WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Forty-fifth Report

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WHO Expert Committee on Biological Standardization
Geneva, 11–18 October 1994

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Introduction

The WHO Expert Committee on Biological Standardization met in Geneva from 11 to 18 October 1994. The meeting was opened on behalf of the Director-General by Dr F.S. Antezana, Assistant Director-General.

Dr Antezana emphasized the contribution of WHO’s biological standardization programme to health programmes in Member States through the provision of biological reference materials and the establishment of minimum requirements for biological substances. WHO was able to provide only limited financial support to the programme, but that reflected the current financial situation rather than a lack of commitment on the part of the Organization. He thanked the International Laboratories for Biological Standards and their governments for the support that they provided to the biological standardization programme. He also drew attention to the rapid and continuing developments, particularly in the fields of recombinant products, cytokines and interferons, and informed the Committee of two WHO informal consultations that had been held recently on the last two topics. He noted that the work of the Committee continued to reflect technical progress in these fields.

General

Development and distribution of International Biological Standards and Reference Reagents

The Committee was informed of the distribution of international reference materials by the International Laboratories for Biological Standards during 1993 (Table 1). It emphasized that the programme was essential for the harmonization of reference materials and requirements internationally, and to facilitate the distribution and use of biological products.

The Committee was concerned to learn of the reduction that WHO had been obliged to make in the budgets of the International Laboratories, and emphasized the continuing and increasing importance of their work. It recognized with gratitude the efforts that the International Laboratories were making, with their own resources and the support of their governments, to maintain their contribution to the biological standardization programme. Nevertheless, the growing demands made on these laboratories and requests that the programme should be expanded increased concern about funding. Possibilities that should be explored included obtaining funding from other sources for specific project areas and recovering some of the costs through more realistic charges for handling and processing requests for reference materials.

The Committee therefore requested the Secretariat to review the priorities and needs for additional standards, to obtain more detailed
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information about the costs incurred by the International Laboratories, and to present a plan of action setting out possible funding options to the next meeting of the Committee.

The Committee was informed that the National Institute for Biological Standards and Control, Potters Bar, had been accredited by the United Kingdom National Measurement Accreditation Service as complying with European Standard EN 45001 for testing laboratories, thus becoming the first European testing laboratory to be awarded accreditation for the control testing of biologicals. Accreditation is an objective recognition of the technical competence of the laboratory for the tests concerned, and the Committee encouraged the other International Laboratories for Biological Standards to consider seeking accreditation for their activities relating to biological reference materials.

Suitability of reference materials for use in different assay systems

In the course of establishing several reference materials, as mentioned elsewhere in this report, the Committee recognized that a reference material might not be suitable for use in assay systems other than those employed during the collaborative study for its establishment. This problem had been recognized previously, and some reference materials had been designated as suitable only for bioassay or only for immunoassay. However, the Committee believed that the development of a wide range of recombinant products had introduced a new factor for consideration. The full spectrum of actions of many of these substances had often not been established at the time that a candidate international reference material was being examined. A reference material for one substance may or may not be appropriate as a calibrant for the assay of an analogue of closely related but not identical structure, as was observed with the interferons and epidermal growth factors. The Committee recommended that the International Laboratories should include, in the memoranda accompanying reference materials, information on the assays in which the reference material had been examined in the relevant collaborative study and a warning that users should validate the applicability of the material in other assay systems.

Regulation and licensing of biological products

The Committee expressed concern about the increasing possibility that products of inadequate quality and potency may reach the market because the requirements of good manufacturing practices and quality assurance are not universally met and, in some countries, there is insufficient control through the regulation and inspection of manufacturers. It urged Member States to recognize the importance of this issue and to address it in an effort to prevent disasters due to faulty products and their consequences for health care systems. The Committee also suggested that the availability of reference materials and
requirements should be more widely publicized, e.g. in WHO drug information, and drawn to the attention of national health and economic authorities.

The Committee noted the document on the regulation and licensing of biological products (BS/94.1759)¹ prepared at a meeting organized by the WHO Regional Office for Europe and held in Vienna in December 1993, and agreed that it should be annexed to this report (Annex 1). It also noted the reliance placed in the document on guidelines previously published by WHO (e.g. Guidelines for National Authorities on Quality Assurance for Biological Products, WHO Technical Report Series, No. 822, 1992, Annex 2, and Good Manufacturing Practices for Biological Products, WHO Technical Report Series, No. 822, 1992, Annex 1). For the convenience of users, and because the contents of these guidelines and related documents are relevant to all biological products, the Committee recommended that consideration be given to issuing a number of relevant documents in the form of a compendium, which might include an introduction drawing attention to incidents that have occurred through failure to implement appropriate controls.

Guidelines for the inspection of manufacturers of biological products

The Committee took note of a document providing guidelines for the inspection of manufacturers of biological products that had been prepared by the Children’s Vaccine Initiative (CVI): Task Force on Quality Control. Although it was concerned primarily with vaccines, much of it is also applicable to other biological and pharmaceutical products. The Committee therefore requested the Secretariat to investigate the possibility of making the document more widely available and suggested that it might be included in the compendium proposed above.

Need for expert advice

The Committee drew attention to the valuable contribution to its work made by the informal consultative groups on cytokines and interferons mentioned in this report, as well as by the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Such groups of experts in particular fields were able to help the Committee in identifying the need for international reference materials, determining priorities, arranging for the preparation of candidate materials, and organizing collaborative studies.

The Committee recognized the rapid increase in areas of biological science in which a need for standardization exists, especially in

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.
diagnostics. It also recognized the difficulty of maintaining a balance of priority between different areas, and the dilemma that this presented to the International Laboratories. The Committee therefore urged the Secretariat to explore ways in which appropriate groups of experts might be consulted informally and, if funding could be obtained, ways in which meetings might be arranged. It would welcome advice from such groups to facilitate its work. The Committee recommended that the establishment of a consultative group to make proposals for standardization and priorities for action in the field of diagnostics should be considered.

**Cytokines**

The Committee was informed that a WHO Informal Consultation on Cytokine Standards and Reference Materials had been held in April 1994 following a recommendation made at its forty-third meeting (WHO Technical Report Series, No. 840, 1994, p. 1). Among the topics discussed were the distribution of existing standards, the need for new reagents and the priority to be given to their establishment, and the status of studies in progress.

The Informal Consultation recommended that a group should be established to monitor developments and establish priorities for developing additional International Standards or Reference Reagents in the field of cytokines. It proposed that interferons should be included within the remit of the group so that developments in this area could also be considered on a regular basis. The Committee endorsed these recommendations and further proposed that any such consultative group should reflect both scientific and regulatory views.

The Committee recommended that the order of priority for the allocation of resources for developing reference materials for cytokines and interferons should be as follows: (i) products approved for marketing; (ii) those undergoing clinical trial; (iii) those nearing clinical evaluation. Although the availability of mouse cytokines had been mentioned at the Consultation, the Committee considered that these had a role in research rather than as therapeutic agents, and reference materials for them should therefore have a lower priority.

The Committee drew attention to the recommendations in the “Guidelines for the preparation, characterization and establishment of International and other Standards and Reference Reagents for biological substances” (WHO Technical Report Series, No. 800, 1990, Annex 4) regarding the number of laboratories participating in collaborative studies. In view of the concern expressed elsewhere in this report about priorities and limited funding, the Committee considered that the resources involved in very large collaborative studies (see interleukin-3 and interleukin-4, p. 18) might be better deployed in smaller studies on more products.
Interferons

The Committee was informed about a WHO Informal Consultation on Standardization of Interferons held in April 1994. It was also informed that, despite a difference of opinion between the participants, a substantial majority had supported the group’s specific recommendations. In view of the apparent problems associated with the assignment of relative potencies to interferons, the Committee requested the National Institute of Biological Standards and Control, Potters Bar, with the cooperation of the Center for Biologicals Evaluation and Research, Rockville, and the proposed consultative group on cytokine reference materials to review the situation and make recommendations.

The Committee noted that the Consultation had recommended that the potency of all new preparations of human interferon alfa should be determined relative to the International Reference Preparation of Interferon, Human Leukocyte, coded 69/19, which is a preparation of natural human interferon. The Committee recommended that this should also apply to existing preparations of human interferon alfa. The Committee also recommended that any distributions of the first International Standard for Interferon, Human, Recombinant, α2(α2b), should be accompanied by a statement that this preparation is not suitable for relative potency assays. It further recommended that the potency of all new preparations of human interferon beta should be determined relative to the second International Standard for Interferon, Human, Fibroblast, β.

The Committee was informed of a proposal by the Consultation that the first International Standard for Interferon, Human (HuIFN-γ), which is of lymphocyte origin, should not be replaced when stocks are exhausted because of the availability of recombinant human interferon gamma (see p. 18).

Acellular pertussis vaccines

The Committee was informed of progress in clinical trials of acellular pertussis vaccines that would be completed during 1995. It recognized the importance of the availability of suitable reference materials in connection with the licensing of such vaccines if the trials are successful. The Committee was therefore pleased that the Secretariat intended to hold a meeting of a consultative group in spring 1995 to review the availability of, and need for, reference materials for acellular pertussis vaccines and to discuss possible requirements for these new preparations.

Priority setting

The Committee considered that criteria should be established and publicized for determining priorities for international reference materials that might be required. It therefore requested the Secretariat to consult widely and prepare a paper on this issue for its consideration.
Publicizing the work of the Committee

Noting that a certain period of time was inevitably required for publication of its reports by WHO, the Committee requested that summary information on the decisions taken at its meetings should be made available to interested parties as rapidly as possible and that such information be widely disseminated. The timely dissemination of information on the Committee’s activities could be expected to enhance not only the ability of all users of reference materials and requirements to do their work but also the Committee’s ability to perform its task.

WHO periodicals, professional publications and society newsletters might be useful in ensuring the rapid dissemination of information on the Committee’s activities. It therefore requested the Secretariat to explore mechanisms for achieving this goal, while recognizing that the final full report would be published by WHO.

Requirements for biological substances¹

Requirements for hepatitis A vaccine (inactivated)

The Committee noted a revised draft of proposed Requirements for Hepatitis A Vaccine (Inactivated) (BS/94.1757) prepared by the participants at a WHO consultation in 1993. It was informed that a revised document had been circulated to members of WHO’s Expert Advisory Panel on Biological Standardization, and a number of comments had been received. After making some modifications, the Committee adopted the revised text as Requirements for Hepatitis A Vaccine (Inactivated), and agreed that it should be annexed to its report (Annex 2).

Requirements for hepatitis B vaccine prepared from plasma

The Committee noted a revised draft of proposed Requirements for Hepatitis B Vaccine Prepared from Plasma (BS/93.1719 Rev.1) prepared by the Secretariat. This document had been revised following extensive discussion at the Committee’s forty-fourth meeting (WHO Technical Report Series, No. 848, 1994, p. 8). A major change had been a reduction in the reliance placed on testing in chimpanzees. The revised document had been circulated to members of WHO’s Expert Advisory Panel on Biological Standardization and a number of comments received. After making some modifications, the Committee adopted the revised text as Requirements for Hepatitis B Vaccine Prepared from Plasma and agreed that it should be annexed to its report (Annex 3).

¹ For a summary of all the requirements for biological substances and other sets of recommendations, see Annex 5.
Requirements for yellow fever vaccine

The Committee noted a revised draft of proposed Requirements for Yellow Fever Vaccine (BS/94.1758) prepared jointly by a consultant and the Secretariat. The revised document had been circulated to members of WHO’s Expert Advisory Panel on Biological Standardization and a number of comments received.

Because of the extensive changes proposed, the Committee requested the Secretariat to circulate a further revision of the draft Requirements to experts in the field for comment. It particularly requested that advice and information should be sought about monkey safety tests currently in use.

International reference materials

Antibiotics

Amphotericin B, vancomycin and spiramycin

The Committee noted that stocks of the International Standards for Amphotericin B and Vancomycin and the International Reference Preparation of Spiramycin were being depleted (BS/94.1766). Demand for the first of these was rising because of the development of new dosage forms of the product, and for the second because generic sources of vancomycin had appeared. The Committee authorized the National Institute for Biological Standards and Control, Potters Bar, to obtain suitable materials to serve as candidate replacement preparations, and arrange collaborative studies. These preparations should be suitable for both microbiological and physicochemical assays.

Gentamicin

The Committee was informed that, in accordance with the request made at its forty-second meeting (WHO Technical Report Series, No. 822, 1992, p. 6), a preparation of gentamicin had been obtained by the National Institute for Biological Standards and Control, Potters Bar, and filled into ampoules. This preparation appeared to be stable. A collaborative study had been performed and the results analysed.

Since stocks of the current International Reference Preparation of Gentamycin (International Nonproprietary Name: gentamicin) would probably be exhausted before its next meeting, the Committee agreed that, pending receipt of the full report of the collaborative study, the candidate replacement could be distributed if the potency was agreed by the participants in the study.
Antibodies

Anti-toxoplasma serum

The Committee noted that the proposed replacement preparation (TOXM 1–85) for the second International Standard for Anti-Toxoplasma Serum, Human, referred to in its forty-third report (WHO Technical Report Series, No. 840, 1994, p. 5) had been submitted to a collaborative study involving 11 laboratories in 6 countries (BS/94.1761). On the basis of the results and in agreement with the participants, the Committee established the preparation coded TOXM 1–85 as the third International Standard for Anti-Toxoplasma Serum, Human, with an assigned potency of 1000 International Units of Anti-Toxoplasma Serum, Human, per ampoule. This value is for total antibodies.

The Committee was informed of the need for a reference material prepared from acute, early-phase serum (predominantly IgM and low-titre, low-avidity IgG antibodies) and one from convalescent serum (high-titre, high-avidity IgG antibodies) for the calibration of diagnostic kits. It was also informed that, since TOXM 1–85 was an early serum, whereas the two preceding International Standards had been convalescent sera, there was some inconsistency in the use of TOXM 1–85 as a replacement preparation for the second International Standard, even for the definition of total antibodies. This inconsistency should be rectified when a new international reference material had been prepared from convalescent serum and two different reference materials, one prepared from acute, early-phase serum and one from convalescent serum, had been established.

Anti-rubella serum and anti-rubella immunoglobulin

The Committee noted that, following recognition of the need for a replacement for the second International Reference Preparation of Anti-Rubella Serum, which had been prepared from normal human immunoglobulin (WHO Technical Report Series, No. 848, 1994, p. 10), a new preparation of normal immunoglobulin had been obtained (BS/94.1762). It was informed that this preparation would be proposed as a replacement preparation for the second International Reference Preparation of Anti-Rubella Serum, and that a limited collaborative study would be arranged.

The Committee further noted that there was a need for a reference material prepared from acute, early-phase serum (predominantly IgM and low-titre, low-avidity IgG antibodies) and one from convalescent serum (high-titre, high-avidity IgG antibodies) for the calibration of diagnostic kits (BS/94.1762). It requested the Statens Serum Institut, Copenhagen, to proceed on this basis in collaboration with the National Institute for Biological Standards and Control, Potters Bar.
The Committee considered that the nomenclature of these preparations should be carefully reviewed to ensure that there was no confusion between them.

**Anti-borrelia serum**

The Committee noted the steady rise in the incidence of Lyme borreliosis in many parts of the world and the increased demand for diagnostic testing (BS/94.1763). It also noted that, because of the number of diagnostic kits available, there was a need for reference materials to permit the direct comparison of results obtained in different laboratories by different methods. The Committee requested the Statens Serum-institut, Copenhagen, to assess the need for both a reference material prepared from acute, early-phase serum (predominantly IgM and low-titre, low-avidity IgG antibodies) and one from convalescent serum (high-titre, high-avidity IgG antibodies), and to obtain suitable materials and organize collaborative studies.

**Anti-streptolysin O**

The Committee noted that a manufacturer had offered a quantity of concentrated human serum that might serve as a replacement for the first International Standard for Anti-Streptolysin O, Human (BS/94.1764). It also noted that previous attempts to obtain such serum had proved fruitless despite a recognized need (WHO Technical Report Series, No. 673, 1982, p. 20). The Committee was informed that the serum would be filled into ampoules and freeze-dried at the National Institute for Biological Standards and Control, Potters Bar, and that a collaborative study would be organized by the Statens Seruminsitut, Copenhagen.

**Anti-parovirus B19 serum**

The Committee noted the need for greater standardization in the testing of anti-parovirus B19 antibodies for diagnostic purposes (BS/94.1765). It also noted that the National Institute for Biological Standards and Control, Potters Bar, in collaboration with the Central Public Health Laboratory, London, had obtained a candidate reference serum containing antibodies to parovirus B19 and that a collaborative study was in progress.

The Committee requested the National Institute for Biological Standards and Control, Potters Bar, to assess the need for both a reference material prepared from acute, early-phase serum (predominantly IgM and low-titre, low-avidity IgG antibodies) and one from convalescent serum (high-titre, high-avidity IgG antibodies).

**Anti-mumps serum**

The Committee was informed that, in accordance with the request made at its forty-fourth meeting (WHO Technical Report Series, No. 848,
1994, p. 11), the Statens Serum Institut, Copenhagen, had obtained an acute, early-phase anti-mumps serum (predominantly IgM and low-titre, low-avidity IgG antibodies) that might serve as a candidate reference material. The Committee was also informed that collection of convalescent serum (high-avidity IgG antibodies) to serve as a candidate reference material was in progress and that collaborative studies would be arranged.

**Anti-cytomegalovirus immunoglobulin**

The Committee was informed that five candidate preparations for a reference material for cytomegalovirus immunoglobulin had now been obtained by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, and that two collaborative studies using enzyme-linked immunosorbent assay (ELISA) and other methods with kits from different sources were under way. Because the results showed some variability, further consultation with the participants was necessary. In view of the urgent need for a reference material, the Committee agreed that, as an interim measure, a selected preparation could be distributed by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, with a unitage agreed by the participants in the collaborative studies.

**Antibodies to human interferon alfa and beta**

The Committee noted the report on a collaborative study of preparations of antibodies to human interferon alfa and beta performed in 14 laboratories in 7 countries (BS/94.1783). On the basis of the results, it established one of the preparations studied, coded G037-501-572, as the first International Reference Reagent for Anti-Interferon Alfa Serum and assigned a content of 8000 neutralizing units to the contents of each ampoule. The Committee also established the preparation coded G038-501-572 as the first International Reference Reagent for Anti-Interferon Beta Serum and assigned a content of 1500 neutralizing units to the contents of each ampoule. It recommended that guidelines on the appropriate assay to be used should be distributed with the preparations since their suitability in all assay systems had not been established.

**Thyroid-stimulating antibody**

The Committee noted the draft report on the collaborative study on the proposed International Standard for Thyroid-stimulating Antibody (BS/94.1769 and Add. 1). It was informed that, when the report was circulated to the participants, some problems had been identified. The Committee requested the National Institute for Biological Standards and Control, Potters Bar, to investigate the situation.
Antigens and related substances

Hepatitis A vaccine (inactivated)

The Committee noted the report on the collaborative study on candidate reference materials for hepatitis A vaccine (inactivated) performed by 9 laboratories in 6 countries (BS/94.1775). It was informed that the preparation selected on the basis of the results of this study had now lost potency for reasons that were not yet clear. The Committee therefore requested the National Institute for Biological Standards and Control, Potters Bar, to investigate the situation and, if necessary, to obtain a further candidate material and arrange a collaborative study.

Measles vaccine (live)

The Committee noted the results of the collaborative study performed in 14 laboratories in 9 countries in parallel with a similar study organized under the auspices of the European Pharmacopoeia Commission (BS/94.1771). It accepted a proposal that the results obtained by the two types of assay system used in the collaborative study (WHO Technical Report Series, No. 848, 1994, p. 12) should be combined.

The Committee established the preparation coded 92/648 as the second International Reference Reagent for Measles Vaccine (Live) and assigned a content of $4.3 \log_{10}$ (equivalent to 20,000) infectious units of measles virus to the contents of each vial. The value assigned to the European Pharmacopoeia Biological Reference Preparation was consistent with this.

The stability of the preparation would continue to be monitored, although the stability of the first International Reference Reagent, also in vials, had proved to be satisfactory. In addition, stability data on comparable vaccines over 5 years are available.

Mumps vaccine (live)

The Committee noted the results of the collaborative study performed in 14 laboratories in 9 countries, in parallel with a similar study organized under the auspices of the European Pharmacopoeia Commission (BS/94.1772). The Committee accepted a proposal that the results obtained by the two types of assay system used in the collaborative study (WHO Technical Report Series, No. 848, 1994, p. 12) should be combined.

On the basis of the results obtained, the Committee established the preparation coded 90/534 as the first International Reference Reagent for Mumps Vaccine (Live) and assigned a content of $4.6 \log_{10}$ (equivalent to 40,000) infectious units of mumps virus to the contents of each ampoule. The value assigned to the European Pharmacopoeia Biological Reference Preparation was consistent with this. The Committee noted that, while the International Reference Reagent was suitable for the assay of the
Urabe and Jeryl Lynn strains of mumps virus, it may not be suitable for the assay of Rubini strain, and further studies are in progress. Stability data on comparable vaccines over 5 years are available.

**Rubella vaccine (live)**

The Committee noted the results of the collaborative study performed in 14 laboratories in 9 countries in parallel with a similar study organized under the auspices of the European Pharmacopoeia Commission (BS/94.1773). It accepted a proposal that the results obtained by the two types of assay system used in the collaborative study (WHO Technical Report Series, No. 848, 1994, p. 12) should be combined.

The results obtained in the two studies were comparable. The Committee established the preparation coded 91/688 as the first International Reference Reagent for Rubella Vaccine (Live) and assigned a content of 3.9 log_{10} (equivalent to 8000) infectious units of rubella virus to the contents of each ampoule. The Committee noted that the International Reference Reagent was suitable for the assay of the RA-27 strain of rubella but might not be suitable for the assay of the Takeda strain.

The Committee was informed that the European Pharmacopoeia Biological Reference Preparation was not the same as the International Reference Reagent but that both were of the same strain. Stability data on comparable vaccines over 5 years are available.

**Pertussis vaccine (whole-cell)**

The Committee noted that a candidate replacement (94/532) for the second International Standard for Pertussis Vaccine, which is a whole-cell material, had been filled into ampoules and freeze-dried by the National Institute for Biological Standards and Control, Potters Bar (BS/94.1760). It was informed that approximately 3100 ampoules of this preparation had been received at the Statens Seruminstitut, Copenhagen, and that a collaborative study would be planned in cooperation with the National Institute for Biological Standards and Control, Potters Bar.

**Polioyelitis vaccine (inactivated)**

The Committee noted the outcome of the collaborative study of candidate reference materials for inactivated polioyelitis vaccine using *in vitro* methods (BS/94.1777 and Corr. 1) and the need for further work. It also noted the results of a reconciliation of the data from the WHO collaborative study and a comparable study of a candidate European Pharmacopoeia Biological Reference Preparation of the same material (BS/94.1779) and a proposal that common potencies should be assigned to both the candidate WHO and the European reference materials (BS/94.1780 and Add. 1). The Committee also noted that no *in vivo* method could be recommended at present for the determination of potency (BS/94.1778). Although there was some correlation between the
results of *in vitro* and *in vivo* assays, more work was required. The Committee established the preparation coded 91/574 as the first International Reference Reagent for Poliomyelitis Vaccine (Inactivated) and assigned the following potencies to the contents of each ampoule for both *in vivo* and *in vitro* assay:

- 430 D-antigen units per ml for type-1 antigen;
- 95 D-antigen units per ml for type-2 antigen;
- 285 D-antigen units per ml for type-3 antigen.

Because the results of the study showed that the correlation between immunogenicity and antigenicity is not necessarily predictable, the Committee did not assign a content in “potency units” to the preparation.

The Committee considered that there might no longer be a need for the International Reference Preparation of Poliomyelitis Vaccine (Inactivated), which was established in 1962, and requested the Secretariat to obtain information with a view to discontinuing this preparation.

**Prostate-specific antigen**

The Committee noted that steps had been taken by the United States National Committee on Clinical Laboratory Standards to prepare a reference material for prostate-specific antigen, which is widely measured as a tumour marker (BS/94.1791), and was informed that similar action was under consideration in Europe. It agreed that there may be a need for an International Standard and requested the National Institute for Biological Standards and Control, Potters Bar, to monitor the situation.

**Blood products and related substances**

**Antithrombin, plasma**

The Committee noted the report on a collaborative study performed by 20 laboratories in 11 countries of a candidate replacement material for the first International Reference Preparation of Antithrombin III (BS/94.1785). It established the preparation of pooled human plasma studied, in ampoules coded 93/768, as the second International Standard for Antithrombin, Plasma, with an assigned potency of 0.85 International Units of Antithrombin to the contents of each ampoule. The Committee was informed that an accelerated degradation study had confirmed the stability of the preparation and agreed that the report on this study should be appended to BS/94.1783.

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1 The new reference material is designated the "second" International Standard in accordance with the provisions agreed by the Committee at its thirty-seventh meeting (WHO Technical Report Series, No. 760, 1987, p. 18).
Factors II, IX and X concentrate

At its forty-fourth meeting (WHO Technical Report Series, No. 848, 1994, p. 14), the Committee had established the second International Standard for Blood Coagulation Factor IX Concentrate, with an assigned potency for factor IX activity. It had requested that the suitability of the material to serve as an International Standard for factors II and X should be reconsidered by the National Institute for Biological Standards and Control, Potters Bar. The Committee noted the results of the collaborative study performed in 6 laboratories in response to this request (BS/94.1784). On the basis of these results, the Committee established the material studied, in ampoules coded 84/683, as the second International Standard for Blood Coagulation Factors II, IX and X Concentrate, Human, and assigned potencies of 9.4 International Units of Factor II Activity and 11.1 International Units of Factor X Activity to the contents of each ampoule, in addition to the potency of 6.3 International Units of Factor IX Activity per ampoule assigned at its forty-fourth meeting (WHO Technical Report Series, No. 848, 1994, p. 14).

The Committee formally discontinued the second International Standard for Blood Coagulation Factor IX Concentrate, established in 1993.

Factor VIII concentrate

The Committee noted that, in accordance with its earlier request (WHO Technical Report Series, No. 848, 1994, p. 14), a collaborative study by 19 laboratories in 13 countries had been performed to calibrate the candidate replacement, coded 88/640, against the fourth International Standard for Blood Coagulation Factor VIII:C Concentrate, and the results had been analysed (BS/94.1776). It also noted that discrepancies arising from assays of this high-purity concentrate against plasma standards had been observed previously when concentrates and plasma were compared.

The Committee established the material studied, in ampoules coded 88/640, as the fifth International Standard for Blood Coagulation Factor VIII:C Concentrate, Human, and assigned a potency of 5.4 International Units of Factor VIII:C Activity to the contents of each ampoule. It requested the National Institute for Biological Standards and Control, Potters Bar, to supply detailed information about the preparation to the Secretariat.

Factor IXa

The Committee was informed of the need for a reference material for an activated factor IX (factor IXa) to improve the reproducibility of assays for factor IXa in monocompontent concentrates of factor IX. It was also informed that a collaborative study to examine the reproducibility of factor IXa assays had been organized by the National Institute for Biological Standards and Control, Potters Bar, and that preliminary results had confirmed the need for a reference material.
Plasma fibrinogen

The Committee had been informed at its forty-fourth meeting (WHO Technical Report Series, No. 848, 1994, p. 16) of problems in the measurement of high fibrinogen levels in plasma using the current International Reference Reagent for Plasma Fibrinogen, Human. It was informed that preliminary studies in one laboratory to examine these problems had found the International Reference Reagent to be satisfactory for this purpose. The Committee nevertheless requested the National Institute for Biological Standards and Control, Potters Bar, to continue these studies.

Plasminogen activators

The Committee was informed that the National Institute for Biological Standards and Control, Potters Bar, had obtained a quantity of recombinant truncated tissue plasminogen activator that had been distributed into ampoules. It was also informed that a stability study was under way, and that the National Institute for Biological Standards and Control, Potters Bar, was investigating the need for reference materials for the various forms of urinary-type plasminogen activator. It appeared that the biological activity in some assay systems differed depending on the presence or absence of glycosylation.

Plasminogen-activator inhibitor 1

The Committee was informed of an interim report on the collaborative study of a candidate reference material, coded 92/654, consisting of recombinant plasminogen-activator inhibitor 1 with human plasma as a carrier referred to in its forty-fourth report (WHO Technical Report Series, No. 848, 1994, p. 15). It was also informed that the Fibrinolysis Sub-Committee of the International Society on Thrombosis and Haemostasis had proposed the use of this preparation as an interim reference material while the situation regarding available diagnostic kits was being clarified.

Protein S in plasma

The Committee was informed that, further to the information provided at its forty-fourth meeting (WHO Technical Report Series, No. 848, 1994, p. 14), a collaborative study of a candidate preparation of normal plasma was in progress in 21 laboratories in 10 countries.

Tissue factor pathway inhibitor

The Committee was informed that, further to the information provided at its forty-fourth meeting (WHO Technical Report Series, No. 848, 1994, p. 16), a collaborative study to investigate the assay of tissue factor pathway inhibitor in plasma samples and recombinant preparations was in progress in 20 laboratories in 10 countries.
Cytokines

**Bactericidal/permeability-increasing protein**

The Committee noted that a recombinant bactericidal/permeability-increasing protein, consisting of the N-terminal half of the native protein (BPI\textsubscript{23}), was undergoing clinical trials and that an International Standard might be required (BS/94.1768). It also noted that one manufacturer had offered a quantity of BPI\textsubscript{23} as a candidate reference material. The Committee expressed concern about the relative priority that should be given to this item in view of the many requests for new biological reference materials and the limited resources of the International Laboratories, but agreed that the National Institute for Biological Standards and Control, Potters Bar, could proceed with its investigations, bearing in mind the question of priority.

**Epidermal growth factor**

The Committee noted the report on the collaborative study on candidate reference materials for epidermal growth factor performed by 12 laboratories in 7 countries (BS/94.1781). It noted that these preparations included both the 53- and the 52-amino-acid forms, and that a preparation of the 53-amino-acid form appeared to be most suitable to serve as an International Standard for bioassay and immunoassay. On the basis of the results of the study, the Committee established one of the 53-amino-acid preparations studied, coded 91/530, as the first International Standard for Epidermal Growth Factor and assigned a potency of 2000 International Units of Epidermal Growth Factor to the contents of each ampoule. The suitability of this preparation in assay systems other than those employed in the collaborative study would have to be established by users. The Committee noted that the availability of a reference reagent for the 52-amino-acid form of epidermal growth factor would help to determine whether a particular assay system could distinguish between the two forms. It therefore established one of the preparations of the 52-amino-acid form of epidermal growth factor studied, in ampoules coded 91/550, as the first International Reference Reagent for Epidermal Growth Factor (1–52) with a nominal content of 1.75 μg of epidermal growth factor (1–52) per ampoule.

**Insulin-like growth factor 1**

The Committee noted the report of the collaborative study performed by 13 laboratories in 7 countries (BS/94.1770). On the basis of the results of the study, it established the preparation coded 91/554 as the first International Standard for Insulin-like Growth Factor 1 and assigned a potency of 150 000 International Units to the contents of each ampoule. The Committee noted that, unlike the existing International Reference Reagent, the new International Standard appeared to be suitable for both biological assays and immunoassays, and it therefore discontinued the
first International Reference Reagent for Insulin-like Growth Factor 1, for Immunoassay. The Committee recognized the problem of the expression of content in terms of mass units but agreed that, for practical purposes, 1 International Unit could be assumed to be equivalent to 1 μg of insulin-like growth factor 1.

**Interferon gamma, recombinant human**

The Committee noted the report on a collaborative study of a preparation of interferon gamma, recombinant human (BS/94.1782). On the basis of the results, it established the preparation studied, coded GXg01-90-535, as the first International Standard for Interferon Gamma, Recombinant Human, and assigned a potency of 80,000 International Units of Interferon Gamma, Recombinant Human, to the contents of each ampoule. The Committee noted that this preparation is suitable for antiviral assay but may not be for other types of assay.

**Interleukin-2 soluble receptor**

The Committee noted that candidate reference materials for interleukin-2 soluble receptor had been obtained by the National Institute for Biological Standards and Control, Potters Bar, and freeze-dried, and that a collaborative study would be initiated when stability data had been obtained.

**Interleukin-3 and interleukin-4**

The Committee noted the report on the collaborative studies performed by 48 laboratories in 16 countries on 5 preparations of interleukin-3 and 5 preparations of interleukin-4 in a variety of in vitro bioassays and immunoassays (BS/94.1788). On the basis of the results of these studies, it established one of the preparations studied, coded 91/510, as the first International Standard for Interleukin-3 and assigned a potency of 1700 International Units of Interleukin-3 to the contents of each ampoule. The Committee also established one of the preparations studied, coded 88/656, as the first International Standard for Interleukin-4 and assigned a potency of 1000 International Units of Interleukin-4 to the contents of each ampoule.

The Committee noted that these preparations are suitable for use only in bioassays. The relative potencies of the candidate preparations included in the collaborative studies were not consistent in the various immunoassay systems employed (BS/94.1788).

**Interleukin-8**

The Committee noted a report on the present status of a collaborative study on preparations of interleukin-8 involving 46 laboratories in 13 countries (BS/94.1786).
Stem cell factor

The Committee noted a report that a collaborative study on preparations of stem cell factor was under way (BS/94.1786).

Endocrinological and related substances

Inhibin, recombinant human

The Committee noted the results of a collaborative study performed in 15 laboratories in 9 countries in which 2 candidate preparations of recombinant inhibin had been compared with natural human inhibin, the International Standard for Inhibin, Porcine, and a preparation of human follicular fluid (BS/94.1787). On the basis of the results of the study, it established one of the preparations studied, coded 91/624, as the first International Standard for Inhibin, Recombinant Human, and assigned a potency of 150 000 International Units of Inhibin to the contents of each ampoule. This assignment was based in part on the aim of maintaining approximate continuity of units of biological activity with the International Standard for Inhibin, Porcine, in bioassays using adult rat pituitary cells, which reflect the inhibition of follicle-stimulating hormone characteristic of the material. Although the collaborative study had involved the use of various bioassays and immunoassays, the Committee emphasized that the suitability of the International Standard for Inhibin, Recombinant Human, in other assay systems would have to be established by the user.

Luteinizing hormone, recombinant human

The Committee noted that the third International Standard for Follicle-stimulating Hormone and Luteinizing Hormone might not be appropriate for the assay of recombinant human luteinizing hormone, currently under development as a therapeutic product (BS/94.1774). It also noted that one manufacturer had offered a quantity of recombinant human luteinizing hormone as a candidate reference material. The Committee requested the National Institute for Biological Standards and Control, Potters Bar, to distribute this material into ampoules and organize a collaborative study.

Somatropin

The Committee noted that problems had arisen following its establishment in 1993 (WHO Technical Report Series, No. 848, 1994, p. 19) of the first International Reference Reagent for Somatropin, in ampoules coded 88/624 (BS/94.1790). The Committee had concluded in 1993 that “it was not appropriate to assign a content of somatropin to the International Reference Reagent because any such value would depend on the method of measurement employed”. This statement had caused widespread confusion and as a result there had been some reluctance by different
authorities and pharmaceutical companies to give the International Reference Reagent official status without an unequivocal statement of content.

Following an international meeting organized by the European Pharmacopoeia in September 1994, WHO was formally requested to re-examine the situation and to assign an ampoule content to the International Reference Reagent.

In view of the arguments presented in BS/94.1790, the Committee established the preparation coded 88/624 as the first International Standard for Somatropin (Recombinant DNA-derived Human Growth Hormone), with a defined content of 2.0 mg of protein per ampoule and a defined specific activity of 3.0 International Units per mg of protein. In making this decision, it noted that the principal use of the Standard will be as the primary reference material for the calibration of secondary reference materials and of therapeutic somatropin preparations. The Committee formally discontinued the first International Reference Reagent for Somatropin, established in 1993.

Toxins

Endotoxin

The Committee had been informed in 1993 that the United States Pharmacopeia (USP) had offered a portion of a candidate USP reference standard to serve as a replacement for the International Standard for Endotoxin for Limulus Gelation Tests because it might be suitable for all types of endotoxin assay (WHO Technical Report Series, No. 848, 1994, p. 21). The Committee noted that a collaborative study of this material was planned (BS/94.1767). Because of the importance of harmonization, the Committee urged the National Institute for Biological Standards and Control, Potters Bar, to design and organize the collaborative study in such a way as to maximize the information to be derived from it.
Annex 1
Regulation and licensing of biological products in countries with newly developing regulatory authorities

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1. **Introduction**

National health authorities have the duty to ensure that available pharmaceutical products, whether imported or manufactured locally, are of good quality, safe and efficacious. This is particularly difficult for vaccines and other biological products, the quality of which cannot be established entirely by tests on the material in the final container. A national control authority should therefore be established that is responsible for ensuring that the manufacturer is adhering to approved standards of good manufacturing practice and quality assurance specific to the product. The procedures through which the national control authority confirms the assurance of quality provided by the manufacturer will depend on the resources available and whether the product is manufactured locally or imported.

In general, biological products are distinguished from other drugs by being derived from living organisms (ranging from normal or genetically modified microorganisms to fluids and tissues derived from various animal and human sources) and frequently have a complex molecular structure. They require special quality considerations because of the biological nature of: (a) the starting materials; and/or (b) the manufacturing process; and/or (c) the test methods needed to characterize batches of the product.

Developments in biological products have been extremely rapid in recent years, and the potential value of such products in improving health care on a global scale is immense. There is an urgent need to match technological advances with appropriate mechanisms for assuring the safety, quality and efficacy of the products.

2. **Scope**

The aim of this Annex is to provide guidance for newly developing national control authorities that may have limited resources to license and regulate biological products, including vaccines. It describes the responsibilities of such authorities and manufacturers and provides references to relevant WHO publications relating to their structure and activities. References to more detailed technical requirements published by WHO, specific for various products including vaccines, are also provided (1, Annex 7).

Much of the material included in this Annex is drawn from guidelines previously published by WHO in the reports of the WHO Expert Committee on Biological Standardization and the WHO Expert Committee on Specifications for Pharmaceutical Preparations (see, in particular, 1, Annex 2; 2, Annex 3; 3, Annexes 5 and 6).
3. **General considerations**

The safety, quality and efficacy of a biological product are primarily the responsibility of the manufacturer; however, the national control authority of each country is responsible for establishing procedures for assuring that biological products intended for use in the country are of adequate quality, safety and efficacy. This responsibility should have a firm statutory basis backed by legislation. Marketing approval (licensing) for a biological product should be granted by a national control authority, which should also be responsible for continued monitoring after licensing. In carrying out these activities, the authority should make use of expert committees and technical advisers and have access to laboratory facilities for the testing of in-process and final product samples.

Countries should have written standards, both general and product-specific, for biological products available for use in them. These should be based on up-to-date standards, such as those available from WHO, and harmonized as far as possible with those of other countries. For new products, WHO, national and pharmacopoeial requirements may not have been established, and the national control authority will need to agree on specifications with the manufacturer on a case-by-case basis. In such cases, WHO or the authority in the country of origin could be consulted and their advice requested.

In general terms, the national control authority should be vested with legal powers to:

(a) issue, vary and revoke licences for biological products on grounds of quality, safety and efficacy;

(b) secure the subsequent safe and effective use of each product by controlling, under the terms of the product licence, the content of all labelling (including package inserts, associated prescribing information and advertising) and the channels through which the product may legitimately be supplied; and

(c) inspect and license all manufacturing premises and importing agents and, where applicable, wholesalers and distributors, hospital dispensaries, independent pharmacies and other retail outlets to ensure that they comply with the relevant regulations and guidelines.

In countries where biological products are manufactured, the national control authority should have the appropriate expertise to evaluate the adequacy of the manufacturer’s establishment and facilities, starting materials, production processes, control-test procedures and product specifications, to determine whether they meet international and/or national requirements. Guidelines for national control authorities on quality assurance for biological products have been published by WHO (1, Annex 2).

Control (laboratory) activities should be fully independent of those of the manufacturer so as to ensure that the national control authority
undertakes its tasks in an independent, authoritative and impartial manner. In some countries, the national laboratory facilities may constitute a separate entity called the national control laboratory. In this case, the laboratory should be administered directly by, or on behalf of, the national control authority.

In countries where biological products are not manufactured, alternative approaches can be defined, e.g. the WHO Certification Scheme (2, Annex 3) for assuring the safety, quality and efficacy of such products (see section 6). However, an approval process limited to a mere listing of facilities and products would not be considered adequate.

In view of the complexity and cost of certain facilities needed for control testing, it may be unavoidable in certain cases that the authority will have to share such facilities with the manufacturer or an academic institution, or to rely on those of an authority or laboratory in another country.

National control authorities should, whenever appropriate, exchange information on safety and other issues, within the normal legal constraints of confidentiality.

4. Structure and function of a national control authority for biological products

The health authorities should establish and maintain a competent national control authority with a defined organizational structure including, if relevant, a competent laboratory facility. The responsibilities, relationships, coordination, and legal status of employees should be specified in the light of their roles in a clearly defined decision-making process.

The authority can be either independent or part of the ministry of health. It is advisable to delegate decision-making responsibilities to those departments within the authority or laboratory with the necessary competence in the field of biologicals so as to facilitate the licensing/regulation process and ensure that it is carried out efficiently.

It is also recommended that the national control authority should make use of external expert advisers and advisory committees with appropriate expertise. Caution should be exercised when identifying such advisers to avoid conflicts of interest.

4.1 Personnel (see also 1, p. 34)

The personnel of the authority or laboratory should include person(s) qualified and experienced in the control of biological products and experts in all appropriate disciplines. The qualifications and experience of the staff at all levels should be appropriate to the review and control activities required for the range of biological products to be controlled.
All staff members of the authority should undergo suitable training and should therefore attend “hands-on” training courses covering both the technical and administrative aspects of licensing and control procedures.

4.2 Administration (see also 1, p. 34)

The national control authority should have established procedures for the receipt and review of manufacturers’ submissions and, if applicable, for testing samples provided in support of applications. When the review procedures, which include the evaluation of detailed reports, have been completed, a notice of approval (licence) or disapproval is sent by the authority to the manufacturer. It may also issue notices of suspension or revocation of approval. Consideration should be given to making appropriate legal expertise available in support of this activity. It is of the utmost importance that proprietary and commercial information is kept confidential.

4.3 Registration documents (see also 1, pp. 34–35)

The national control authorities should provide guidance on the information to be provided, the format to be used, and the acceptability of specific forms, and should maintain adequate filing and archiving facilities so that all submissions, evaluations, records and correspondence are available and kept up to date. A computer program for drug regulatory authorities can be obtained from WHO.¹

The national control authority should possess, or have access to, library facilities appropriate to its fields of activity. The documents available should include current national and international requirements for biological substances, and other relevant specifications and recommendations published by WHO or other official bodies.

4.4 Good manufacturing practices inspectorate

The national control authority should have access either to suitably qualified inspectors who are independent of manufacturers or to recent inspection reports from qualified inspectors. The purpose of inspections is to ensure that each manufacturer’s facilities and procedures comply with the principles of good manufacturing practice as described in national or WHO publications (1, Annex 1) and with the requirements and/or conditions for the approval of the product concerned. Guidelines are available on the conduct of inspections of manufacturers of drugs and biologicals (1, Annex 2; 2, Annex 2).

¹ Available on request, together with a user manual, from the Division of Drug Management and Policies, World Health Organization, 1211 Geneva 27, Switzerland.
5. **Aspects of the licensing process**

5.1 **Establishing a registration system for existing medical (biological) products** (see also 3, p. 71)

Before any system of control can be effective, it is necessary to identify and catalogue all the products already sold or otherwise supplied on the domestic market, in both the public and the private sectors, that qualify for control. Guiding principles for national drug regulatory authorities that have yet to introduce comprehensive legal provisions on drug regulation have been published by WHO (3, Annex 6). An appointed day should be established after which no existing biological products may be lawfully distributed or supplied unless they have been notified to the authority, and no new product may be introduced until a request for a product and establishment licence have been granted by it.

The effective administration of the provisional registration procedure depends on:

(a) the prior identification of all interested manufacturers and importers;
(b) a precise definition of a notifiable biological product based primarily on the claims made for it on the label and the indications for use;
(c) the issue of guidelines on the procedure to be followed.

Each notified product, as a minimum, must be identified by name, the names and full addresses of the manufacturer and of the responsible agent, if any, representing the manufacturer, the name and full address of an importing agent, a brief description of the manufacturing procedure including a declaration by the manufacturer that good manufacturing practices have been followed and a manufacturing flow chart, a description of the dosage form, its composition (including active and inactive ingredients (using International Nonproprietary Names where appropriate)), the therapeutic class, the indications, a copy of all labelling, including any package insert, a copy of any relevant certificates and warranties relating to the product or its components, and appropriate shipment conditions.

It is desirable for an updated compendium to be available containing information for the physician on approved products. This should distinguish those products given provisional approval from those given final approval (new product licences).

5.2 **Screening of provisionally registered products**

(see also 3, pp. 71–72)

A rapid screening of all provisionally registered products should be undertaken at the earliest opportunity with a view to securing the withdrawal from the market of any product which, simply on the basis of a review of its ingredients and indications, is judged not to meet appropriate safety standards.
After this preliminary review, a set of longer-term priorities needs to be set for the definitive assessment of provisionally registered products. Consideration needs to be given to the resources required, in terms of both personnel and information. The review should be adapted to a proposed time-schedule, depending on which, additional information may be requested by the national control authority from provisionally registered companies, e.g. periodic safety updates or evidence of lack of efficacy.

5.3 New product licences (see also 3, pp. 73–74)

The licensing of a vaccine or other biological product requires the issue of licences for both the manufacturing establishment and the product. The approval or licensing of a manufacturing establishment for the production of biological products should be granted only if the manufacturer complies with the relevant international or equivalent national standards for good manufacturing practice.

A licence for a given biological product will be issued by the national control authority only when it is satisfied that the product is in conformity with the relevant national and/or international requirements, including the manufacturer’s specifications, applicable to it.

The normal procedure for the issue of a product licence consists of the following three stages:

(a) the manufacturing establishment and product licence applications are received from the manufacturer, screened for completeness, and then reviewed for evidence of compliance with good manufacturing practices and for safety, quality and efficacy by the authority’s technical staff;

(b) the authority may perform laboratory tests, review reports of or perform pre-licensing inspections, and seek the advice of external experts on specific technical questions when deciding whether or not to authorize the marketing of the product;

(c) the formal administrative action to grant or refuse a licence is then taken by the designated authorized person.

The assessment of the product must be based on its safety, quality and efficacy when used as intended. However, the availability of the product may be dictated by national policy considerations, such as the national need for comparative efficacy and/or safety, or cost-effectiveness. Advice on basic or essential drugs is published periodically by WHO (4).

5.4 Renewal and variation of licences (see also 3, pp. 74–75)

The precise circumstances under which licence-holders are required to apply for a renewal or variation in a product licence differ from country to country and should be clearly defined by the national authority. In general, if a manufacturer wishes to vary the conditions of the approved
licence to any significant extent, the variations must be submitted to the authority for approval. Significant changes might include changes in aspects of the manufacturing procedures or the facility, or in the product specifications, dosage forms or labelling. The procedure for the renewal of licences is more variable. In many countries, reregistration, but not licence renewal, is required annually. In others, licences must be renewed every 5 or 7 years.

Licence-holders should be required, in all circumstances, to inform regulatory authorities immediately of unanticipated adverse effects that could possibly be associated with a licensed product and that might call for the licence to be made subject to certain restrictions or withdrawn (see section 8).

5.5 Information on the manufacturing establishment
(see also 7, pp. 35–36)

The manufacturer should provide sufficient information to demonstrate compliance with the principles of good manufacturing practices, including the existence of adequate quality-assurance systems. Plans, diagrams, flow charts, standard operating procedures and texts may be used to convey the necessary information in relation to (but not limited to):

- Personnel, and in particular their qualifications and experience, organization and reporting relationships, training schedules and record-keeping systems.
- The location and construction of the building used for manufacture and control.
- The flow of raw materials, personnel and manufactured product through the facility.
- The animal facilities.
- Air, water and steam systems and power supply.
- Drainage and effluent systems.
- The segregation of operations.
- Lists of major equipment.
- Maintenance schedules for equipment and building services.
- Cleaning procedures, schedules and control measures.
- Quality-assurance and quality-control procedures.
- Storage and quarantine facilities, and procedures for raw materials, packaging materials, in-process and bulk materials, and final product.
- Validation procedures.
- Documentation and record-keeping systems.
- Labelling and packaging facilities and procedures.
- Recall and retrieval procedures.

To be certain that the buildings, facilities, personnel, procedures and practices comply with the description in the licence application, the national control authority should review the availability of the
manufacturer's inspection reports. If a report is available from the
country of origin, this should suffice. Alternatively, if no report is
available, or no inspection has been performed, an inspection should be
conducted before a licence is issued. The inspectors selected should be
independent of the manufacturer and have sufficient expertise to conduct
a meaningful review in accordance with the system of good
manufacturing practices in use; this should include buildings, facilities,
procedures, personnel and quality assurance.

5.6 **Information on the product** (see also 1, pp. 36-37)

The manufacturer should provide sufficient information to demonstrate
the safety, quality and effectiveness of the product as manufactured and
controlled in the establishment described above, and should refer for
guidance to the national requirements or, if these are not available, the
relevant technical requirements published by WHO. The submission
should include the following, if appropriate:

- Information on the source materials (e.g. microorganisms,
  blood/plasma donations, cells/cell substrate, pollen), including their
  specifications and the tests used to demonstrate compliance with the
  specifications.
- A description of the cold-chain procedures employed.
- Information on the raw materials and packaging materials, including
  their specifications and the tests used to demonstrate compliance.
- Information on the methods of manufacture, including a description of
  the seed lot and cell-substrate systems used, together with in-process,
  bulk and final product specifications, and the tests employed to
demonstrate compliance.
- The demonstration of the consistency of manufacture, for which the
  results of tests on a minimum of three satisfactory and consecutive
  production batches, ideally of different bulk lots, of a size
  corresponding to that contemplated for routine production, are
  normally required.
- Any proposal for the reprocessing of the product.
- The results of stability studies undertaken to justify the proposed
  validity period for the product under the indicated storage conditions.
- The labels and package inserts.
- The documentation used in the manufacturing and control procedures,
  including standard operating procedures and protocols containing
details of production and quality control testing.
- Reports of preclinical studies.
- Clinical trial data.
- A list of countries in which the product is approved for use.

The nature and extent of pre-licensing testing undertaken by the national
control authority should reflect any particular considerations relevant to
the product. Chemical, physical and biological tests additional to those
specified in national or international requirements may also be performed.
For imported products, results of testing may be obtained under the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce (see section 6).

6. Alternative procedures for approval of imported products – the WHO Certification Scheme

The national authorities of countries wishing to import biological products can simplify the licensing formalities, and reduce the need for testing, by accepting certificates issued by the responsible authorities in the country of manufacture, stating that the quality of the product meets a certain standard. The WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce, as summarized in this section, provides a suitable basis for the approval and release of imported products (2, Annex 3; 3, Annex 5). For the purpose of this Certification Scheme, “biological product” refers to a product presented in its finished dosage form and to the bulk material that is processed to produce this dosage form.

6.1 Participating countries

Each country participating in the Certification Scheme should communicate to WHO the name and address of the department of its national control authority dealing with biological products and, if appropriate, any significant reservations relating to its participation. WHO would then notify all other countries.

Exporting countries participating in the Certification Scheme should ensure that:

- the approval of biological products is subject to appropriate control testing by the national control authority to guarantee safety and efficacy, and that adequate facilities are available for such testing;
- the manufacturer complies with the requirements for good manufacturing practices and quality assurance of biological products as recommended by WHO, or with equivalent national standards;
- the national control authority conducts appropriate inspections including, for example, the examination of records and samples, so as to ensure that manufacturers comply with these requirements;
- the inspectors in the service of the authority possess the appropriate expertise.

Exporting countries participating in the Certification Scheme should, whenever possible, ensure that International Nonproprietary Names are used on certificates and for labelling the biological product (5).
6.2 Certification of products

Biological products exported under the Certification Scheme should be certified by the national control authority of the exporting country by means of certificates to be sent to the corresponding authority of the importing country. The importing country can then either license the product or make licensing conditional on the submission and approval of supplementary data.

The issue of certificates for a biological product would be subject to the conditions set by the authority of the exporting country. Certificates would, however, be expected to state that:

- the product is approved for use within the exporting country or, if not, the reason for which approval has not been obtained;
- the manufacturing establishment in which the product is produced is inspected at suitable intervals to check that the manufacturer complies with the principles of good manufacturing practices and quality assurance (see I, Annex 2).

For many biological products, certification on an individual lot basis is necessary because of the difficulty of controlling the starting materials and ensuring that batch-to-batch variation is within acceptable limits.

6.3 Requests for additional information

Additional information may be requested by the national control authority of the importing country from the corresponding authority of the exporting country. This information may be provided directly by the latter, or through the manufacturer, and may include:

- Information showing that the requirements for good manufacturing practices and quality assurance of biological products have been satisfied.
- Information on control tests performed on the product by the authority of the exporting country.
- The names and functions of the persons officially designated to sign release certificates for individual batches of the product.
- Copies of all documentation and labels supplied with the product on packaging materials and package inserts and approved by the authority in the exporting country, together with the date(s) on which such approval was accorded.

Information on general and specific standards for quality assurance of the biological product to be exported may also be requested if so required under the legislation of the importing country. Of concern, in particular, is information that will ensure that the quality of the product will not be adversely affected by the storage and shipment conditions. The consent of the manufacturer to the provision of such information should be obtained by the national control authority of the exporting country.
6.4 Reporting of defects and adverse reactions

Defects may occur in biological products imported under the Certification Scheme. If they are considered to be of a serious nature by the importing country, and are not attributable to local conditions of storage and transport, the national control authority of the importing country should notify the corresponding authority of the exporting country and provide the relevant data. Adverse reactions of unexpected severity or frequency should also be notified to the authority of the exporting country. Similarly, if the authority of the exporting country discovers quality defects or receives reports of unexpected adverse reactions, it should inform the corresponding authority of the importing country of the problem and any action taken. Guidance on the reporting of adverse drug reactions is contained in the report of a Working Group of the Council for International Organizations of Medical Sciences (CIOMS) (6, Annex 1).

7. Authorization of clinical trials

An authority may occasionally need to consider an application to conduct a clinical trial of an unapproved product for the prevention or treatment of a condition. To provide for this contingency, the registration system should include provision for the importation of the necessary materials, subject to appropriate controls. Such trials should take place only after formal clearance has been obtained from the competent registration authority and after assurances have been obtained that they will be conducted in conformity with the principles contained in the World Medical Assembly’s Declaration of Helsinki, the good clinical practices guidelines of national authorities, and the CIOMS guidelines (7, Annex 1).

8. Post-licensing monitoring

(see also 1, pp. 39–40)

8.1 Product release

At the time a product is approved, the national control authority should decide what controls are to be applied to the release of batches of the product. This decision will be influenced by the nature of the product and the resources available for laboratory testing. Controls will usually be imposed on complex products, e.g. vaccines, and on those obtained by complex manufacturing procedures. The control system may involve the activities described below, and may be reviewed and revised once satisfactory and consistent production has been demonstrated:
The testing of samples of intermediate, bulk or final product should confirm compliance with the requirements and agreed specifications (see section 5). The nature and frequency of the tests to be carried out are decided by the national control authority.

The evaluation of the manufacturer's protocols for the manufacture and control of each batch will be undertaken by the national control authority. Examples of model summary protocols are annexed to the individual requirements for biological substances published by WHO. The critical review of batch protocols by the authority is a most important part of the control of biological products. The information provided should make it possible to review the manufacture and testing of each batch of a particular product, including all required in-process controls and control tests on final products to confirm compliance with the approved specifications.

8.2 Inspections

Periodic inspections of the manufacturing facility should be carried out on behalf of the national control authority to assure continued compliance with good manufacturing practices and with the specifications established for the product at the time of approval. Records of complaints and reports of adverse reactions should be examined.

8.3 Post-licensing surveillance

The procedures described in sections 8.1 and 8.2 above do not preclude the need for a post-licensing sampling and surveillance system. Countries should establish a national system for the post-licensing surveillance of biological products. Clinicians and other health workers should be encouraged to report to national control authorities and manufacturers any unexpected adverse events occurring after the administration of biological products.

The mechanisms for reporting (e.g. standardized forms), the receiving body (e.g. the national control authority), the deadline for reporting (e.g. 48 hours), and the types of adverse events reportable need to be clearly defined by the authority and will depend on its structure and resources. The manufacturers and the authorities should assess these reports and, in consultation with each other, attempt to evaluate their significance. This assessment may require the testing of products already released and the inspection of production and control facilities and local distribution channels. If an imported product is associated with adverse reactions, the manufacturer and, where appropriate, other national control authorities and WHO should be notified.

Guidance on the operation of a monitoring system for adverse reactions is provided in a CIOMS report (6). The national control authority or another government body may decide to perform epidemiological studies to obtain further information on the performance of products in actual use.
8.4 Recall and revocation
National control authorities should have a system for enforcing the recall of batches, revoking approvals, communicating such decisions to users, and ensuring that the authorities of other receiving countries importing the product are notified accordingly.

8.5 Approval of manufacturing changes
Any significant change in the manufacturing establishment, source materials, production process, quality-assurance procedures, product specifications or labelling is subject to the prior approval of the national control authority. Significant changes in the manufacturing process may require major modifications to the existing licence, and the submission of new lots of product to demonstrate consistency of manufacture and new clinical data.

8.6 Approval of new indications
National control authorities should require manufacturers to submit significant proposed changes in product indications for use for evaluation and approval. An authority may request additional clinical data if changes in the dosage regimen, including the dose, route, frequency or timing, are proposed.

9. Powers of enforcement
In order to assume its responsibilities, an authority must possess powers of enforcement backed by legal provisions for imposing penalties for offences such as misbranding, adulteration and the sale of counterfeit products. It should also have the power to order the suspension of lot release, seizure or recall when necessary to prevent the release of suspect lots or to ensure that suspected hazardous materials are withdrawn.

In establishing administrative decision-making mechanisms, the authority should not lose flexibility. In particular, it should make provision for:

- implementing decisions regarded as urgent in the interest of public safety;
- formal consultation (usually through representative bodies) with pharmaceutical companies and other interested parties, including pharmacists, doctors, nurses and patients.

The authority should publish a list of licensed products and sources at regular intervals.
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References


Other documents


Annex 2

Requirements for hepatitis A vaccine (inactivated)
(Requirements for Biological Substances No. 49)

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Introduction

Hepatitis A virus (HAV) infection is a significant cause of morbidity and attendant economic loss in many parts of the world. The virus is present worldwide and rates of infection are inversely associated with levels of environmental sanitation and personal hygiene. Improvements in sanitation and hygiene can reduce the transmission of HAV. However, in less developed countries, an improvement in sanitary conditions may result in an increase in the burden of clinical disease as peak rates of infection shift from early childhood, when infection is largely asymptomatic, to older age groups in which it is more often symptomatic. Passive immunoprophylaxis using immune globulin can prevent disease in individuals who are exposed to the virus; however, the protective effect is temporary and immune globulin is not suitable for the control of HAV in large populations. Within the past few years, several vaccines have been developed that provide active immunity and potentially long-lasting protection against HAV. The development of these vaccines represents a major advance in the ability to control HAV infection and reduce the burden of disease.

General considerations

Hepatitis A is caused by HAV, a non-enveloped virus with positive-sense single-stranded RNA, belonging to the genus Hepatovirus (Heparnavirus) of the Picornaviridae family. All human isolates of HAV, while having up to 20–25% variation in nucleic acid sequence, belong to a single serotype. Epidemiological evidence in humans and in vitro and in vivo studies support the concept that antibodies to HAV induced by any isolate of the virus will protect against all viral strains.

The isolation and propagation of HAV in cell culture were critical steps in the successful development of hepatitis A vaccines. Several HAV isolates have been propagated in cell culture and have been used to make both inactivated and attenuated vaccines. These Requirements relate only to the production of hepatitis A vaccine (inactivated) in human diploid cells or continuous cell lines. If any other production process is used, different requirements may apply.

Inactivated hepatitis A vaccines are prepared by the purification of virus propagated in cell culture followed by formalin inactivation, an approach similar to that used for inactivated poliovirus vaccines. Inactivation is one of the critical production steps, and the inactivation process should be carefully monitored. The vaccines produced to date have been shown to be highly immunogenic when two different adjuvants (aluminium and liposomal formulation) were used. Two large studies have demonstrated the efficacy of the vaccines obtained from two manufacturers.
Each of the following sections constitutes a recommendation. The parts of each section printed in normal type have been written in the form of requirements so that, if a national control authority so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments or recommendations for guidance. To facilitate the international distribution of vaccine made in accordance with these Requirements, an example of a summary protocol for recording the results of tests is provided as Appendix 1.

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

**Part A. Manufacturing requirements**

**A.1 Definitions**

**A.1.1 International name and proper name**

The international name shall be “Vaccinum hepatitidis A inactivatum”. 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.

**A.1.2 Descriptive definition**

“Vaccinum hepatitidis A inactivatum” shall consist of hepatitis A virus grown in cell cultures and inactivated. The preparation shall satisfy all the requirements formulated below.

**A.1.3 International reference materials**

An International Reference Preparation of Hepatitis A Immunoglobulin is available from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands. Samples are distributed free of charge, on request, to national control laboratories.

**A.1.4 Terminology**

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

*Master cell bank:* A quantity of adequately characterized cells stored at −60 °C or below in aliquots of uniform composition, one or more of which may be used for the production of a manufacturer’s working cell bank.
Manufacturer's working cell bank (MWCB): A quantity of cells of uniform composition derived from one or more ampoules of the master cell bank, which may be used for the production of cell cultures. A master cell bank 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 in aliquots to form the MWCB. One or more of the ampoules from such a pool may be used for the production cell culture.

Production cell culture: A cell culture derived from one or more ampoules of the MWCB and used for the production of HAV.

Adventitious agents: Contaminating microorganisms of the cell substrate or materials used in its culture, including bacteria, fungi, mycoplasmas and endogenous and exogenous viruses.

Virus master seed lot: A quantity of virus that has been prepared as a single lot and has a uniform composition. It is used for the preparation of working seed lots.

Virus working seed lot: A quantity of virus of uniform composition derived by passage from the master seed lot, by a method and at a passage level approved by the national control authority.

Single virus harvest: A virus suspension harvested from production cell cultures that were processed together. Multiple harvests from the same production cell culture may be pooled and considered a single virus harvest.

Purified pool: A purified single virus harvest or a pool of single virus harvests purified at the same time.

Final bulk: The preparation present in the container from which the final containers are filled. The final bulk is prepared from one or more purified pool(s) or part of a pool after inactivation.

Final lot: A collection of sealed final containers of vaccine that are homogeneous with respect to the risk of contamination during the preparation of finished vaccine, including the filling process and, if applicable, the freeze-drying. The containers of a final lot must therefore have been filled, or the final lot prepared in one working session.

A.2 General manufacturing requirements

The general manufacturing requirements contained in Good Manufacturing Practices for Pharmaceutical (1) and Biological (2) Products shall apply to hepatitis A vaccine with the following addition: all staff involved in the production of hepatitis A vaccine should be shown to be immune to hepatitis A.
A.3 Control of source materials

A.3.1 Cell cultures for virus propagation

A.3.1.1 Cell banks used for providing production cell cultures
If human diploid cells are used for the propagation of HAV, they shall meet the Requirements for Human Diploid Cells used for the Production of Varicella Vaccine (Live) (3) and shall be approved by, and registered with, the national control authority.

If continuous cell lines are used for the propagation of HAV, they shall be approved by, and registered with, the national control authority and meet the Requirements for Continuous Cell Lines used for Biologicals Production (4).

The cells in any cell bank shall have been characterized with respect to their genealogy, growth characteristics, and viability during storage and shall have been shown to be free from detectable adventitious agents.

A.3.1.2 Cell culture medium
Serum used for the propagation of cells for hepatitis A vaccine production shall be tested to demonstrate freedom from bacteria, fungi and mycoplasmas as specified 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) (5) and to demonstrate freedom from viruses. Serum of bovine origin must come from countries or herds certified to be free of bovine spongiform encephalopathy.

Some countries require that bovine serum should come from herds that have not been given feed derived from ruminant protein.

Suitable tests for detecting viruses in calf or newborn-calf serum are given in Appendix 1 of the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (6). In some countries sera are also examined for freedom from certain phages.

Penicillin and other β-lactam antibiotics shall not be used at any stage of manufacture.

Minimal concentrations of other antibiotics may be used if approved by the national control authority.

A.3.1.3 Trypsin used for preparing cell cultures
Trypsin used for preparing cell cultures shall be bacteriologically sterile and free from mycoplasmas and viruses, especially porcine parvoviruses. If bovine trypsin is used, it must come from countries or herds certified to be free of bovine spongiform encephalopathy. The methods used to ensure this shall be approved by the national control authority.

A.3.2 Virus seed

A.3.2.1 Strain of virus
Strains of HAV used in the production of vaccine shall be approved by the national control authority. Each strain shall be identified by means of
historical records that include information on the origin of the virus. Any strain that will yield a vaccine meeting the requirements set forth in the present document, and that has been shown by clinical trials to produce a safe and effective vaccine, may be used.

A.3.2.2 Virus seed lot system
The production of vaccine shall be based on the virus seed lot system. The virus working seed lot used for the production of vaccine batches shall be prepared from a master seed lot by a method approved by the national control authority. All virus seed lots shall be stored at a temperature of −60 °C or below.

A.3.2.3 Tests on virus seed lots
The seed lot used for the production of vaccine shall be free from detectable adventitious agents and shall be produced in conditions that satisfy the requirements of sections A.4.1 and A.4.2.1.

Identity and infectivity. Each virus seed lot shall be identified as HAV by appropriate serological methods.

Appropriate serological methods include enzyme immunoassay or hepatitis A neutralization assay, using a reference serum or a monoclonal antibody known to neutralize HAV.

The infectivity of each seed lot shall be established in the cell culture system selected for production.

Freedom from bacteria, fungi and mycoplasmas. Each virus seed lot shall be shown to be free from bacterial, mycotic and mycoplasmal contamination by appropriate tests as specified 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) (5).

Tests for adventitious viruses. Each virus seed lot shall be tested in cell cultures for adventitious viruses. Neutralization of HAV may not be necessary for many tests because the virus is generally not cytopathogenic and has a limited host range.

A volume of each seed lot of at least 10 ml shall be tested for adventitious viruses by inoculation into simian cells. Similar volumes shall likewise be tested in human cell cultures and also in cell cultures of the same type but not the same batch as that used in the preparation of the virus seed. Uninoculated control cell cultures shall be included in the tests. All cell cultures shall be incubated at 35–37 °C and observed for at least 14 days.

The cells shall be observed microscopically for cytopathic changes. At the end of the observation period, the cells or fluids shall be tested for haemadsorbing viruses and other adventitious agents as specified in sections A.4.1.1 and A.4.1.2. For a test to be valid, no more than 20% of the culture vessels should have been discarded for non-specific reasons by the end of the test period. For the seed virus to be satisfactory, no cytopathic changes or adventitious agents shall be detected.
If primary monkey kidney cultures were used in the adaptation of the virus strain to cell culture, tests for simian viruses should be done.

Specific tests may be required for simian immunodeficiency virus (SIV), simian virus 40 (SV40), Marburg virus and B virus. In some countries virus seed lots are also tested in animals.

Tests for SIV may include reverse transcriptase assay, infectivity in a sensitive cell line, hybridization tests with appropriate recombinant DNA probes or the polymerase chain reaction (PCR), inoculation of a sensitive host monkey, and determination of infection by seroconversion or molecular diagnostic tests.

A.4 Control of vaccine production

A.4.1 Control cell cultures

An amount of the production cell suspension equivalent to at least 5% of the total volume but not more than 1000 ml shall be used to prepare control cultures of uninfected cells.

These cells should be maintained under similar conditions of time, temperature and media as the infected cells. The control cells shall be maintained until the time of harvest of the production cells. At the time of the HAV harvest (usually 2–4 weeks after inoculation), the control cells shall be examined for degeneration caused by adventitious agents.

In addition, at the end of the observation period, fluids collected from the control culture shall be pooled and tested for the presence of adventitious agents as described below. Samples that are not tested immediately shall be stored at −60 °C or below.

If multiple virus harvests are made from the same production cell culture lot, the control fluid taken at the time of each harvest shall be frozen and stored at or below −60 °C until the last virus harvesting is completed. The control fluids shall then be pooled in proportion to their amounts and submitted to the required tests.

In some countries, samples of fluid from each control vessel are collected at the same time as fluid is harvested from the corresponding production vessels.

If any such tests show evidence of the presence in control cultures of any adventitious agents, the harvest of virus shall not be used for vaccine production.

For the test to be valid, no more than 20% of the control culture vessels should have been discarded for non-specific reasons by the end of the test period.

In some countries, the national control authority may permit reduced testing for adventitious agents where the manufacturer has demonstrated that:

(a) all raw materials of animal origin used in the cell culture process, the MWCB and the virus seed lots are free from detectable adventitious agents;
(b) at the end of the cell culture, the cell culture system is free from bacterial, mycotic and mycoplasmal contamination as specified 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) (5);
(c) the manufacturing process is consistent and validated to remove/inactivate a panel of representative adventitious agents.

A.4.1.1 Test for haemadsorbing adventitious viruses
At the end of the observation period, 25% of the control cells shall be tested for the presence of haemadsorbing viruses, using guinea-pig red cells. If the red cells have been stored, the duration of storage shall not have exceeded 7 days, and the temperature of storage shall have been in the range of 2–8 °C.

Some national control authorities require that the cell cultures described in section A.4.1.2 should be tested for the presence of haemadsorbing viruses at the end of the incubation period(s). If this is the case, the test for haemadsorbing viruses described here may be deleted.

In some countries, the national control authority requires that tests for haemadsorbing viruses should also be made on control cultures 3–5 days and 12 days after inoculation of the production cultures, and that other types of red cells, including cells from humans (blood group O), monkeys, and chickens (or other avian species) should be used in addition to guinea-pig cells. In all tests readings should be taken after incubation for 30 minutes at 0–4 °C, and again after a further incubation for 30 minutes at 20–25 °C. For the test with monkey red cells readings should also be taken after a final incubation for 30 minutes at 34–37 °C.

For the tests to be valid, no more than 20% of the control culture vessels should have been discarded for non-specific reasons by the end of the test period.

A.4.1.2 Tests for non-haemadsorbing adventitious agents
At 14 days after the day of inoculation of the production cultures or at the time of final virus harvest, a sample of cell culture fluid shall be taken from each control culture and pooled. Ten millilitres of the pool shall be tested in the same cell cultures, but not the same batch, as that used for virus growth. Additional 10 ml samples of each pool shall be tested in both human and simian cells.

Each sample shall be inoculated into bottles of cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm² per ml of pooled fluid. At least one bottle of the cell culture shall remain uninoculated as a control.

The inoculated cultures shall be incubated at a temperature of 35–37 °C for a period of at least 14 days and shall be examined for abnormal morphology.

The cell culture safety tests are satisfactory if no cytopathic changes attributable to adventitious agents in the test sample are detected.
Some national control authorities require that, at the end of this observation period, a subculture is made in the same culture system and observed for at least 7 days. Furthermore, some national control authorities require that these cells should be tested for the presence of haemadsorbing viruses.

For the tests to be valid, no more than 20% of the control culture vessels should have been discarded for non-specific reasons by the end of the test period.

A.4.1.3 Identity test
The cells used in hepatitis A vaccine production should be documented to be derived from the characterized MWCB.

An identity test shall be performed on the control cell cultures by a method approved by the national control authority, as specified in Appendix 1, section 2.2.2 of the Requirements for Human Diploid Cells used for the Production of Varicella Vaccine (Live) (3) or section 3.4 of the Requirements for Continuous Cell Lines used for Biologicals Production (4).

Methods used for identity testing include biochemical (e.g. isoenzyme analysis), immunological (e.g. histocompatibility antigen assays), and cytogenic marker tests or, for diploid cells, karyotyping of at least one metaphase spread of chromosomes.

A.4.2 Control of single virus harvests

A.4.2.1 Sterility tests
A sample of each single virus harvest or virus culture supernatant shall be tested for bacterial, fungal and mycoplasma contamination by appropriate tests as specified 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) (5). Any single harvest in which contamination is detected shall be discarded.

A.4.2.2 Virus content
A sample removed from each virus harvest shall be tested for antigen content by an immunological assay and/or for virus content by an infectivity assay to confirm consistency of production.

A.4.3 Preparation and control of purified pool

A.4.3.1 Pooling of single harvests
Only virus harvests meeting the requirements for sterility and virus content of sections A.4.2.1 and A.4.2.2 shall be pooled.

A.4.3.2 Purification procedures
Each pool of virus shall be purified before inactivation. The methods used to purify HAV from virus harvests shall be approved by the national control authority.
Each manufacturer shall demonstrate, by testing each lot, or by validation of the purification process, that residual contaminants are consistently reduced to a level acceptable to the national control authority.

The protein content per human dose and the minimum purity shall be approved by the national control authority.

Animal serum albumin shall be reduced to less than 50 ng per human dose.

The upper limit of cellular DNA for vaccine produced in continuous cell lines shall be 100 pg per dose.

Consistency of purification within the limits approved by the national control authority shall be demonstrated. For each lot there shall be a residual marker substance whose clearance is monitored.

Serum albumin is an appropriate candidate for a marker, though other components may be more appropriate for specific manufacturing processes.

A.4.3.3 Inactivation of virus

The virus in the purified pools shall be inactivated by a method that has been validated. The kinetics of inactivation shall be suitably monitored and demonstrated by the manufacturer to be consistently effective. Virus aggregates, if present, shall be removed before inactivation. The method shall be approved by the national control authority.

Chemical or physical steps, such as filtration, may be used to remove aggregates.

The importance of filtration or clarification of the crude virus suspensions as a means of improving the consistency of the inactivation process has been clearly established. Satisfactory results have been reported with several types of filter but a 0.2 \( \mu \text{m} \) filter should be used for the final filtration.

If the crude virus suspension is filtered, it is preferable to start inactivation within 24 hours. Since the purpose of the filtration step is to remove particulate matter and other interfering substances that may diminish the effectiveness of the inactivation process, and since aggregates tend to form if the filtrate is left to stand after filtration, efforts should be made to keep within this time limit.

Formaldehyde may be used as an inactivating agent in the production of vaccines. It has been recommended that tests for free formaldehyde should be performed at intervals and the concentration maintained at the desired level by intermittent readjustments. Some manufacturers use a combination of initial formaldehyde treatment with some other method of inactivation.

If the virus pool is filtered before inactivation is initiated, a second filtration shall be carried out during the process of inactivation.

This is done after the infective virus titre has fallen below detectable levels but before the first sample for the safety test is taken.

Test for effective inactivation. Each undiluted bulk suspension shall be tested for effective inactivation of HAV by a method approved by the national control authority. Two samples shall be tested, one of which
shall be taken at the end of the inactivation period and the other not later than three-quarters of the way through this period. The total sample size for these two samples shall be 5% of the batch or not more than 1500 adult doses. After removal or neutralization of the inactivating agent, the samples shall be tested by inoculation into cell cultures for the absence of infective HAV. The test samples shall be inoculated into sensitive cell cultures preferably of the same type as that from which the vaccine was prepared. The inoculated cells shall be incubated for a validated period of time at the optimum growth temperature of the particular strain of HAV used in the vaccine. Since HAV is relatively difficult to detect in cell culture when present in small amounts, any residual virus shall be amplified by a blind passage. A portion of the entire cell lysate from the first passage shall be used as the inoculum for the second passage in similar cultures, which shall be incubated for a period equal to that used for the first passage. Absence of HAV replication shall be monitored by an assay approved by the national control authority.

The approved assay could be an immunofluorescence assay, radioimmuno-focus assay, enzyme-linked immunosorbent assay, direct radioimmunoassay or possibly an assay for HAV-specific RNA replication.

Suitable infectious HAV incula shall be used concurrently as positive controls to demonstrate cellular susceptibility and absence of interference.

Some strains of cell-culture-adapted HAV are essentially non-pathogenic in humans at a dose many log_{10} infectious units (powers of 10) greater than any that could conceivably be present as residual infectious virus after inactivation.

A record of consistency shall be established by the production of five consecutive lots. If any bulk suspension fails the test for effective inactivation, a further five consecutive lots shall be prepared and shown to be satisfactory for re-establishing production.

*Antigen content of bulk suspension.* The antigen content of the vaccine shall be assayed prior to the addition of adjuvant (see section A.4.2.2). The assay for antigen content shall be approved by the national control authority.

### A.4.4 Preparation and control of final bulk

#### A.4.4.1 Preservatives

Preservatives that might be added to the vaccine shall have been shown to have no deleterious effect on the immunizing potency of the product. The preservatives used and their concentrations shall be approved by the national control authority.

#### A.4.4.2 Addition of adjuvant

Where the final bulk contains an adjuvant, the adjuvant and the concentration used shall be approved by the national control authority.

Where aluminium salts are used, the concentration of aluminium should not exceed 1.25 mg per single human dose.
A.4.4.3 Test for completeness of adsorption to adjuvant
Tests shall be carried out to confirm that hepatitis A antigen is adsorbed to the adjuvant. The tests and limits shall be approved by the national control authority.

A.4.4.4 Sterility tests
Each final bulk shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified 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) (5), or by a method approved by the national control authority.

A.4.4.5 Tests for chemicals used in production
The concentration of any organic solvent and inactivating agent remaining in the final vaccine shall be determined by methods approved by the national control authority. These concentrations shall not exceed upper limits specified by the national control authority.

A.4.4.6 Potency tests
A potency test on the final bulk may be performed. The required potency and the assay method shall be approved by the national control authority.

A.5 Filling and containers
The requirements concerning filling and containers in Good Manufacturing Practices for Biological Products (2, section 4) shall apply.

A.6 Control tests on final lot
The national control authority may permit tests for endotoxin, protein content, preservatives and adjuvants to be performed on the final bulk instead of on the final lot.

A.6.1 Identity test
An identity test based on immunological reactivity shall be performed on the vaccine in the final labelled container.

The potency test may serve as an identity test.

A.6.2 Potency test
A potency test on the vaccine in the final container shall be performed if it has not been performed on the final bulk. The required potency of the vaccine and the assay method shall be based on evidence submitted to prove efficacy in clinical trials and shall be approved by the national control authority.

Potency assays for hepatitis A vaccinee have not been worked out in detail.
Manufacturers should devise a validated assay for their product.
A.6.3 Sterility test
Each final lot shall be tested for sterility as specified in Part A, sections 5.1 and 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (5).

A.6.4 General safety tests
Each final lot shall be tested for the absence of abnormal toxicity in mice and guinea-pigs by parenteral injection. The test procedures shall be approved by the national control authority.

A.6.5 Test for endotoxin
Each final lot shall be tested for endotoxin. The test and limits shall be approved by the national control authority.

A.6.6 Test for preservative
Each final lot shall be tested for the presence of preservative, if used. The test used and the permitted concentration shall be approved by the national control authority.

A.6.7 Protein content
Limits shall be defined for the protein content of the final product.

A.6.8 Adjuvants
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.

The formulation shall be such that a homogeneous suspension is administered to the patient.

A.6.9 Residual moisture
If the vaccine is freeze-dried, the residual moisture shall be determined in the final product by a method and with an upper limit approved by the national control authority.

A.6.10 Inspection of final containers
Every container in each final lot shall be inspected visually and those showing abnormalities shall be discarded.

A.7 Records
The requirements in Good Manufacturing Practices for Biological Products (2, section 8) shall apply.
A.8 **Samples**

The requirements in Good Manufacturing Practices for Biological Products (2, section 9) shall apply.

A.9 **Labelling**

The requirements in Good Manufacturing Practices for Biological Products (2, section 7) shall apply, with the addition of the following:

The leaflet accompanying the package shall state:

- the nature of the cell culture used;
- the virus strain used for the production of the vaccine;
- the method used for inactivating the virus;
- the nature and amount of adjuvant and preservative present.

A.10 **Distribution and shipping**

The requirements in Good Manufacturing Practices for Biological Products (2, section 8) shall apply.

A.11 **Storage and expiry date**

The requirements in Good Manufacturing Practices for Biological Products (2) shall apply.

A.11.1 **Storage conditions**

Hepatitis A vaccine (inactivated) shall be stored at all times at a temperature between 2 °C and 8 °C. Alternative storage temperatures shall be justified and approved by national control authorities. Aluminium-absorbed vaccines shall not be frozen.

A.11.2 **Stability of vaccine and expiry date**

The stability of the vaccine shall be established at the recommended storage temperature. The expiry date shall be fixed with the approval of the national control authority.

Accelerated degradation studies at 37°C may provide useful additional information.

**Part B. National control requirements**

B.1 **General**

The general requirements for control laboratories in the Guidelines for National Authorities on Quality Assurance for Biological Products (7) shall apply with the addition of the following:
The national control authority shall approve the strains to be used and the cell substrate, and specify the potency requirements, and shall be satisfied that the results of all tests, including those done on pools during the process of manufacture, are satisfactory and that consistency has been established.

B.2 Release and certification

A vaccine lot shall be released only if it fulfils the national requirements and/or Part A of the present Requirements. A protocol based on the model given in Appendix 1, signed by the responsible official of the manufacturing establishment, shall be prepared and submitted to the national control authority in support of a request for release of vaccine for use.

At the request of the manufacturing establishment, the national control authority shall provide a certificate that states whether the vaccine meets all national requirements and/or Part A of the present Requirements. The certificate shall be based on the model given in Appendix 2.

The purpose of the certificate is to facilitate the exchange of hepatitis A vaccines among countries.

Authors

The first draft of these Requirements was prepared in February 1993 by Dr S. Feinstone, Division of Viral Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA, and by Dr V. Grachev, Scientist, Biologicals, and Dr D. Magrath, Chief, Biologicals, World Health Organization, Geneva, Switzerland. A revised draft was formulated at an informal consultation held in Geneva from 15 to 17 November 1993, attended by the following people:

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References


Appendix 1
Example, for guidance, of a summary protocol for the production and testing of hepatitis A vaccine (inactivated)

Data on the control of source materials are required only on first submission of a product and whenever a change is made.

**Identification of final lot**
- Name and address of manufacturer
- Lot no. of vaccine
- Date of manufacture of final lot
- Expiry date
- Total volume of final lot
- Number of containers and doses

**Control of source materials (A.3)**

*Cell cultures for virus propagation (A.3.1)*

*Cell banks used for providing production cell cultures (A.3.1.1)*
- Origin and short history of cell bank
- Authority that approved cell seed
- Characteristics of cell bank

*Cell culture medium (A.3.1.2)*
- Origin of serum used for cell cultures
- Results of tests performed on serum

*Trypsin used for preparing cell cultures (A.3.1.3)*
- Results of tests performed on trypsin

*Virus seed (A.3.2)*

*Strain of virus (A.3.2.1)*
- Short history
- Authority that approved strain of HAV

---


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Virus seed lot system (A.3.2.2)

Date of preparation of virus master seed lot

Date of preparation of virus working seed lot

Number of passages between master and working seed lots

Number of subcultures between working seed lot and production

Tests on virus seed lots (A.3.2.3)

Identification of the virus seed lot

Method used

Results

Infectivity of seed lot

Method used

Results

Freedom from bacteria, fungi and mycoplasmas

<table>
<thead>
<tr>
<th>bacteria</th>
<th>fungi</th>
<th>mycoplasmas</th>
</tr>
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<tbody>
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<tr>
<td>Media used</td>
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<tr>
<td>Observation period</td>
<td></td>
<td></td>
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<tr>
<td>Results</td>
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</tbody>
</table>

Tests in cell cultures for adventitious viruses

Methods used

Results

Control of vaccine production (A.4)

Control cell cultures (A.4.1)

Observation period

Percentage of culture vessels discarded for non-specific reasons

Results

Tests for haemadsorbing adventitious viruses (A.4.1.1)

Methods

Results
Tests for non-haemadsorbing adventitious viruses (A.4.1.2)
Methods
Results

Identity test (A.4.1.3)
Methods
Results

Control of single virus harvests (A.4.2)
Sterility tests (A.4.2.1)

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<thead>
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<th>mycoplasmas</th>
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<tr>
<td>Results</td>
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Virus content (A.4.2.2)
Method
Results

Preparation and control of purified pool (A.4.3)
Pooling of single harvests (A.4.3.1)
Number of single harvests
Volume of bulk material

Purification procedures (A.4.3.2)
Protein nitrogen test
Method
Results

Animal serum test
Method
Results

Residual DNA test
Method
Results
**Inactivation of virus (A.4.3.3)**

Treatment before inactivation

Method of inactivation
  Agent and concentration
  Temperature
  Date of start of inactivation
  Second sterile filtration
    (if applicable)
  Date of completion of inactivation

Test for effective inactivation
  Method
  Results

Antigen content of bulk suspension
  Method
  Results

**Preparation and control of final bulk (A.4.4)**

Composition of final bulk
(after mixing of all ingredients)
and identification number

**Preservatives (A.4.4.1)**

Concentrations

**Addition of adjuvant (A.4.4.2)**

Volume of bulk
Nature and volume of adjuvant added
and final concentration

**Tests for completeness of adsorption to adjuvant (A.4.4.3)**

Method
Results
Date
Sterility tests (A.4.4.4)

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Tests for chemicals used in production (A.4.4.5)

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Potency tests (A.4.4.6)

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Control tests on final lot (A.6)

Identity test (A.6.1)

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Potency test (A.6.2)

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Sterility test (A.6.3)

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General safety tests (A.6.4)

Test in mice

Date of inoculation

57
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<tr>
<td>Results (give details of deaths)</td>
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<tr>
<td><strong>Test in guinea-pigs</strong></td>
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<td>Date of inoculation</td>
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<tr>
<td>No. of guinea-pigs tested</td>
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<td>Volume and route of injection</td>
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<td>Observation period</td>
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<td>Results (give details of deaths)</td>
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<td><strong>Test for endotoxin</strong> (A.6.5)</td>
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<td><strong>Protein content</strong> (A.6.7)</td>
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<td><strong>Adjuvants</strong> (A.6.8)</td>
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<td><strong>Residual moisture (if applicable)</strong> (A.6.9)</td>
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<td><strong>Inspection of final containers</strong> (A.6.10)</td>
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</table>
Submission addressed to national control authority for batch release

Name (typed) and signature
of head of production laboratory

____________________________________

Date

____________________________________

Certification by person taking overall responsibility for production and control of the vaccine:

I certify that lot no. _____ of hepatitis A vaccine (inactivated) satisfies national requirements and/or Part A of the Requirements for Biological Substances No. 49 (Requirements for Hepatitis A Vaccine (Inactivated)).

Signature

____________________________________

Name (typed)

____________________________________

Date

____________________________________
Appendix 2

**Model certificate for the release of hepatitis A vaccine (inactivated) by national control authorities**


<table>
<thead>
<tr>
<th>Lot no.</th>
<th>Date of last potency test by manufacturer</th>
<th>Expiry date</th>
<th>Lot no.</th>
<th>Date of last potency test by manufacturer</th>
<th>Expiry date</th>
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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): [7]

Name (typed) ________________________________________________________________

Signature ________________________________________________________________

Date ________________________________________________________________

---

1. To be completed by the national control authority of the country where the vaccine has been manufactured, and to be provided by the vaccine manufacturer to importers.
2. Name of manufacturer.
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 distribution and shipping, which the national control authority may not be in a position to assess.
9. Or his or her representative.
Annex 3

Requirements for hepatitis B vaccine prepared from plasma
(Requirements for Biological Substances No. 31, revised 1994)

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Introduction

Viral hepatitis type B is a major public health problem occurring endemically in all parts of the world, more than 2000 million people having been infected with the hepatitis B virus (HBV). The disease is characterized by a relatively long incubation period, high carrier rate, and diverse manifestations, including the serious consequences associated with cirrhosis and hepatocellular carcinoma. More than 80% of all cases of the latter are attributable to HBV, making it second only to tobacco among the known causes of human cancer. In some regions of the world, coinfection and superinfection with hepatitis delta virus have been associated with high morbidity and mortality in HBV-positive individuals. Although rare in infants and children, severe fulminant hepatitis B with hepatic failure and coma may develop in adults. Approximately 300 million people, most of whom live in developing countries, are lifelong chronic carriers of HBV. Such carriers in adulthood are at high risk of serious illness and death from cirrhosis of the liver and primary liver cancer, diseases that kill 1-2 million carriers per year. Primary liver cancer caused by HBV infection is one of the top three causes of cancer-related deaths in much of Africa, Asia and the Pacific Basin. In addition, carriers constitute a permanent reservoir of infected individuals who perpetuate the infection from generation to generation. HBV infection occurring at, or shortly after, birth or in early childhood is especially likely to lead to the chronic carrier state.

In the absence of any specific antiviral therapy for HBV infection, immunization, both passive and active, is the only available means of controlling the disease. Hyperimmune gamma globulin in combination with hepatitis B vaccine has been shown to be extremely effective in preventing infection in children born to HBV-positive mothers. The use of the vaccine alone, although slightly less effective, has also been shown to be capable of reducing this mode of infection in many countries throughout the world. In addition, the widespread use of hepatitis B vaccine in newborn children has been shown to reduce the rate of transmission among older children in the household.

Hepatitis B vaccine, if given before exposure, can prevent infection and disease and the development of a carrier state in almost all individuals. The vaccine is highly effective when included in the infant immunization schedule, although it can be used at any age. Two types of hepatitis B vaccine exist, namely plasma-derived vaccine and recombinant DNA-derived vaccine. Immunity in individuals given the recombinant DNA vaccine can be boosted with plasma-derived vaccine and vice versa. Seroconversion rates with the two vaccines are comparable. Plasma-derived vaccines have been shown to be safe, and transmission of HBV and other viruses, including the human immunodeficiency virus (HIV) and hepatitis C virus (HCV), has not been documented following extensive surveillance. The present document concerns only the plasma-derived vaccine.
HBV is a 42-nm enveloped particle containing a partially double-stranded DNA genome. The envelope consists of host lipids and virus-specific proteins encoded by a large open reading frame that has three in-phase translation start codons. In addition to the infectious virus, two non-infectious morphological forms are produced during infection. All three morphological forms share common antigenic epitopes referred to as the hepatitis B surface antigen (HBsAg), which is antigenically complex and is composed of proteins (75% protein by weight), carbohydrates (in the form of glycoproteins) and host-derived lipids (25%) (1). During infection, hepatocytes synthesize and secrete HBsAg in excess, mainly as lipoprotein in the form of 22-nm diameter particles (2, 3). All HBsAg-containing particles have a group-specific determinant “a”, which confers protection. Additional complexities have led to the recognition of 10 different serotypes of HBsAg (4). However, there is no known improvement in vaccine efficacy when all of the various subtypes are included in the final vaccine formulation. The small 22-nm spherical morphological form can circulate in chronic carriers in very high concentrations sometimes exceeding 100 µg/ml. This form is the source antigen from which plasma-derived HBV vaccine is prepared.

**General considerations**

Because of the worldwide prevalence of hepatitis B infection (5), Requirements for Hepatitis B Vaccine Prepared from Plasma were formulated in 1980 (6), and revised in 1984 (7), and again in 1987 (8). Since immunization with purified 22-nm spherical particles containing HBsAg leads to the production of protective antibody against the group “a” determinant, these particles represent a convenient source of starting material for the production of hepatitis B vaccines.

HBsAg-positive source plasma may contain infectious agents possessing a wide range of physicochemical and biological characteristics, and of various degrees of resistance to different modes of inactivation. Consequently, it is essential that, in addition to the careful selection of donors and methods of separating and purifying the 22-nm HBsAg-containing particles, procedures that are known to inactivate all infectious agents potentially present in human plasma should be applied during the vaccine manufacturing process. Inactivation steps such as heat and formalin treatment have been successfully used alone or in combination to render the purified material free from infectious agents including HIV. Whatever the procedures used to purify HBsAg particles and to inactivate the final product, the resultant vaccines must be demonstrated to be safe (i.e. free from demonstrable virus and other microbial agents), immunogenic (i.e. capable of eliciting antibody against the virus in both animals and humans by the administration of a standardized dose of antigen), and efficacious (i.e. protective against the disease).
Because HBsAg particles are relatively stable, harsh and rigorous treatment can be employed during the purification and inactivation steps. This permits the production of an essentially pure vaccine antigen of uniform composition. Most manufacturers employ an initial fractionation step followed by either chromatographic or ultracentrifugal separation before inactivation. All these procedures are known to be capable of purifying the particles by removing any contaminating blood products and other infectious agents that may be present in the starting material.

For vaccines prepared by urea, pepsin and formalin treatment, it has been shown that each of the three steps can inactivate at least $10^5 \ (5 \ \log_{10})$ infectious units of HBV infectivity (9). Moreover, these processes also inactivate members of all known groups of animal viruses (10). Examination of vaccines prepared by two-step heat inactivation has demonstrated that each step can inactivate at least $10^6 \ (6 \ \log_{10})$ infectious units of HBV (11), and that these procedures are capable of inactivating 12 different animal viruses (12). No transmission of HIV, HBV, HCV or any other virus has been documented following the use of the vaccines currently available.

The safety of most plasma-derived hepatitis B vaccines was initially ensured by safety tests in two to four hepatitis B antibody-free chimpanzees per lot. Because of the safety record of these vaccines in humans, the shortage of animals, and the costs associated with chimpanzee safety trials, any requirement to continue to use chimpanzees to demonstrate safety must be fully justified. Model viruses such as duck hepatitis B virus may be used in validation studies. Nevertheless, the national control authority must be satisfied that any production process used will yield a safe and effective vaccine. This may mean that a safety test using chimpanzees has to form part of the validation of a process proposed by a new manufacturer, or a revised process proposed by an existing one.

The immunogenicity of plasma-derived hepatitis B vaccines has been routinely assessed in mice by a quantitative assay performed with each lot by injecting serial dilutions of alum-adjuvanted vaccine intra-peritoneally (13, 14). This procedure provides an effective dose 50% (ED$_{50}$), defined as the dose at which 50% of the animals seroconvert, which can be compared with that of a simultaneously titrated standard preparation. When carried out properly this test can provide a useful measure of immunogenicity, but there is considerable variation from laboratory to laboratory (15, 16). Thus, if this test is used, it should be highly standardized, and the immunogenicity results for each new lot of vaccine should be compared with those for an internal standard, measured at the same time, rather than with a historical ED$_{50}$ value. An International Reference Reagent has been established for this purpose. It is acknowledged that vaccines that are immunogenic in mice may not be highly immunogenic in humans. However, correlations between the responses in humans and mice should be assessed during clinical trials.
Immunogenicity in humans is expressed as the geometric mean level of anti-HBs antibodies in terms of milli-International Units per ml using an international reference material for comparison.

The efficacy of HBV vaccines can be assessed in terms of their ability to protect against infection, disease or the development of a chronic carrier state. These three end-points are different and may require different levels of immunity. This distinction is important since dosage and schedule regimens designed on the basis of clinical trials and using only the prevention of HBV infection as the end-point may overestimate the dosage and schedule required to prevent the chronic carrier state. This may be the primary goal of immunization in countries of high HBV endemcity, where infections in childhood seldom cause disease but rather lead to the development of the chronic carrier state which accounts for most of the morbidity and mortality in later life.

In the first large-scale studies of the efficacy of a hepatitis B vaccine, a classical randomized double-blind placebo-controlled trial was carried out in a high-risk population of homosexual men (16). This showed a 90% reduction in the incidence of HBsAg antigenaemia and hepatitis that correlated with a 95% seroconversion rate.

New data have suggested that some of the initial requirements for vaccine production and testing, although appropriate at the time, may no longer be relevant. In this revision, the Requirements for Hepatitis B Vaccine Prepared from Plasma (8) have been modified in the light of experience with the use of seven different vaccines over several years.

Each of the following sections constitutes a recommendation. The parts of each section printed in normal type have been written in the form of requirements, so that, if a national control authority so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments and recommendations for guidance. To facilitate the international distribution of vaccine made in accordance with these Requirements, a summary protocol for recording the results of tests is provided as Appendix 1.

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

A.1 Definitions

A.1.1 International name and proper name

The international name shall be “Vaccinum hepatitidis B explasma humanum”. 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.

A.1.2 Descriptive definition

“Vaccinum hepatitidis B explasma humanum” is a preparation of purified hepatitis B surface antigen (HBsAg) that has been treated to inactivate HBV and other pathogens known to be present in human blood. The preparation shall satisfy all the requirements formulated below.

A.1.3 International reference materials

An International Reference Reagent for Plasma-derived Hepatitis B Vaccine for Immunogenicity Studies is available from the National Institute for Biological Standards and Control, Potters Bar, Herts., England.

An International Reference Preparation of Anti-Hepatitis B Immunoglobulin is available from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands.

These materials are distributed free of charge, on request, to national control authorities.

A.1.4 Terminology

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

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.

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

HBV: hepatitis B virus, a 42-nm enveloped virus.

HBsAg: hepatitis B surface antigen, the complex of antigens associated with the virus envelope and subviral forms (22-nm spherical and tubular particles). Native HBsAg is coded for by envelope sequences encoded by the S gene plus pre-S2 and pre-S1.

Anti-HBs: antibody to hepatitis B surface antigen.

Single-donor plasma: plasma obtained from a single donation of whole blood or obtained from a single donor by plasmapheresis.
Plasma pools: pools of single-donor plasma that have been shown to be satisfactory before pooling.

Purified HBsAg batch: purified HBsAg prepared from one or more plasma pools by suitable procedures.

Purified, inactivated HBsAg batch: purified HBsAg prepared from one or more plasma pools by suitable procedures and further treated to inactivate HBV and other pathogens that may be present in human blood or plasma.

Final aqueous bulk: the final bulk before the addition of adjuvant. It consists of one or more purified, concentrated HBsAg batches that have been treated to inactivate infectious agents that may be present in human blood.

Final bulk: The homogeneous adjuvanted finished material prepared from one or more batches of final aqueous bulk 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 vaccine. A final lot must therefore consist of finished material dispensed into containers in one working session from a single final bulk under standardized conditions in a common chamber.

A.2 General manufacturing requirements

The general manufacturing requirements contained in Good Manufacturing Practices for Pharmaceutical (17) and Biological (18) Products shall apply to establishments manufacturing hepatitis B vaccine, with the addition of the following directives:

Completely separate areas shall be used for the separation and inactivation steps. All separation and inactivation steps shall be carried out in closed systems and closely monitored.

Steps should be taken to ensure that no persons in the production areas are chronic carriers of HBsAg and that all such persons are immune to HBV as shown by the presence of anti-HBs, either as a result of natural infection or by immunization.

A.3 Production control

The general production precautions formulated in Good Manufacturing Practices for Biological Products (18, section 6) shall apply to the manufacture of hepatitis B vaccine.

A.3.1 Collection of blood and plasma and selection of donors

Appropriate information is given in Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives (revised 1992) (19).
The criteria for the selection of donors of whole blood or plasma by plasmapheresis, their physical fitness, including a medical history and physical examination, are described in the above-mentioned Requirements, except that the donors must be HBsAg-positive and are not subject to the exclusions relating to HBV. Plasmapheresis of donors of HBsAg-positive plasma shall be permitted, even if the liver function test values are above normal limits in individual donors, provided that the values obtained are stable.

In some countries plasma from donors who are seropositive for malaria, sexually transmitted diseases, human (gamma) herpes virus 4 (Epstein-Barr virus) and cytomegalovirus is excluded from use for hepatitis B vaccine production.

In some countries separate areas or special times are set aside for the collection of plasma known to be HBsAg-positive.

Records should be kept of the identity of the donors for the identification of each batch of vaccine.

When plasma is collected in regions or from populations with a high prevalence of hepatitis delta virus infection, screening of individual donors for evidence of chronic delta virus infection should be considered, although the presence of delta infection need not disqualify a donor.

The acceptable upper limits of liver function tests for donor exclusion should be specified by the national control authority.

Appropriate guidelines to define donor changes of such significance as to justify discontinuation of plasmapheresis should be established by the responsible physician.

No guidelines have been established for the maximum volume of plasma that can be taken in any one year from a chronic HBV carrier, but this volume should not exceed the limits imposed in certain countries for healthy HBsAg-negative donors (19).

Where plasmapheresis is regularly performed on HBsAg-positive donors, it is advisable for equipment to be dedicated specifically for this purpose.

### A.3.2 Tests on single-donor plasma

Each single-donor plasma shall be shown by sensitive tests to be seronegative for HIV-1 and HIV-2 (20) and for HCV. Each single-donor plasma, whether obtained from whole blood or by plasmapheresis, shall be tested for HBsAg content by a method approved by the national control authority.

The subtype of HBsAg is irrelevant since protective immunity is not related to it. Thus, mixing of whole blood or plasma containing different subtypes of HBV is of no concern and may even be desirable.

Many countries have minimum standards of HBsAg content for the acceptance of whole blood or single-donor plasma as the starting material. A convenient and suitable method is to make a 1:100,000 dilution of test plasma in human serum seronegative for HBsAg and anti-HBs and retain only those plasma units that test positive by radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).
immunoassay (EIA) at this dilution. In countries where RIA and EIA are not readily available, a titre of greater than or equal to 1:16 by countercurrent immunoelectrophoresis is acceptable.

A.3.3 **Pooling of single-donor plasma**

Only plasma meeting the requirements of sections A.3.1 and A.3.2 and having a defined minimum amount of HBsAg shall be included in a plasma pool.

A.3.4 **Tests on plasma pools**

Some countries may require the tests of section A.3.2 to be repeated on the plasma pools.

A.3.4.1 **Sterility tests**

Each plasma pool shall be tested for microbial contamination as specified in Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (21). If testing demonstrates microbial contamination, the pool shall be destroyed. The final decision on sample size shall be taken by the national control authority.

A.3.4.2 **Tests for adventitious viruses**

*Tests in cell cultures.* A sample of at least 5 ml of each plasma pool shall be tested for adventitious viruses by inoculation into simian cell cultures. Similar volumes of the plasma pool shall likewise be tested in human diploid cell cultures. The inoculated cell cultures and uninoculated control cultures shall be observed for at least 14 days. At the end of this observation period a subculture in the same cell system shall be made and cultures observed for at least 14 days.

In some countries larger volumes of the plasma pool are required to be tested.

Simian cell cultures are prepared from the kidneys of Cercopithecus monkeys or from Vero cells. For the human cell cultures, either WI-38 or MRC-5 may be used.

The plasma pool passes the test if none of the cell cultures show evidence of the presence of any adventitious virus attributable to the plasma pool. For a test to be valid, no more than 20% of the culture vessels should have been discarded for non-specific reasons by the end of the test period.

*Tests in adult mice.* Each plasma pool shall be tested in adult mice for adventitious viruses pathogenic to mice. Each of at least 10 adult mice, of 15-20 g weight, shall be inoculated intracerebrally with 0.03 ml, and at least 10 mice intraperitoneally with at least 0.5 ml of the plasma pool. The mice shall be observed for at least 21 days. All mice that die after the first 24 hours of the test or that show signs of illness shall be autopsied and examined for evidence of viral infection, both macroscopically by direct observation and by subinoculation of appropriate tissue suspensions by the intracerebral and intraperitoneal route into at least five additional mice, which shall be observed for 21 days.
The plasma pool shall pass the test if at least 80% of the original inoculated mice survive the observation period and if no mice show evidence of infection with adventitious transmissible viruses attributable to the plasma pool.

**Tests in suckling mice.** Each plasma pool shall be tested in suckling mice for adventitious viruses pathogenic to mice. Each of at least 20 mice less than 24 hours old shall be inoculated intracerebrally with 0.01 ml and intraperitoneally with at least 0.1 ml of the plasma pool. The mice shall be observed daily for at least 14 days. All mice that die after the first 24 hours of the test or that show signs of illness shall be autopsied and examined to determine the cause of death or illness.

In some countries a subinoculation (second passage) is suggested and in others an additional blind passage is made of a suspension of the pooled emulsified tissue (minus skin and viscera) of all mice surviving the original 14-day test.

The plasma pool passes the test if at least 80% of the original inoculated mice survive the observation period and if no mice show evidence of infection with adventitious transmissible viruses attributable to the plasma pool.

**Tests in embryonated eggs.** A sample of at least 5 ml of each plasma pool shall be tested in a group of embryonated hens’ eggs by the allantoic route of inoculation and a similar sample in a separate group of eggs by the yolk-sac route of inoculation, using at least 0.25 ml of the plasma pool per egg for each route of inoculation. The incubation of the eggs and the observation time shall be approved by the national control authority. The embryo shall remain normal throughout the observation period.

The plasma pool passes the test if there is no evidence of the presence of any adventitious agents attributable to it.

**Other tests**

In some countries the national control authority may require tests on the plasma pools for HBV DNA to be carried out by sensitive DNA hybridization assays to monitor the elimination of HBV DNA during the subsequent purification steps.

### A.3.5 Concentration, purification and inactivation

Each plasma pool shall be subjected to procedures that concentrate and purify HBsAg reproducibly and result in the inactivation of residual HBV. The methods used shall remove the bulk of extraneous substances and inactivate infectious agents so that the resultant purified product is safe when administered to humans.

#### A.3.5.1 Concentration and purification

Since the purity of the final aqueous bulk is highly dependent on the method of purification, the national control authority shall approve the degree of purity.
The many licensed vaccines that exist worldwide are all freed from contaminating substances by means of similar biochemical and biophysical separation methods. Various combinations of biophysical, biochemical and chemical treatments may be used to concentrate and purify HBsAg from the plasma pool (22-33). The national control authority shall approve the exact combination of methods and shall ensure that the production process is reproducible and will give rise to consecutive lots that do not substantially differ with respect to degree of purity.

In some countries the required HBsAg content of vaccines is not less than 95% of the total protein content of the finished vaccine.

When a new process for the concentration and purification of HBsAg from the plasma pool is introduced, the efficiency of HBV DNA removal at each step during the purification process shall be validated. The national control authority shall approve the acceptable level of residual HBV DNA, the permitted level of contaminating plasma proteins, and the method for determining the identity of non-HBsAg proteins.

Removal of HBV DNA (3.2 kilobases) can be monitored by Southern blot hybridization assay. This assay should be sensitive to 1 pg of HBV DNA, equivalent to approximately 300,000 copies of HBV DNA.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions followed by staining with a sensitive silver stain will permit the detection of all polypeptides. The electropherogram may be scanned by a densitometer to determine what proportion of the total polypeptides is accounted for by each individual polypeptide.

Native PAGE under non-denaturing conditions followed by staining with a sensitive silver stain will permit the detection of all polypeptides not associated with the purified HBsAg; this will remain at the top of the gel while the free polypeptides will enter the gel.

A convenient and suitable way of measuring purity is to determine the specific activity of the purified HBsAg (34). This can be done by dividing the reciprocal of the end-point dilution per ml, as determined by a sensitive RIA or EIA, by the protein concentration quantified by the Lowry method. This method can be used to determine the degree of purity as compared with that of the starting material and of other purified, inactivated HBsAg batches. The higher the specific activity, the greater the degree of purity.

A.3.5.2 Inactivation

The national control authority shall approve the method for the inactivation of HBV and other potential contaminating agents in the purified HBsAg batch. It shall also approve the number and types of inactivation steps that shall be used and the procedures to be used in validating the method. In assessing the efficiency of a procedure or procedures for inactivation the national control authority shall take into consideration data demonstrating the ability of each method to inactivate infectious agents that may be found in human blood. Each of the procedures shall be validated in order to demonstrate lot-to-lot consistency.
A procedure involving two heating cycles, namely 90 seconds at 103 °C followed by 10 hours at 65 °C, has been shown to inactivate members of 12 different virus families (29). A period of warming up to 65 °C has been shown to inactivate representatives of the following virus families: Poxviridae (vaccinia), Picornaviridae (encephalomyocarditis virus), Togaviridae (sindbis virus), Coronaviridae (murine hepatitis virus), Orthomyxoviridae (influenza virus), Rhabdoviridae (vesicular stomatitis virus), Herpesviridae (cytomegalovirus), and Retroviridae (HIV-1, murine leukemia virus). After prolonged heating at 65 °C or heating for 90 seconds at 103 °C, paroviruses and the phage ΦX174 were also completely inactivated (29). Papovaviruses, represented by simian virus 40, were the most heat-resistant, with a reduction of only 10⁴ tissue culture infectious doses (TCID₅₀) per ml after 90 seconds at 103 °C.

In a study of the safety of plasma-derived hepatitis B vaccine prepared following two heating cycles, 10⁶ chimpanzee infectious doses (CID₅₀) of HBV heated at 103 °C for 90 seconds and another 10⁶ doses heated at 65 °C for 10 hours were not infectious in two hepatitis B antibody-free chimpanzees. Thus, the two-step heat inactivation process may be expected to inactivate 10¹² CID₅₀ of HBV DNA (71), which is approximately equivalent to 3 μg of HBV DNA on the assumption that 1 CID₅₀ contains one copy of HBV DNA.

Dilutions of human sera containing between 10⁵ and 10⁶ CID₅₀ of HBV per ml were treated with 1 μg/ml pepsin at pH 2.0 for 18 hours, urea (8 mol/litre) for 4 hours, or 1:4000 formalin for 72 hours, after which 1 ml of each treated serum was inoculated intravenously into one or two chimpanzees. No evidence of HBV infection was seen during a 6-month observation period. Thus, each of these procedures inactivated a minimum of 10⁹ CID₅₀ of HBV DNA in the presence of unfractionated human serum (9), which is approximately equivalent to 3 fg of HBV DNA on the assumption that 1 CID₅₀ contains one copy of HBV DNA.

Inactivation procedures successfully employed in the production of plasma-derived hepatitis B vaccine include: (a) 1:4000 formalin at 37 °C for 48 hours (9); (b) 1:4000 formalin at 30 °C for 48 hours (31); (c) 1:4000 formalin at 36 °C for 72 hours (20); (d) heat treatment at 101–104 °C for 1.5 minutes followed by pasteurization for 10 hours at 65 °C (22); (e) 1:500 formalin at 37 °C for 4 days (35); (f) continuous-flow flash heating at 102 °C for 160 seconds followed by pasteurization for 10 hours at 65 °C (36); and (g) heat inactivation at 101 °C for 90 seconds and, after adsorption to aluminium phosphate, an additional heat inactivation at 65 °C for 10 hours (37).

Heat-inactivation procedures used in some vaccine manufacturing processes induce particle aggregation, which prevents subsequent accurate determination of the HBsAg content. Such aggregation may result in enhanced immunogenicity in vivo, but decreased antigenicity in vitro, as measured by immunological assays, presumably because of steric hindrance of antibody-binding activity. Therefore, if in vitro methods are being used, the HBsAg content of the purified HBsAg batch should be determined before the inactivation step.

A decrease in the concentration of free formaldehyde occurs in the course of the inactivation process. Tests for residual free formaldehyde should therefore be performed at intervals, and the concentration maintained at the desired level by intermittent readjustments.

Some authorities have suggested the use of model viruses, such as duck hepatitis B virus, in validation studies.
The production of hepatitis B vaccine shall be based on a method of inactivation shown to give a consistently safe product. A record of consistency shall have been established by the testing of five consecutive lots (see section A.3.7.1).

The method of inactivation is independent of the method of purification. The national control authority shall approve the combination of procedures used for purification and for inactivation.

A.3.6 Tests on purified, inactivated HBsAg batches

A.3.6.1 Sterility tests
A volume at least 10 ml of the purified, inactivated HBsAg batch shall be tested for sterility as specified in Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (21).

A.3.6.2 Tests for HBsAg and total protein
After purification, the protein content shall be measured for both total protein and HBsAg-specific protein. The latter shall be compared with a suitable reference preparation. If the procedure used for inactivation can interfere with the tests described in sections A.3.6.3 to A.3.6.5, these tests shall be performed before the inactivation step.

It has been found suitable to measure the total concentration of protein by means of extinction coefficient or by the micro-Kjeldahl technique, the Lowry test or another appropriate reproducible method.

The content of HBsAg shall be determined by a sensitive serological test such as EIA or RIA for which the level of sensitivity is known. The concentration of HBsAg shall be related to the total protein. The purity and minimum content of HBsAg shall be approved by the national control authority.

By making limiting two-fold dilutions of the purified, inactivated batch in human sera seronegative for HBsAg and anti-HBs, it is possible to determine the concentration of the starting material by multiplying the limit of sensitivity of the test by the dilution factor.

The degree of purity of the purified, inactivated HBsAg batch can be determined by the methods described in section A.3.5.1.

For the assay of antigenic content by standard immunological tests, a purified International Reference Reagent without adjuvant is recommended. Although such a reference reagent is not currently available, an estimate of the antigenic content may be obtained by preparing serial two-fold dilutions of the purified antigen in normal human serum and determining the end-point by any immunological test for which the sensitivity is known.

A.3.6.3 Tests for extraneous substances
Tests shall be made for the presence of blood group substances and other blood proteins, including liver-specific membrane proteins, by methods approved by the national control authority.
Agglutinins and agglutinogens are tested for by haemagglutination. Immunoassays, such as immunoelectrophoresis, agar gel diffusion, RIA, enzyme-linked immunosorbent assay (ELISA) and PAGE have been used to test for other extraneous proteins.

The preparations shall be free from detectable blood group substances. The permitted concentration of non-HBsAg proteins present in the vaccine shall be determined by the national control authority.

A.3.6.4 Test for HBV DNA
The preparation shall be tested for residual HBV DNA using a sensitive test approved by the national control authority and the level shown to be less than 1 pg HBV DNA per 50 doses.

A.3.6.5 Test for antigen purity
The degree of purity of the purified, inactivated HBsAg batch shall be determined.

The methods described in section A.3.5.1 (25) may be used.

The national control authority shall determine the electrophoretic pattern permitted as a demonstration of purity.

A.3.6.6 Test for reagents used during manufacture
A test shall be made for the presence of any potentially hazardous reagent, including inactivating reagents such as free formaldehyde, that may have been used during the manufacture of the purified, inactivated HBsAg batch.

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

A suitable procedure for the detection of free formaldehyde is described by Freireich & Chandler (38).

A.3.7 Final aqueous bulk

Only batches that have satisfied the requirements in sections A.3.5 and A.3.6 shall be included in the final aqueous bulk.

A.3.7.1 Safety test
In view of the concern about the possibility of infectious agents being present in the plasma used for the production of hepatitis B vaccine, the national control authority shall ensure that the procedures for the collection of plasma and the production process, including purification and inactivation, are well regulated, validated and reproducible, and will give rise to consecutive lots that will not differ with respect to safety.

In the light of the known safety record of currently licensed hepatitis B vaccine when validated inactivation procedures are used, and the high cost and shortage of chimpanzees, the testing of initial lots for the presence of infectious HBV in chimpanzees is no longer considered essential in the control of the vaccine produced by these methods, provided that an alternative procedure is approved by the national control authority.
A.3.7.2 Sterility tests and tests for adventitious viruses
All final aqueous bulks shall be tested for sterility as described in section A.3.4.1 and for adventitious viruses as described in section A.3.4.2.

A.3.7.3 Test for HBsAg
The quantity of HBsAg compared with the total protein in the final aqueous bulk shall be determined by a quantitative serological procedure in comparison with a suitable reference reagent (see section A.1.3). The purity and minimum content of HBsAg shall be approved by the national control authority.

The tests referred to in section A.3.6.2 have been found to be suitable. The samples for this test should be taken before the addition of preservative.

A.3.7.4 Pyrogenicity test
In order to detect non-endotoxin pyrogens, each final aqueous bulk shall be tested for pyrogenicity by a suitable test. The test shall be approved by the national control authority.

A.3.8 Final bulk

A.3.8.1 Addition of adjuvant
Where the final bulk contains an adjuvant, each final bulk or final lot shall be assayed for the content of adjuvant. The adjuvant and the concentration used shall be approved by the national control authority. Where aluminium salts are used, the concentration of aluminium shall not exceed 1.25 mg per single human dose. The national control authority shall determine the method used to quantify aluminium (39-43).

A.3.8.2 Tests for completeness of adsorption to adjuvant
Tests shall be carried out to confirm that HBsAg is adsorbed to the adjuvant. The tests and limits shall be approved by the national control authority.

A convenient and suitable method is to centrifuge the preparation and determine the end-point titre of HBsAg in the supernatant by EIA or RIA. The amount of HBsAg not bound to the adjuvant can be determined by multiplying the end-point dilution by the known sensitivity of the EIA or RIA. This value can be compared with the value obtained before adding adjuvant.

A.3.8.3 Tests for sterility
A volume of at least 10 ml of the final aqueous bulk shall be tested for bacterial and mycotic sterility as specified in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (21).

A.3.8.4 Tests for preservative
Each final bulk or final lot shall be tested for the presence of preservative. The method used and the permitted concentration shall be approved by the national control authority.
The most common preservative used for hepatitis B vaccine is thiomersal. Early analytical methods for organic mercurials involved the decomposition of the metallo-organic compounds (46) and detection with a complexing agent such as diphenylthiocarbazone (47, 48). Greater specificity was achieved by the use of better extraction procedures (49) or column chromatography (50). More sophisticated analytical methods, such as atomic absorption (51), neutron activation (52), gas-liquid chromatography (53) and high-pressure liquid chromatography (54), detect degradation products as well as intact thiomersal. A suitable and simple procedure described by Christensen & Weis Bentzon (55) relies on microbiological methods using agar diffusion from paper discs.

A.3.8.5 Tests for inactivating agent
The concentration of any inactivating agent remaining in the final vaccine shall be determined by the methods approved by the national control authority. This concentration shall not exceed a specified upper limit.

If the manufacturer has validated a process for the total clearance of inactivating agent and if this has been approved by the national control authority, a test may not be required on each batch.

A.3.8.6 Test for potency
An appropriate quantitative potency assay shall be performed on each final bulk or final lot. The vaccine potency shall be compared with that of a reference preparation calibrated against the International Reference Reagent (see section A.1.3). The required potency and the assay method shall be approved by the national control authority. The national control authority may approve the use of a validated in vitro assay.

The following is an example of a method that has been found suitable. Groups of at least 20 suitable mice, 5 weeks of age, are vaccinated intraperitoneally with graded doses of adjuvanted hepatitis B vaccine diluted in the adjuvant used in the vaccine. Similar groups of mice are inoculated with the adjuvanted reference preparation. The mice are bled 28 days later and the sera are kept separate. Antibody determinations are performed by a sensitive quantitative method such as RIA or EIA. The data are analysed according to seroconversion and the geometric mean titre of anti-HBs for each antigen dose. The strain of mice used for this test must give a steep dose-response curve with the reference antigen.

A collaborative study was carried out to examine the suitability of plasma-derived hepatitis B vaccine as an immunogenicity reference (14, 16). The dilution of vaccine required to induce antibodies in 50% of test animals and potency estimates varied widely between laboratories.

In some countries a quantitative extinction test in guineas-pigs has been shown to be suitable.

The potency in an in vivo assay should be measured in terms of the quantity of vaccine giving an antibody response in 50% of the animals (ED50). Since it has been shown that seroconversion in mice is influenced by the amount of pre-S2 polypeptide in the vaccine, the potency in mice may not reflect immunogenicity in humans. The purpose of the potency test is thus to establish an ED50 value to quantify lot-to-lot variation and ensure conformity with the material used in the clinical trial that showed the product to be suitable.
A.4 **Filling and containers**

The requirements concerning filling and containers in Good Manufacturing Practices for Biological Products (18, section 4) 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 conditions of storage.

A.5 **Control tests on final product**

A.5.1 **Identity test**

An identity test using an appropriate method shall be performed on the vaccine in the final container.

ELISA, immunoblot or single-dose immunogenicity tests have been found suitable, depending on whether the antigen can be removed readily from the adjuvant.

A.5.2 **Potency test**

An appropriate quantitative potency assay shall be performed on each final lot or final bulk by the methods described in section A.3.8.6.

A.5.3 **Sterility tests**

The final lot shall be tested for sterility as specified in Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (21).

A.5.4 **General safety tests**

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

A.5.5 **Pyrogenicity test**

Each final lot shall be tested for pyrogenicity by a suitable test. The test shall be approved by the national control authority.

The national control authority may approve replacement of the pyrogenicity test on the final lot by a validated test for endotoxin.

A.5.6 **Test for preservative**

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

Suitable procedures for quantifying thiomersal, the most common preservative, are described in section A.3.8.4.
A.5.7 Assay of adjuvant

Each final lot or final bulk shall be assayed for the content of adjuvant. The method used and 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.

A.6 Records

The requirements in Good Manufacturing Practices for Biological Products (18, section 8) shall apply.

A.7 Samples

The requirements in Good Manufacturing Practices for Biological Products (18, section 9) shall apply.

A.8 Labelling

The requirements in Good Manufacturing Practices for Biological Products (18, section 7) shall apply, with the addition of the following directive:

The leaflet accompanying the package shall include the following information:

– the method used to inactivate HBV;
– the nature and amount of any preservative, adjuvant or stabilizer present in the vaccine;
– the volume of one recommended human dose, immunization schedules and the recommended routes of administration; these 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 HBsAg contained in one recommended human dose.

A.9 Distribution and shipping

The requirements in Good Manufacturing Practices for Biological Products (18, section 8) shall apply. In addition, the conditions of shipping shall be such that the vaccine does not freeze.

Temperature indicators should be packaged with each vaccine shipment to show that freezing did not occur.

If freezing has occurred, the vaccine should not be used.

A.10 Storage and expiry date

The requirements in Good Manufacturing Practices for Biological Products (18) shall apply.
A.10.1 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 vaccines in bulk form or in final containers shall be kept at 2–8 °C. After distribution or issue, the vaccine shall be stored at a temperature not exceeding 8 °C. The vaccine shall have been shown to maintain potency for a period equal to that between the date of issue and the expiry date. During storage the vaccine shall not be frozen.

A.10.2 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, the date of this test being that on which the test system was inoculated.

Part B. National control requirements

B.1 General

The general requirements for control laboratories in the Guidelines for National Authorities on Quality Assurance for Biological Products (56) and the revised Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives (19) shall apply.

The national control authority shall:

– approve the degree of purity;
– approve the method for inactivation and the permitted level of extraneous protein;
– approve the method for determining potency;
– ensure that a reference preparation is available for the expression of activity of HBsAg contained in a given quantity of protein.

B.2 Release and certification

A vaccine lot shall be released only if it fulfils the national requirements and/or part A of the present Requirements. A protocol based on the model given in Appendix 1, signed by the responsible official of the manufacturing establishment, shall be prepared and submitted to the national control authority in support of a request for release of vaccine for use.

At the request of the manufacturing establishment, the national control authority shall provide a certificate that states whether the vaccine meets all national requirements and/or Part A of the present Requirements. The certificate shall be based on the model given in Appendix 2.

The purpose of the certificate is to facilitate the exchange of hepatitis B vaccines among countries.
Authors

The draft of these revised Requirements was prepared by Dr H.A. Fields, Chief, Molecular and Immunodiagnostic Section, Hepatitis Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA, and by Dr V. Grachev, Scientist, Biologicals, and Dr D. Magrath, Chief, Biologicals, WHO, Geneva, Switzerland.

Acknowledgements

Acknowledgements are due to the following experts for their comments and advice: Professor J. P. Cano, Director, National Health Laboratory, Montpellier, France; Ms M. Cona, Vice-President for Scientific Affairs, International Federation of Pharmaceutical Manufactures Associations, Geneva, Switzerland; Dr Darodjatun, President Director, Perum Bio Farma (Pasteur Institute), Bandung, Indonesia; Dr M. Ferguson, Division of Virology, National Institute for Biological Standards and Control, Potters Bar, Herts., England; Professor D. K. Hazra, Chief Consultant, Nuclear Medicine and Radioimmunoassay Unit, Post Graduate Department of Medicine, S.N. Medical College, Agra, India; Dr K. J. Healy, Head, Quality Assurance, CSL Ltd, Victoria, Australia; Professor M. Kantoch, Head, Virology Department, National Institute of Hygiene, Warsaw, Poland; Mr P. E. Lamoine, formerly Head, Biological Standardization, Institute of Hygiene and Epidemiology, Brussels, Belgium; Dr M. Mirchamsy, Associate Director, Razi State Institute of Sera and Vaccines, Teheran, Islamic Republic of Iran; Dr R. Netter, formerly General Inspectorate of Social Affairs, Ministry of Solidarity, Health and Social Protection, Paris, France; Dr J. C. Petricciani, Vice President, Regulatory Affairs, Genetic Institute, Cambridge, MA, USA; Professor G. R. E. Swanker, Division of Biochemistry, Department of Basic Medical Sciences, University of Papua New Guinea, Boroko, Papua New Guinea; Dr M. Weeke-Lüttmann, Paul Ehrlich Institute, Langen, Germany.

References


Appendix 1

Example, for guidance, of a summary protocol for the production and testing of hepatitis B vaccine prepared from plasma

Identification of final lot

Name and address of manufacturer

International name and proprietary name of vaccine

Lot no. of final product

Date of manufacture of final lot

Date containers were filled

Number of containers and nature (ampoules or vials)

Date of last potency test

Number of doses in each container

Volume of single dose

Expiry date

Production control (A.3)

Collection of blood and plasma and selection of donors (A.3.1)

Results

Tests on single-donor plasma (A.3.2)

Methods

Results

HBsAg content

Pooling of single-donor plasma (A.3.3)

Volume of plasma pools

Number of single-donor plasmas pooled

---

Tests on plasma pools (A.3.4)

Sterility tests (A.3.4.1)

<table>
<thead>
<tr>
<th></th>
<th>bacteria</th>
<th>fungi</th>
<th>mycoplasmas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of inoculation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media used</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tests for adventitious viruses (A.3.4.2)

Tests in cell cultures

  Method
  Results
  Date

Tests in adult mice

  Method
  Results
  Date

Tests in suckling mice

  Method
  Results
  Date

Tests in embryonated eggs

  Method
  Results
  Date

Other tests (if applicable)

  Method
  Results
  Date

Concentration, purification and inactivation (A.3.5)

Concentration and purification (A.3.5.1)

Method

85
Results
Date

**Inactivation** (A.3.5.2)
Method
Concentration of inactivator
Date of starting inactivation
Date of finishing inactivation
Results

**Tests on purified, inactivated HBsAg** (A.3.6)

**Tests for sterility** (A.3.6.1)
Date of inoculation
Media used
Observation period
Results

<table>
<thead>
<tr>
<th>bacteria</th>
<th>fungi</th>
<th>mycoplasmas</th>
</tr>
</thead>
</table>

**Tests for HBsAg and total protein** (A.3.6.2)
Method
Results
Date

**Tests for extraneous substances** (A.3.6.3)
Method
Results
Date

**Tests for HBV DNA** (A.3.6.4)
Method
Results
Date

**Tests for antigen purity** (A.3.6.5)
Method
Results
Date
Tests for reagents used during manufacture (A.3.6.6)

Method
Results
Date

Final aqueous bulk (A.3.7)
Safety test (A.3.7.1)

Method
Results
Date

Sterility tests and tests for adventitious viruses (A.3.7.2)

Sterility tests

<table>
<thead>
<tr>
<th>bacteria</th>
<th>fungi</th>
<th>mycoplasmas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Date of inoculation
Media used
Observation period

Results

Tests for adventitious viruses

Tests in cell cultures:
Method
Results
Date

Tests in adult mice:
Method
Results
Date

Tests in suckling mice:
Method
Results
Date

Tests in embryonated eggs:
Method
Results
<table>
<thead>
<tr>
<th>Date</th>
<th>Other tests (if applicable):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method</td>
</tr>
<tr>
<td></td>
<td>Results</td>
</tr>
<tr>
<td></td>
<td>Date</td>
</tr>
</tbody>
</table>

**Test for HBsAg (A.3.7.3)**

<table>
<thead>
<tr>
<th>Method</th>
<th>Results</th>
<th>Date</th>
</tr>
</thead>
</table>

**Pyrogenicity test (A.3.7.4)**

<table>
<thead>
<tr>
<th>Method</th>
<th>Results</th>
<th>Date</th>
</tr>
</thead>
</table>

**Final bulk (A.3.8)**

Composition of final bulk (after mixing of all ingredients) and identification number

Addition of adjuvant (A.3.8.1)

<table>
<thead>
<tr>
<th>Volume of bulk</th>
<th>Nature and volume of adjuvant added and final concentration</th>
</tr>
</thead>
</table>

Tests for completeness of adsorption to adjuvant (A.3.8.2)

<table>
<thead>
<tr>
<th>Method</th>
<th>Results</th>
<th>Date</th>
</tr>
</thead>
</table>

Tests for sterility (A.3.8.3)

<table>
<thead>
<tr>
<th>bacteria</th>
<th>fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of inoculation</td>
<td>Media used</td>
</tr>
<tr>
<td>Observation period</td>
<td>Results</td>
</tr>
</tbody>
</table>
Tests for preservative (A.3.8.4)
Method
Results
Date

Tests for inactivating agent (A.3.8.5)
Method
Results
Date

Tests for potency (A.3.8.6)
Method
Results
Date

Filling and containers (A.4)
Date of filling
Quantity of containers
Volume of vaccine per container
Control for defective containers

Control tests on final product (A.5)
Identity test (A.5.1)
Method
Results
Date

Potency test (A.5.2)\(^1\)
Method
Results
Date

Sterility tests (A.5.3)  
<table>
<thead>
<tr>
<th></th>
<th>bacteria</th>
<th>fungi</th>
<th>mycoplasmas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of inoculation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media used</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) If not done on final bulk.
**General safety tests** (A.5.4)

*Test in mice*
- Date of inoculation
- No. of mice tested
- Volume and route of injection
- Observation period
- Results (give details of deaths)

*Test in guinea-pigs*
- Date of inoculation
- No. of guinea-pigs tested
- Volume and route of injection
- Observation period
- Results (give details of deaths)

**Pyrogenicity test** (A.5.5)
- Method
- Results
- Date

**Test for preservative** (A.5.6)
- Method
- Results
- Date

**Assay of adjuvant** (A.5.7)
- Method
- Results
- Date

---

1 If not done on final bulk.
Submission addressed to national control authority for batch release

Name (typed) and signature of head of production laboratory

________________________________________

Date

________________________________________

Certification by person taking overall responsibility for production and control of the vaccine:

I certify that lot no. _____ of hepatitis B vaccine satisfies national requirements and/or Part A of the Requirements for Biological Substances No. 31 (Requirements for Hepatitis B Vaccine Prepared from Plasma), revised 1994.

Signature

________________________________________

Name (typed)

________________________________________

Date
Appendix 2

**Model certificate for the release of hepatitis B vaccine prepared from plasma by national control authorities**

The following lots of hepatitis B vaccine produced by _______\(^2\) in _______\(^3\), whose numbers appear on the labels of the final containers, meet all national requirements\(^4\) and Part A\(^5\) of Requirements for Biological Substances No. 31 (Requirements for Hepatitis B Vaccine Prepared from Plasma), revised 1994\(^6\) and comply with Good Manufacturing Practices for Pharmaceutical\(^7\) and Biological\(^8\) Products.

<table>
<thead>
<tr>
<th>Lot no.</th>
<th>Date of last potency test by manufacturer</th>
<th>Expiry date</th>
<th>Lot no.</th>
<th>Date of last potency test by manufacturer</th>
<th>Expiry date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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

The Director of the National Control Laboratory (or Authority as appropriate)\(^9\).

Name (typed) ______________________________________________

Signature ________________________________________________

Date ______________________________________________________

---

\(^1\) To be completed by the national control authority of the country where the vaccine has been manufactured, and to be provided by the vaccine manufacturer to importers.

\(^2\) 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 distribution and shipping, which the national control authority may not be in a position to assess.


\(^9\) Or his or her representative.
Annex 4

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. Copies may be obtained from appointed sales agents for WHO publications or from: Distribution and Sales, World Health Organization, 1211 Geneva 27, Switzerland.

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

Additions

**Antibodies**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Quantity/Units</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-toxoplasma human serum</td>
<td>1000 IU/ampoule</td>
<td>Third International Standard 1994</td>
</tr>
</tbody>
</table>

This substance is held and distributed by the International Laboratory for Biological Standards, Statens Serum Institute, 5 Artillerivej, 2300 Copenhagen S, Denmark.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Quantity/Units</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-interferon alpha serum</td>
<td>8000 neutralizing units/ampoule</td>
<td>First International Reference Reagent 1994</td>
</tr>
<tr>
<td>Anti-interferon beta serum</td>
<td>1500 neutralizing units/ampoule</td>
<td>First International Reference Reagent 1994</td>
</tr>
</tbody>
</table>

These substances are held and distributed by the International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Potters Bar, Herts., EN6 3QG, England.

**Antigens and related substances**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Logarithmic Value</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles vaccine (live)</td>
<td>$4.3 \log_{10}$ (20,000)</td>
<td>Second International Reference Reagent 1994</td>
</tr>
<tr>
<td>Mumps vaccine (live)</td>
<td>$4.3 \log_{10}$ (40,000)</td>
<td>First International Reference Reagent 1994</td>
</tr>
<tr>
<td>Rubella vaccine (live)</td>
<td>$3.9 \log_{10}$ (8000)</td>
<td>First International Reference Reagent 1994</td>
</tr>
</tbody>
</table>

---

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poliomyelitis vaccine (inactivated)</td>
<td>430 D-antigen units/ml for type-1 antigen</td>
<td>First International Reference Reagent 1994</td>
</tr>
<tr>
<td></td>
<td>95 D-antigen units/ml for type-2 antigen</td>
<td>First International Reference Reagent 1994</td>
</tr>
<tr>
<td></td>
<td>285 D-antigen units/ml for type-3 antigen</td>
<td>First International Reference Reagent 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood products and related substances</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antithrombin, plasma</td>
<td>0.85 IU/ampoule</td>
<td>Second International Standard 1994</td>
</tr>
<tr>
<td>concentrate, human</td>
<td>6.3 IU/ampoule</td>
<td>Second International Standard 1994</td>
</tr>
<tr>
<td></td>
<td>11.1 IU/ampoule</td>
<td>Second International Standard 1994</td>
</tr>
<tr>
<td>Blood coagulation factor VIII:C</td>
<td>5.4 IU/ampoule</td>
<td>Fifth International Standard 1994</td>
</tr>
<tr>
<td>concentrate, human</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytokines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>2000 IU/ampoule</td>
<td>First International Standard 1994</td>
</tr>
<tr>
<td>Epidermal growth factor (1-52)</td>
<td>1.75 µg/ampoule</td>
<td>First International Reference Reagent 1994</td>
</tr>
<tr>
<td>Insulin-like growth factor 1</td>
<td>150 000 IU/ampoule</td>
<td>First International Standard 1994</td>
</tr>
<tr>
<td>Interferon gamma, recombinant human</td>
<td>80 000 IU/ampoule</td>
<td>First International Standard 1994</td>
</tr>
<tr>
<td>Interleukin-3</td>
<td>1700 IU/ampoule</td>
<td>First International Standard 1994</td>
</tr>
<tr>
<td>Interleukin-4</td>
<td>1000 IU/ampoule</td>
<td>First International Standard 1994</td>
</tr>
</tbody>
</table>

These substances are held and distributed by the International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Potters Bar, Herts., EN6 3QG, England.
These substances are held and distributed by the International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Potters Bar, Herts., EN6 3QG, England.

**Endocrinological and related substances**

Inhibin, recombinant human 150,000 IU/ampoule First International Standard 1994

Somatropin (recombinant DNA-derived human growth hormone) 2.0 mg protein/ampoule First International Standard 1994

Discontinuation

Blood coagulation factor IX concentrate Second International Standard 1993

Insulin-like growth factor 1, for immunoassay First International Reference Reagent 1988

Somatropin (recombinant DNA-derived human growth hormone) First International Reference Reagent 1993
Annex 5

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,¹ as listed here.

Requirements

1. General Requirements for Manufacturing Establishments and Control Laboratories
   Revised 1965, TRS 323 (1966)
   Replaced by “Good manufacturing practices for biological products”, TRS 822 (1992) and “Guidelines for national authorities on quality assurance for biological products”, TRS 822 (1992)

2. Requirements for Poliomyelitis Vaccine (Inactivated)
   Addendum 1985, TRS 745 (1987)

3. Requirements for Yellow Fever Vaccine
   Revised 1975, TRS 594 (1976)
   Addendum 1987, TRS 771 (1988)

4. Requirements for Cholera Vaccine
   Revised 1968, TRS 413 (1969)
   Addendum 1973, TRS 530 (1973)

5. Requirements for Smallpox Vaccine
   Adopted 1966, TRS 323 (1966)

6. General Requirements for the Sterility of Biological Substances
   Revised 1973, TRS 530 (1973)

¹ Abbreviated here as TRS.
7. Requirements for Poliomyelitis Vaccine, Oral
   Revised 1989, TRS 800 (1990)
8. Requirements for Diphtheria, Tetanus, Pertussis and Combined
   Vaccines
   Revised 1989, TRS 800 (1990)
9. Requirements for Procaine Benzylpenicillin in Oil with Aluminium
   Monostearate
   Revised 1966, TRS 361 (1967)
   Discontinued 1989, TRS 800 (1990)
11. Requirements for Dried BCG Vaccine
    Revised 1985, TRS 745 (1987)
    Amendment 1987, TRS 771 (1988)
12. Requirements for Measles Vaccine (Live)
    Revised 1987, TRS 771 (1988)
    Replaced by Requirements No. 47
13. Requirements for Anthrax Spore Vaccine (Live, for Veterinary Use)
    Adopted 1966, TRS 361 (1967)
14. Requirements for Human Immunoglobulin
    Adopted 1966, TRS 361 (1967)
    Replaced by Requirements No. 27
15. Requirements for Typhoid Vaccine
    Adopted 1966, TRS 361 (1967)
16. Requirements for Tuberculins
    Revised 1985, TRS 745 (1987)
17. Requirements for Influenza Vaccine (Inactivated)
    Revised 1990, TRS 814 (1991)
18. Requirements for Immune Sera of Animal Origin
    Adopted 1968, TRS 413 (1969)
19. Requirements for Rinderpest Cell Culture Vaccine (Live) and
    Rinderpest Vaccine (Live)
    Adopted 1969, TRS 444 (1970)
20. Requirements for Brucella abortus Strain 19 Vaccine (Live, for
    Veterinary Use)
    Adopted 1969, TRS 444 (1970)
    Addendum 1975, TRS 594 (1976)
21. Requirements for Snake Antivenins
    Adopted 1970, TRS 463 (1971)
22. Requirements for Rabies Vaccine for Human Use
    Amendment 1992, TRS 840 (1994)
23. Requirements for Meningococcal Polysaccharide Vaccine
    Adopted 1975, TRS 594 (1976)
24. Requirements for Rubella Vaccine (Live)
   Adopted 1976, TRS 610 (1977)
   Replaced by Requirements No. 47

25. Requirements for Brucella melitensis Strain Rev. 1 Vaccine (Live, for Veterinary Use)
   Adopted 1976, TRS 610 (1977)

26. Requirements for Antimicrobial Susceptibility Tests
   I. Agar Diffusion Tests Using Antimicrobial Susceptibility Discs
      Addendum 1982, TRS 687 (1983)
      Addendum 1985, TRS 745 (1987)
      Addendum 1987, TRS 771 (1988)
      Addendum 1989, TRS 800 (1990)
      Addendum 1990, TRS 814 (1991)

27. Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives

28. Requirements for Influenza Vaccine (Live)
   Adopted 1978, TRS 638 (1979)

29. Requirements for Rabies Vaccine for Veterinary Use
   Amendment 1992, TRS 840 (1994)

30. Requirements for Thromboplastins and Plasma Used to Control Oral Anticoagulant Therapy
    Revised 1982, TRS 678 (1983)

31. Requirements for Hepatitis B Vaccine Prepared from Plasma

32. Requirements for Rift Valley Fever Vaccine

33. Requirements for Louse-Borne Human Typhus Vaccine (Live)

34. Requirements for Typhoid Vaccine (Live, Attenuated, Ty 21a, Oral)

35. Requirements for Rift Valley Fever Vaccine (Live, Attenuated) for Veterinary Use

36. Requirements for Varicella Vaccine (Live)
    Revised 1993, TRS. 848 (1994)
37. Requirements for Continuous Cell Lines Used for Biologicals Production
   Adopted 1985, TRS 745 (1987)

38. Requirements for Mumps Vaccine (Live)
   Adopted 1986, TRS 760 (1987)
   Replaced by Requirements No. 47

39. Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques in Yeast
   Adopted 1986, TRS 760 (1987)
   Replaced by Requirements No. 45

40. Requirements for Rabies Vaccine (Inactivated) for Human Use Produced in Continuous Cell Lines
   Adopted 1986, TRS 760 (1987)
   Amendment 1992, TRS 840 (1994)

41. Requirements for Human Interferons Made by Recombinant DNA Techniques

42. Requirements for Human Interferons Prepared from Lymphoblastoid Cells
   Adopted 1988, TRS 786 (1989)

43. Requirements for Japanese Encephalitis Vaccine (Inactivated) for Human Use

44. Requirements for Haemorrhagic Fever with Renal Syndrome (HFRS) Vaccine (Inactivated)
   Adopted 1993, TRS 848 (1994)

45. Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques
   Adopted 1988, TRS 786 (1989)

46. Requirements for Haemophilus Type b Conjugate Vaccines

47. Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)
   Note, TRS 848 (1994)

48. Requirements for Vi Polysaccharide Typhoid Vaccine

49. Requirements for Hepatitis A Vaccine (Inactivated)
   Requirements for Immunoassay Kits [unnumbered]
Other documents

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)
TRS 565 (1975)

Development of national assay services for hormones and other substances in community health care
TRS 565 (1975)

TRS 610 (1977)

Guidelines for quality assessment of antitumour antibiotics
TRS 658 (1981)

The national control of vaccines and sera
TRS 658 (1981)
Replaced by “Guidelines for national authorities on quality assurance for biological products”, TRS 822 (1992)

Procedure for approval by WHO of yellow fever vaccines in connexion with the issue of international vaccination certificates
TRS 658 (1981)

A review of tests on virus vaccines
TRS 673 (1982)

Standardization of interferons (reports of WHO informal consultations)
TRS 687 (1983)
TRS 725 (1985)
TRS 771 (1988)

Production and testing of the WHO yellow fever virus primary seed lot 213-77 and reference batch 168-73
TRS 745 (1987)

Report of a WHO Meeting on Hepatitis B Vaccines Produced by Recombinant DNA Techniques
TRS 760 (1987)

Procedure for evaluating the acceptability in principle of vaccines proposed to United Nations agencies for use in immunization programmes, revised 1988
TRS 786 (1989)

Guidelines for the preparation, characterization and establishment of international and other standards and reference reagents for biological substances, revised 1989
TRS 800 (1990)
Guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology
   TRS 814 (1991)

Good manufacturing practices for biological products
   TRS 822 (1992)

Guidelines for national authorities on quality assurance for biological products
   TRS 822 (1992)

Guidelines for assuring the quality of monoclonal antibodies for use in humans
   TRS 822 (1992)

Laboratories approved by WHO for the production of yellow fever vaccine, revised 1991
   TRS 822 (1992)

Regulation and licensing of biological products in countries with newly developing regulatory authorities
   TRS 858 (1995)
Annex 6

Corrigenda: reference materials for apolipoproteins A-1 and B and haemoglobins A₂ and F

Apolipoproteins A-1 and B

The International Reference Reagent for Apolipoprotein A-1 in Human Serum, established in 1992, and the International Reference Reagent for Apolipoprotein B, established in 1993, are held and distributed by:

WHO Collaborating Center for Reference and Research on Blood Lipids
Centers for Disease Control and Prevention
4770 Buford Highway F20
Atlanta
Georgia 30341-3724
USA.


Haemoglobins A₂ and F

The identification codes given in WHO Technical Report Series, No. 848, 1994, pp. 16-17 for the International Reference Reagents for Raised Haemoglobin A₂ and for Raised Haemoglobin F, established in 1993, should be amended as follows:

**Haemoglobin A₂**
Page 16, line 37: replace ...98/574... with ...89/666...

**Haemoglobin F**
Page 17, line 7: replace ...88/570... with ...85/616...