that they are stored at a temperature not higher than 30°C, and of not more than five years provided that they are stored at 5°C ± 3°C.

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

Liquid immunoglobulin shall be stored 5°C ± 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.

12. Control of preparations of coagulation-factor concentrates

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 ten plasma donations is covered in Part B, section 6.5.

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 ten donations may be purified.

Source material for factor VIII preparations shall meet the criteria for donor selection and testing for disease markers as specified in Parts A and B for whole blood donors and preferably be
plasma frozen within 6 h of collection from the donor (or from frozen cryoprecipitate). Such material shall be kept frozen at such a temperature that the activity of the factor VIII is maintained. Processing of the freshly thawed pooled cryoprecipitate material shall be completed within a few hours in order to preserve factor VIII activity.

12.1 Tests on final containers common to other blood fractions

The following tests common to other blood products shall apply to the final containers of factor VIII and factor IX concentrates and fibrinogen:

(1) sterility (see section 6.6);
(2) freedom from abnormal toxicity (see section 6.7).

12.2 Special tests for coagulation-factor concentrates

12.2.1 Solubility and clarity

Factor VIII preparations and fibrinogen 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.

12.2.2 Protein content

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

12.2.3 Tests for additives

Tests to determine the concentration of additives (such as heparin, polyethylene glycol, citrate, sodium, and glycine) used during production shall be carried out if required by the national control authority.
12.2.4 *Determination of 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: (1) drying over phosphorus pentoxide for 24 hours at a pressure not exceeding 2.7 Pa (0.02 mm Hg); and (2) the Karl Fischer method.

12.2.5 *Determination of hydrogen ion concentration*

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

In some countries, different pH-values are approved.

12.3 *Special tests applicable to factor VIII concentrates*

12.3.1 *Potency test*

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.

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

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 stated potency.

12.3.2 *Test for alloagglutinins*

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

Although it is not possible to be specific about the tests for alloagglutinins or to specify an upper limit of titre in the coagulation-factor preparations, a test for alloagglutinins should nevertheless be made and the test results mentioned on the package insert (instructions for use).
12.4 Special tests applicable to factor IX concentrates

12.4.1 Potency test

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.

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

12.4.2 Test for the 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 concentration of fibrinogen solution is 3–10 g/l.

12.5 Special 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 shall be clottable by thrombin.
12.6 Records

See section 7.

12.7 Samples

See section 8.

12.8 Labelling

The requirements given in section 9 shall apply, with the addition of the following information:

— the content of the factor expressed in international units;
— the amount of protein in the container;
— the volume of diluent required for reconstitution;
— a reference to a package insert giving the instructions for use, warnings about the possible transmission of infectious agents, and precautions.

12.9 Distribution and shipping

See section 10.

12.10 Storage and expiry date

The requirements given in Part A, section 10 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (5, p. 19) shall apply.

Final containers of freeze-dried preparations of factor VIII and factor IX shall have a shelf-life of not more than two years provided that they are stored at 5°C ± 3°C.

Final containers of fibrinogen shall have a shelf-life of not more than five years provided that they are stored at 5°C ± 3°C.

For fibrinogen, the shelf-life is usually five years at 5°C ± 3°C.

Other storage conditions and expiry dates may be approved by the national control authority provided that they are consistent with the data on the stability of the products.
PART E. NATIONAL CONTROL REQUIREMENTS

1. General

The general requirements for control laboratories in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (5, p. 19) 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, C, and D.

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

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

2. Release and certification

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

A statement signed by the appropriate official of the national control authority shall be provided at the request of the manufacturing establishment and shall certify whether or not the product in question meets all national requirements as well as those of Parts A, B, C, and D, whichever is relevant, of the present requirements. The certificate shall further 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.
AUTHORS

The first draft of the revised Requirements for the Collection, Processing, and Quality Control of Blood, Blood Components, and Plasma Derivatives was prepared in June 1987 by a WHO consultant, Mrs P.A. Hoppe, Deputy Director, Division of Blood and Blood Products, Office of Biologics Research and Review, Food and Drug Administration, Bethesda, MD, USA.

The second draft of the revised Requirements was formulated by the following participants at the WHO Consultations on Requirements for Blood and Blood Products held in Geneva and Amsterdam:

*Geneva, 9–11 December 1987*

Dr J. Fischer, Behringwerke AG, Marburg, Federal Republic of Germany  
Dr J. Furesz, Bureau of Biologics, Drugs Directorate, Ottawa, Canada  
Dr A.E. Gonzaga, Chief, Brazilian Red Cross Blood Program, Rio de Janeiro, Brazil  
Dr C. Guthrie, Commonwealth Serum Laboratories, Parkville, Victoria, Australia  
Dr B. Habibi, National Blood Transfusion Centre, Paris, France  
Professor W. Hennessen, Bern, Switzerland  
Mrs P.A. Hoppe, Deputy Director, Division of Blood and Blood Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA  
Dr H. Krijnen, Director, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands  
Dr D. Magrath, National Institute for Biological Standards and Control, Potters Bar, Herts., England  
Dr H. Mirchamsy, Associate Director, Razi State Institute of Sera and Vaccines, Teheran, Islamic Republic of Iran  
Dr R. Netter, Director-General, National Health Laboratory, Ministry of Social Affairs and Employment, Paris, France  
Dr J.C. Petricciani, Deputy PHS AIDS Coordinator, Office of the Assistant Secretary for Health, Department of Health and Human Services, Washington, DC, USA  
Dr L. Pushkar, Chief, Central Laboratory for State Control and Standardization of Blood Derivatives, Central Research Institute of Haematology and Blood Transfusion, Moscow, USSR  
Dr S.N. Saxena, Director, Central Research Institute, Kasauli, India  
Professor G. Swaniker, Department of Basic Medical Science, Faculty of Medicine, University of Papua New Guinea, Boroko, Papua New Guinea  
Dr D.P. Thomas, Head, Division of Haematology, National Institute for Biological Standards and Control, Potters Bar, Herts., England  
Dr Xiang Jian-zhi, Chairman, Expert Committee on Science and Technology, Shanghai Institute of Biological Products, Shanghai (Western), China  
Dr D. Zewdie, Deputy Director, National Research Institute of Health, Addis Ababa, Ethiopia

*WHO secretariat*

Dr V. Grachev, Biologicals, World Health Organization, Geneva, Switzerland  
Dr P. Sizarot, Acting Chief, Biologicals, World Health Organization, Geneva, Switzerland

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Amsterdam, 14-18 March 1988

Professor W. van Aken, Medical Director, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands
Dr D. Boucher, Chief, Blood Products Division, Bureau of Biologics, Drugs Directorate, Virus Laboratory, Tunney's Pasture, Ottawa, Canada
Dr Cheng Yagin, National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Health, Temple of Heaven, Beijing, China
Dr J.S. Finlayson, Division of Blood and Blood Products, Office of Biologies Research and Review, Food and Drug Administration, Bethesda, MD, USA
Dr S. Funakoshi, Director, Development, Research and Development Division, The Green Cross Corporation, Osaka, Japan
Professor H. Heininger, Director, Central Laboratory, Blood Transfusion Service, Red Cross Foundation, Bern, Switzerland
Dr J.A. Hooper, Director Immunoglobulin, Scientific Affairs, Hyland Therapeutic Division, Travenol Laboratories, Glendale, CA, USA
Dr K. Komuro, Director, Department of Blood Products, National Institute of Health, Tokyo, Japan
Dr R. Kotitschke, Biowest Pharma GmbH, Frankfurt, Federal Republic of Germany
Dr H.W. Krijnen, Director, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands
Dr D. Magrath, Chief, Biologics, World Health Organization, Geneva, Switzerland
Dr D. Menache-Aronson, Medical Operations, American Red Cross National Headquarters, Washington, DC, USA
Dr M. Mikaelson, Director Research and Development, Plasma Products, Kabi Vitrum AB, Stockholm, Sweden
Dr P. Schiff, Executive Director, Blood Products Division, Commonwealth Serum Laboratories, Parkville, Victoria, Australia
Dr F. Seiler, Behringwerke AG, Marburg, Federal Republic of Germany
Dr D.F. Tankersley, Division of Blood and Blood Products, Office of Biologies Research and Review, Food and Drug Administration, Bethesda, MD, USA
Dr D.P. Thomas, Head, Division of Haematology, National Institute for Biological Standards and Control, Potters Bar, Herts., England
Dr A.D.B. Webster, Division of Immunological Medicine, Clinical Research Centre, Harrow, England
Professor R.J. Wedgwood, Department of Paediatrics RD-20, University of Medicine, Seattle, WA, USA

The third draft of the revised Requirements was formulated in January 1988 by the following WHO Headquarters staff and consultants, taking into consideration the second draft and the need for the Requirements to be consistent with other WHO requirements:

Dr V. Grachev, Scientist, Biologicals, World Health Organization, Geneva, Switzerland
Mrs P.A. Hoppe, Deputy Director, Division of Blood and Blood Products, Office of Biologies Research and Review, Food and Drug Administration, Bethesda, MD, USA (Consultant)
Dr H. Krijnen, Director, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands (Consultant)
ACKNOWLEDGEMENTS

Acknowledgements are due to the following experts for their comments and advice on the third draft of these Requirements: Dr N. Charriott, Director, Swiss Serum and Vaccine Institute, Bern, Switzerland; Ms M. Cone, Vice President for Scientific Affairs, International Federation of Pharmaceutical Manufacturers Associations (IFPMA), Geneva, Switzerland; Dr W.N. Gibbs, Chief, Health Laboratory Technology, World Health Organization, Geneva, Switzerland; Dr A. Ganzago, Chief, Brazilian Red Cross Blood Program, Rio de Janeiro, Brazil; Dr C. Guthrie, Operation Director, Commonwealth Serum Laboratory, Parkville Victoria, Australia; Dr B. Habibi, National Blood Transfusion Centre, Paris, France; Dr K. Hannegren, National Bacteriological Laboratory, Stockholm, Sweden; Professor S. R. Hollán, National Institute of Haematology and Blood Transfusion, Budapest, Hungary; Dr R.M. Lequin, Manager, Evaluation and Training Group, Organon Teknika, Turnhout, Belgium; Dr T. Matuhasi, Professor Emeritus, University of Tokyo, Okinaka Memorial Institute for Medical Research, Tokyo, Japan; Dr D.E. Smith, Medical Director, The Blood Center, New Orleans, Louisiana, LA, USA; Dr G.R.E. Swaniker, Faculty of Medicine, Department of Basic Medical Sciences, University of Papua New Guinea, Boroko, Papua New Guinea; Dr I. Di Tommaso, Tuscan Institute for Serotherapy and Vaccine Production (SCLAPO), Siena, Italy; Dr Xiang Jun-zhi, Head, Division of Science and Technology, Shanghai Institute of Biological Products, Ministry of Public Health, China.

REFERENCES

Appendix 1

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

If applicable, results of tests for antibody to hepatitis B core antigen and for alanine aminotransferase

4. Special information
   (a) Anticoagulant used
   (b) Whether collected for special purposes (e.g., antibody-specific)
   (c) Precautions in use of 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 published by WHO?

Name and signature of responsible person       Date
Appendix 2

COMPOSITION OF COMMONLY USED ANTICOAGULANTS FOR BLOOD COLLECTION

Acid–citrate–glucose solution

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisodium citrate (dihydrate)</td>
<td>22.0 g</td>
</tr>
<tr>
<td>Citric acid (monohydrate)</td>
<td>8.0 g</td>
</tr>
<tr>
<td>Glucose (monohydrate)</td>
<td>24.5 g</td>
</tr>
<tr>
<td>Water for injection to</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

15 ml of this solution are used for 100 ml of blood.

Citrate–phosphate–glucose solution

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisodium citrate (dihydrate)</td>
<td>26.3 g</td>
</tr>
<tr>
<td>Citric acid (monohydrate)</td>
<td>3.27 g</td>
</tr>
<tr>
<td>Sodium dihydrogen phosphate (monohydrate)</td>
<td>2.22 g</td>
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<tr>
<td>Glucose (monohydrate)</td>
<td>25.5 g</td>
</tr>
<tr>
<td>Water for injection to</td>
<td>1000 ml</td>
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</table>

14 ml of this solution are used for 100 ml of blood.

Citrate–phosphate–glucose–adenine

<table>
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<th>Ingredient</th>
<th>Amount</th>
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<tr>
<td>Trisodium citrate (dihydrate)</td>
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<tr>
<td>Citric acid (monohydrate)</td>
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<tr>
<td>Glucose (monohydrate)</td>
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<td>Sodium dihydrogen phosphate (monohydrate)</td>
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<td>Adenine</td>
<td>0.275 g</td>
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<td>Water for injection to</td>
<td>1000 ml</td>
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</table>

14 ml of this solution are used for 100 ml of blood.

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Annex 5

BIOLOGICAL SUBSTANCES: INTERNATIONAL STANDARDS AND REFERENCE REAGENTS

A list of international biological standards, international biological reference preparations, and international biological reference reagents is issued as a separate publication.1 Copies may be obtained from appointed sales agents for WHO publications or from: World Health Organization, Distribution and Sales Service, 1211 Geneva 27, Switzerland.

The Expert Committee made the following changes to the previous list.

Additions

### Allergens

<table>
<thead>
<tr>
<th>Substance</th>
<th>Standard</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bermuda grass (Cynodon dactylon) pollen extract</td>
<td>100 000 RAST inhibition units/ampoule</td>
<td>First International Reference Reagent 1988</td>
</tr>
</tbody>
</table>

(This substance is held and distributed by the Paul Ehrlich Institute, Frankfurt, Federal Republic of Germany.)

### Toxoids

<table>
<thead>
<tr>
<th>Substance</th>
<th>Standard</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Diphtheria toxoid for flocculation tests</td>
<td>900 Lf units/ampoule</td>
<td>First International Reference Reagent 1988</td>
</tr>
<tr>
<td>Tetanus toxoid for flocculation tests</td>
<td>1000 Lf units/ampoule</td>
<td>First International Reference Reagent 1988</td>
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</tbody>
</table>

(These substances are held and distributed by the State Serum Institute, Copenhagen, Denmark.)

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Blood products

Factor VIII and antigen:
- 0.91 IU/ampoule
- 0.60 IU/ampoule clotting activity:
- 0.91 IU/ampoule antigen:
- 0.84 IU/ampoule

Von Willebrand factor in plasma
- ristocetin cofactor activity:
  - 0.84 IU/ampoule

Endocrinological and related substances

Follicle stimulating hormone, urinary, human, and Luteinizing hormone, urinary, human
- 54 IU/ampoule
- 46 IU/ampoule

Insulin-like growth factor I for immunoassay
- 3.1 μg/ampoule

Luteinizing hormone, pituitary, human
- 35 IU/ampoule

Prolactin, human
- 0.053 IU/ampoule

(These substances are held and distributed by the National Institute for Biological Standards and Control, Potters Bar, Herts., EN6 3QG, England.)

Discontinued

Digitalis
- 76.0 mg/IU

(This substance was held and distributed by the National Institute for Biological Standards and Control, Potters Bar, Herts., EN6 3QG, England.)
Annex 6

REQUIREMENTS FOR BIOLOGICAL SUBSTANCES
AND OTHER SETS OF RECOMMENDATIONS

The specification of requirements to be fulfilled by preparations of biological substances is necessary in order to ensure that these products are safe, reliable, and potent prophylactic or therapeutic agents. International recommendations on requirements are intended to facilitate the exchange of biological substances between different countries and to provide guidance to workers responsible for the production of these substances as well as to others who may have to decide upon appropriate methods of assay and control.

Recommended requirements and sets of recommendations concerned with biological substances are formulated by international groups of experts and are published in the Technical Report Series of the World Health Organization, as listed here.

<table>
<thead>
<tr>
<th>No.</th>
<th>Year of publication</th>
<th>Requirements for Biological Substances:</th>
</tr>
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<tbody>
<tr>
<td>178</td>
<td>1959</td>
<td>* 1. General Requirements for Manufacturing Establishments and Control Laboratories</td>
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<tr>
<td></td>
<td></td>
<td>* 2. Requirements for Poliomyelitis Vaccine (Inactivated)</td>
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<tr>
<td>179</td>
<td>1959</td>
<td>* 3. Requirements for Yellow Fever Vaccine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>* 4. Requirements for Cholera Vaccine</td>
</tr>
<tr>
<td>180</td>
<td>1959</td>
<td>* 5. Requirements for Smallpox Vaccine</td>
</tr>
<tr>
<td>200</td>
<td>1960</td>
<td>* 6. General Requirements for the Sterility of Biological Substances</td>
</tr>
<tr>
<td>237</td>
<td>1962</td>
<td>* 7. Requirements for Poliomyelitis Vaccine (Oral)</td>
</tr>
<tr>
<td>274</td>
<td>1964</td>
<td>* 8. Requirements for Pertussis Vaccine</td>
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<td>* 9. Requirements for Procaine Benzylpenicillin in Oil with Aluminium Monostearate</td>
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</tbody>
</table>

* Replaced by revised Requirements.
293 1964 WHO Expert Committee on Biological Standardization:
* 10. Requirements for Diphtheria Toxoid and Tetanus Toxoid

322 1966 WHO Expert Group:
Requirements for Biological Substances (Revised 1965):
* 1. General Requirements for Manufacturing Establishments and Control Laboratories
* 2. Requirements for Poliomyelitis Vaccine (Inactivated)
* 5. Requirements for Smallpox Vaccine
* 7. Requirements for Poliomyelitis Vaccine (Oral)

329 1966 WHO Expert Committee on Biological Standardization:
* 11. Requirements for Dried BCG Vaccine
* 12. Requirements for Measles Vaccine (Live) and Measles Vaccine (Inactivated)

361 1967 WHO Expert Committee on Biological Standardization:
* 9. Requirements for Procaine Benzylpenicillin in Oil with Aluminium Monostearate (Revisions adopted 1966)
13. Requirements for Anthrax Spore Vaccine (Live—for Veterinary Use).
† 14. Requirements for Human Immunoglobulin
15. Requirements for Typhoid Vaccine

384 1968 WHO Expert Committee on Biological Standardization:
* 16. Requirements for Tuberculins
* 17. Requirements for Inactivated Influenza Vaccine

413 1969 WHO Expert Committee on Biological Standardization:
† 4. Requirements for Cholera Vaccine (Revised 1968)
18. Requirements for Immune Sera of Animal Origin

444 1970 WHO Expert Committee on Biological Standardization:
* 19. Requirements for Rinderpest Cell Culture Vaccine (Live) and Rinderpest Vaccine (Live)
† 20. Requirements for Brucella abortus Strain 19 Vaccine (Live—for Veterinary Use)
* Development of a National Control Laboratory for Biological Substances
(A guide to the provision of technical facilities)

463 1971 WHO Expert Committee on Biological Standardization:
21. Requirements for Snake Antivenins

486 1972 WHO Expert Committee on Biological Standardization:
* 7. Requirements for Poliomyelitis Vaccine (Oral) (Revised 1971)

530 1973 WHO Expert Committee on Biological Standardization:
* 4. Requirements for Cholera Vaccine (Revised 1968) (Addendum 1973)

* Replaced by revised Requirements.
† Replaced by revised Requirements No. 27.
‡ Refer also to subsequent Addendum.
6. General Requirements for the Sterility of Biological Substances
   (Revised 1973)
* 17. Requirements for Inactivated Influenza Vaccine (Addendum 1973)
* 22. Requirements for Rabies Vaccine for Human Use

565 1975 WHO Expert Committee on Biological Standardization:
   Recommendations for the Assessment of Binding-Assay Systems
   (Including Immunoassay and Receptor Assay Systems) for Human
   Hormones and their Binding Proteins (A guide to the formulation
   of requirements for reagents and assay kits for the above assays and
   notes on cytochemical bioassay systems)
   Development of national assay services for hormones and other
   substances in community health care

594 1976 WHO Expert Committee on Biological Standardization:
   † 3. Requirements for Yellow Fever Vaccine (Revised 1975)
   20. Requirements for Brucella abortus Strain 19 Vaccine (Live—for
   Veterinary Use) (Specification of tests used in the Requirements)
   (Addendum 1975)
   † 23. Requirements for Meningococcal Polysaccharide Vaccine

610 1977 WHO Expert Committee on Biological Standardization:
   Report of a WHO Working Group on the Standardization of Human
   Blood Products and Related Substances
   † 23. Requirements for Meningococcal Polysaccharide Vaccine
   (Addendum 1976)
   † 24. Requirements for Rubella Vaccine (Live)
   25. Requirements for Brucella melitensis Strain Rev. 1 Vaccine (Live
   —for Veterinary Use)
   † 26. Requirements for Antibiotic Susceptibility Tests. I. Agar Diffusion
   Tests using Antibiotic Susceptibility Discs

626 1978 WHO Expert Committee on Biological Standardization:
* 17. Requirements for Inactivated Influenza Vaccine (Addendum 1977)
† 23. Requirements for Meningococcal Polysaccharide Vaccine
   (Addendum 1977, incorporating Addendum 1976)
* 27. Requirements for the Collection, Processing, and Quality Control
   of Human Blood and Blood Products
* Guidelines for the Preparation and Establishment of Reference
   Materials for Biological Substances

638 1979 WHO Expert Committee on Biological Standardization:
   † 8 & 10. Requirements for Diphtheria Toxoid, Pertussis Vaccine,
   Tetanus Toxoid, and Combined Vaccines (Revised 1978)
* 11. Requirements for Dried BCG Vaccine (Revised 1978)
17. Requirements for Influenza Vaccine (Inactivated) (Revised 1978)
28. Requirements for Influenza Vaccine (Live)

* Replaced by revised Requirements.
† Refer also to subsequent Addendum.
WHO Expert Committee on Biological Standardization:

1. Requirements for Poliomyelitis Vaccine (Oral) (Addendum 1980)
2. Requirements for Diphtheria Toxoid, Pertussis Vaccine, Tetanus Toxoid, and Combined Vaccines  (Addendum 1980)
3. Requirements for Rabies Vaccine for Human Use (Revised 1980)
4. Requirements for Meningococcal Polysaccharide Vaccine (Addendum 1980)
5. Requirements for Rabies Vaccine for Veterinary Use
6. Requirements for Hepatitis B Vaccine
7. Requirements for Antibiotic Susceptibility Tests (Suggested changes 1980)
8. Requirements for Rubella Vaccine (Live) (Addendum 1980)
9. Requirements for Thromboplastins and Plasma used to Control Oral Anticoagulant Therapy
10. Guidelines for Quality Assessment of Antitumour Antibiotics
11. The National Control of Vaccines and Sera
12. Requirements for Immunoassay Kits

† Procedure for Approval by WHO of Yellow Fever Vaccines in Connection with the Issue of International Vaccination Certificates

WHO Expert Committee on Biological Standardization:

1. Requirements for Poliomyelitis Vaccines (Inactivated) (Revised 1981)
2. Requirements for Diphtheria Toxoid, Pertussis Vaccine, Tetanus Toxoid, and Combined Vaccines (Addendum 1981)
3. Requirements for Measles Vaccines (Live) (Addendum 1981)
4. Requirements for Antimicrobial Susceptibility Tests (Revised 1981)
5. Requirements for Rift Valley Fever Vaccine
6. A Review of Tests on Virus Vaccines
7. The Standardization of Interferons
8. Requirements for Louse-Borne Human Typhus Vaccine (Live)

† Requires also to subsequent Addendum.

Requirements for Typhoid Vaccine (Live Attenuated, Ty 21a, Oral)
WHO Expert Committee on Biological Standardization:

* Replaced by revised Requirements.
† Refer also to subsequent Addendum.
‡ Replaced by Requirements No. 45.
26. Requirements for Antimicrobial Susceptibility Tests
   1. Agar Diffusion Tests Using Antimicrobial Susceptibility Disks
      (Addendum 1987)
   31. Requirements for Hepatitis B Vaccine Prepared from Plasma
      (Revised 1987)
   41. Requirements for Human Interferons Made by Recombinant DNA
       Techniques
   43. Requirements for Japanese Encephalitis Vaccine (Inactivated) for
       Human Use
   Laboratories approved by WHO for the production of yellow fever
   vaccines (revision of appendix to the Procedure for Approval by
   WHO of Yellow Fever Vaccines in Connexion with the Issue of
   International Vaccination Certificates)
   * Modification for Lyophilized BCG Vaccine of the Procedure for
     Evaluating the Acceptability in Principle of Vaccines Proposed to
     United Nations Agencies for Use in Immunization Programmes
   * Model Certificate for the Release of BCG Vaccines Acquired by United
     Nations Agencies

Standardization of Interferons: report of a WHO informal consultation

786 1989    WHO Expert Committee on Biological Standardization:
Procedure for Evaluating the Acceptability in Principle of Vaccines
Proposed to United Nations Agencies for Use in Immunization
Programmes (Revised 1988)

27. Requirements for the Collection, Processing, and Quality Control
    of Blood, Blood Components, and Plasma Derivatives (Revised
    1988)

42. Requirements for Human Interferons Prepared from
    Lymphoblastoid Cells

45. Requirements for Hepatitis B Vaccines Made by Recombinant
    DNA Techniques

* Replaced by the Procedure for Evaluating the Acceptability in Principle of
  Vaccines Proposed to United Nations Agencies for Use in Immunization Programmes
  (Revised 1988).

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