WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Forty-first Report

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WHO Expert Committee on Biological Standardization
Geneva, 16–23 October 1990

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Introduction

The WHO Expert Committee on Biological Standardization met in Geneva from 16 October to 23 October 1990. The meeting was opened on behalf of the Director-General by Dr J. Idänpää-Heikkilä, Deputy Director, Division of Drug Management and Policies.

General

Growth factors and cytokines

At its thirty-ninth meeting (WHO Technical Report Series, No. 786, 1989, p. 12) the Committee discussed recombinant cytokines. More recently, polypeptide growth factors have assumed increasing importance. Taking this into account, the Committee discussed issues associated with the classification and standardization of various recombinant-DNA-derived products, including growth factors, cytokines and other biological response modifiers. It requested the WHO Secretariat to review the situation and evaluate how WHO should interact with other groups interested in the same issues.

The term “polypeptide growth factors” refers to polypeptides (of relative molecular mass up to 80,000) having a regulatory action on cell differentiation or proliferation through interaction with specific high-affinity cell-surface receptors, and active only at short range (as opposed to classic hormones which act on specific distant targets). Research in this field is expanding rapidly with the discovery both of new growth factors and of new effects of those already known; at the same time, there is increasing evidence of the complexity of the interactions between different growth factors.

The therapeutic and diagnostic potential of a number of growth factors is being investigated, and the need for international reference materials is becoming evident. The major potential therapeutic applications of such growth factors are as anticancer, anti-inflammatory, immunomodulatory and wound-healing agents, and these are currently being investigated. Priority is being given to: (i) epidermal growth factor (EGF), currently undergoing clinical trials as a wound-healing agent and being evaluated in cancer chemotherapy; (ii) acidic and basic fibroblast growth factors (aFGF, bFGF), a group of polypeptides with potential as wound-healing agents; and (iii) platelet-derived growth factor (PDGF) for use in wound-healing and the treatment of chronic ulcers (e.g., varicose, diabetic).

International reference preparations derived from materials of human origin

The Committee noted that the Guidelines for the Preparation, Characterization and Establishment of International and Other Standards
and Reference Reagents for Biological Substances, revised in 1989 (WHO Technical Report Series, No. 800, p. 181), required that, for safety reasons, biological materials of human origin being considered for the preparation of international reference materials should be tested to ensure the absence of infectivity markers for hepatitis B virus human immunodeficiency virus (HIV) and other pathogens.

In considering the establishment of new or replacement international reference materials derived from materials of human origin, and in reviewing the present status of candidate materials that may later be proposed for establishment, the Committee emphasized the need for the starting materials to be screened for hepatitis C virus antibody as well, since tests licensed for that purpose were now available.

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

The Committee noted that the distribution of international reference materials by the four International Laboratories for Biological Standards had continued in 1989 (Table 1) (BS/90.1644). It also noted that the proportions of recipient laboratories in different categories (national control authorities, manufacturers, research laboratories and universities) had been tabulated (Table 2). In view of the widespread use of, and increasing need for, international reference materials, particularly for the products of biotechnology, the Committee stressed the importance of the distribution of such materials in promoting the international standardization of biological products, thus facilitating their free circulation between countries, to the benefit of the health of the peoples of the world.

The Committee agreed that, in order to assign priorities, it would be useful to gather information, through a questionnaire circulated to recipients, on the ways in which the international reference materials are used. The Committee therefore requested the WHO Secretariat to obtain such information and to identify trends in the distribution and use of these materials.

**WHO bank of Vero cells for the production of biologicals**

The Committee was informed of progress in the distribution of ampoules from the WHO bank of Vero cells referred to in its fortieth report (WHO Technical Report Series, No. 800, 1990, p. 11), and noted that, since the cell bank was established in 1989, 26 requests for such ampoules had been received. The Committee was also informed that the WHO Secretariat planned to establish a WHO bank of BHK-21 cells.

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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.
Table 1
International biological standards and reference reagents distributed in 1989 by the International Laboratories for Biological Standards

<table>
<thead>
<tr>
<th>WHO region</th>
<th>Number of ampoules distributed by International Laboratories for Biological Standards</th>
<th>Amsterdam</th>
<th>Copenhagen</th>
<th>Potters Bar</th>
<th>Weybridge</th>
<th>Total</th>
<th>% of total for all regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td></td>
<td>27</td>
<td>25</td>
<td>34</td>
<td>0</td>
<td>86</td>
<td>0.6</td>
</tr>
<tr>
<td>Americas</td>
<td></td>
<td>227</td>
<td>553</td>
<td>1473</td>
<td>18</td>
<td>2271</td>
<td>15.4</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td></td>
<td>20</td>
<td>30</td>
<td>20</td>
<td>3</td>
<td>73</td>
<td>0.5</td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td>1222</td>
<td>1754</td>
<td>6966</td>
<td>187</td>
<td>10129</td>
<td>68.5</td>
</tr>
<tr>
<td>South-East Asia</td>
<td></td>
<td>34</td>
<td>163</td>
<td>612</td>
<td>15</td>
<td>824</td>
<td>5.6</td>
</tr>
<tr>
<td>Western Pacific</td>
<td></td>
<td>54</td>
<td>408</td>
<td>884</td>
<td>59</td>
<td>1405</td>
<td>9.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>1584</strong></td>
<td><strong>2933</strong></td>
<td><strong>9989</strong></td>
<td><strong>282</strong></td>
<td><strong>14788</strong></td>
<td></td>
</tr>
</tbody>
</table>


Table 2
Recipients of international reference materials in 1989 by category

<table>
<thead>
<tr>
<th>Category</th>
<th>Parcels dispatched to each category by the International Laboratories for Biological Standards</th>
<th>Amsterdam&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Potters Bar&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Weybridge&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>National control authorities</td>
<td>526</td>
<td>33</td>
<td>321</td>
<td>21</td>
</tr>
<tr>
<td>Manufacturers</td>
<td>1058</td>
<td>67</td>
<td>1236</td>
<td>79</td>
</tr>
<tr>
<td>Research laboratories</td>
<td>1058</td>
<td>67</td>
<td>1236</td>
<td>79</td>
</tr>
<tr>
<td>Universities</td>
<td>1058</td>
<td>67</td>
<td>1236</td>
<td>79</td>
</tr>
</tbody>
</table>

<sup>a</sup> Figures were not available for parcels dispatched by the State Serum Institute, Copenhagen, Denmark.
<sup>b</sup> Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands.
<sup>c</sup> National Institute for Biological Standards and Control, Potters Bar, Herts., England.
<sup>d</sup> Central Veterinary Laboratory, Weybridge, Surrey, England.
Revision of the publication *Biological substances: international standards and reference reagents, 1986*

The Committee was informed that, because stocks were exhausted, the WHO Secretariat had prepared a revised version of the WHO publication *Biological substances: international standards and reference reagents, 1986.* The Committee agreed that the publication continued to be very useful, and discussed the future organization of materials within it. In particular, the WHO Secretariat was asked to review ways in which substances currently available for distribution could be clearly distinguished from those that had been discontinued or replaced.

The Committee also drew attention to the fact that new infectious agents, such as HIV and hepatitis C virus, continue to be identified, and that there is an ongoing need for the users of biological substances to be advised to take steps to reduce their risk of exposure to any human pathogens that might be present.

**Diphtheria toxoid potency assays**

The Committee noted that, in accordance with the request made in its fortieth report (Technical Report Series, No. 800, 1990, p.13), the Secretariat had revised the document describing the potency determination in mice of diphtheria toxoid in vaccines by means of serum neutralization of diphtheria toxin in Vero cell cultures (BS/89.1613). It agreed that the method would be a useful contribution towards decreasing the cost of using laboratory animals by enabling mice to be used instead of guinea-pigs for the potency testing of adsorbed diphtheria toxoid in vaccines. The Committee nevertheless stressed that laboratories wishing to use the method should first validate it on vaccines of the same composition as those to be tested. The Committee requested the Secretariat to make the revised document (BS/89.1613 rev.1) available on request.

**Antivenoms — guidelines for potency assay**

The Committee recognized that little progress had been made in 40 years of work with the characterization of reference materials for snake venoms and antivenoms, as discussed in its thirty-ninth report (WHO Technical Report Series, No. 786, 1989, p. 14). It recognized further that this was due to a number of factors, of which the most important was the variation in the biological activities of venoms from closely related species and even of venom collected from the same animal at different times of the year. The Committee therefore welcomed the document (BS/90.1641) on guidelines for the assay of antivenoms. After making some changes, the Committee
requested the WHO Secretariat to make copies of the document available
on request.

Residual DNA in biological products from continuous cell lines

The Committee was informed that the results of a collaborative study to
examine the reliability of assays for the measurement of residual DNA in
biological products derived from continuous cell lines revealed both a
marked degree of inaccuracy and large differences in the estimates made
by different laboratories. A total of 15 laboratories in ten countries,
including nine manufacturers and six control laboratories, were asked to
assay DNA in aqueous media to which known amounts of DNA had been
added. In general, there was a tendency to obtain false-positive results in
DNA-free samples and to underestimate the amounts of DNA in positive
samples. This resulted in poor discrimination between DNA-free samples
and those containing low levels of DNA.

Several laboratories obtained values of less than 100 pg of DNA for
samples to which up to 425 pg had been added, and would therefore have
erroneously accepted them as complying with the relevant requirements
published by WHO. One source of error was the processing of the samples
for testing, which can either result in losses or produce false-positive
results due to the introduction of contaminated materials.

All the laboratories used hybridization methods, though there appeared to
be no correlation between specific methodological details and the
accuracy of the results obtained.

Since no laboratory obtained consistently acceptable results, the
Committee concluded that there was clearly a need for an intensive effort
to standardize and improve the reliability of assays for residual DNA.

Potency of oral poliomyelitis vaccine

The Committee noted that the report on the second phase of the
collaborative study on the reliability of laboratory estimates of the
potency of oral poliomyelitis vaccine (BS/90.1651) confirmed the
conclusions reached at its fortieth meeting (WHO Technical Report
Series, No. 800, 1990, p.13), which had been based on the preliminary
data showing that the use of a reference preparation was of greater
benefit than that of a standard method.

The Committee noted that analysis of the results from laboratories
participating in both phases of the study showed that expressing viral
infectivity in terms of that of a reference preparation reduced the
between-laboratory variation in potency estimates to 5.6-fold, as
compared with 8.6-fold when the local method was used. When a common
method was used, the variation was reduced to 6.0-fold, with a further
slight reduction to 4.4-fold when infectivity was expressed in terms of the
reference.
The Committee was informed that, in accordance with the request made in its fortieth report (WHO Technical Report Series, No. 800, 1990, p. 14), a new working reference material was being prepared for distribution on request to determine, in cell cultures, the actual or relative infectivity of oral poliomyelitis vaccines.

**Potency of live measles vaccine**

The Committee noted the results of the collaborative study, referred to in its fortieth report (WHO Technical Report Series, No. 800, 1990, p. 17), to assess consistency within, and variation between, laboratories in estimates of the potency of live measles vaccine (BS/90.1643). This showed that, at least for the strains covered by the study (Schwarz, Edmonston-Zagreb, CAM-70 and Moraten), expressing the potency of vaccines in terms of that of the reference preparation reduced the between-laboratory variation in potency estimates from 80-fold to 10-fold. The Committee therefore recognized that comparison of estimates of the potency of vaccine samples is improved by the inclusion in assays of the International Reference Reagent for the Assay of Measles Vaccine (Live).

**Common matrix for immunoassays of thyroid-stimulating hormone**

The Committee noted the final report (BS/90.1947) of the joint WHO/International Federation of Clinical Chemistry collaborative study organized by the National Institute for Biological Standards and Control, Potters Bar, on the use of a common matrix for assays of thyroid-stimulating hormone, referred to in its thirty-ninth report (WHO Technical Report Series, No. 786, 1989, p. 22). It noted that the main conclusion of the study was that, for well-controlled two-site immuno-metric assays of thyroid-stimulating hormone, agreement between the estimates in serum made by different laboratories was not likely to be substantially improved by the use of a common matrix.

**Antibiotics**

**Teicoplanin**

The Committee noted that the preparation of teicoplanin referred to at its thirty-ninth meeting (WHO Technical Report Series, No. 786, 1989, p. 15) had been subjected to a collaborative study organized by the National Institute for Biological Standards and Control, Potters Bar, in five laboratories in five countries (BS/90.1642). The Committee established the preparation studied, in ampoules coded AES 0029, as the International Standard for Teicoplanin and assigned an activity of 51 550 International Units of Teicoplanin to the contents of each ampoule.
Antibodies

Rabies immunoglobulin

The Committee noted that a replacement was needed for the International Standard for Rabies Immunoglobulin (BS/90.1639), and was informed that a candidate material had been offered. It therefore requested the State Serum Institute, Copenhagen, to obtain this material and to organize a collaborative study.

Anti-measles serum

The Committee was informed that there was an urgent need to replace the International Reference Preparation of Anti-Measles Serum. It noted that the National Institute for Biological Standards and Control, Potters Bar, had identified a candidate material and completed a collaborative study (BS/90.1636). The Committee also noted that the material, which had been ampouled in 1966, appeared to be stable, but requested the National Institute for Biological Standards and Control, Potters Bar, to conduct accelerated stability studies to check that it had adequate stability. Nevertheless, on the basis of the results of the study, it established the preparation, in ampoules coded 66/202, as the second International Standard for Anti-Measles Serum, and assigned an activity of 5 International Units of Anti-Measles Serum to the contents of each ampoule.

Anti-poliovirus serum

The Committee was informed that the three International Standards for Anti-Poliovirus Serum Types 1, 2 and 3 were virtually exhausted and that attempts to replace them by animal sera had been unsuccessful. It was also informed that the National Institute for Biological Standards and Control, Potters Bar, had acquired a quantity of trivalent human antiserum freeze-dried in ampoules which might be suitable as a replacement. The Committee was further informed that a collaborative study was being organized, and expressed the hope that the report on that study would be finalized as a matter of urgency.

Cytomegalovirus immunoglobulin

The Committee was informed that the collaborative study of the candidate international reference material for cytomegalovirus immunoglobulin referred to in its thirty-ninth report (WHO Technical Report Series, No. 786, 1989, p. 15) was in progress in ten laboratories in seven countries, and that cytomegalovirus immunoglobulin preparations from two sources were included in the study.
Anti-\textit{Haemophilus influenzae} type b polysaccharide serum

The Committee noted that the Center for Biologics Evaluation and Research, Bethesda, had completed studies of a preparation of anti-\textit{Haemophilus influenzae} type b polysaccharide serum (BS/90.1640). It also noted that the data available on the stability of the preparation were limited and that the collaborative study did not cover a wide range of international laboratories. The Committee, therefore, while expressing its appreciation to the Center for making this reference material available, agreed that further studies were needed both on stability and the mode of expression of results, to support acceptance of the preparation as an international reference material.

Tetanus immunoglobulin

The Committee was informed that preliminary evidence indicated that the candidate reference material for tetanus immunoglobulin referred to in its thirty-ninth report (WHO Technical Report Series, No. 786, 1989, p.15) was suitable as regards both precision of fill and thermal stability. It was also informed that the National Institute for Biological Standards and Control, Potters Bar, had arranged a collaborative study in 18 laboratories.

Antigens

\textbf{International Reference Preparation of BCG Vaccine}

The Committee noted that supplies of the current International Reference Preparation of BCG Vaccine might be sufficient to last for another ten years (BS/90.1638). Nevertheless, because of a potential increase in demand, it requested the Secretariat to monitor the situation. The Committee also noted that a working reference material had been available for comparing BCG vaccines in clinical trials. However, there was a demand for a working reference material of the same strain as the International Reference Preparation. A candidate material had been acquired for this purpose, and collaborative studies were being organized by WHO and by the State Serum Institute, Copenhagen.
Blood products and related substances

Anti-D (anti-Rhₐ) complete blood-typing serum
(chemically modified)

The Committee noted that the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, had obtained material suitable to serve as a candidate international standard for anti-D (anti-Rhₐ) complete blood-typing serum (chemically modified), and had completed a collaborative study in nine laboratories in eight countries (BS/90.1645).

The Committee established the material studied as the International Standard for Anti-D (Anti-Rhₐ) Complete Blood-Typing Serum (Chemically Modified) and, on the basis of the results, assigned an activity of 128 International Units of Anti-D (Anti-Rhₐ) Complete Blood-Typing Serum (Chemically Modified) to the contents of each ampoule.

The Committee requested the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, to continue studies to evaluate the stability of the new International Standard, in view of the instability of unmodified anti-D complete blood-typing serum (WHO Technical Report Series, No. 745, 1987, p. 21).

Antithrombin III concentrate

The Committee noted that the National Institute for Biological Standards and Control, Potters Bar, had obtained material suitable to serve as a candidate international standard for antithrombin III concentrate, and that a collaborative study in 14 laboratories in 11 countries had been completed (BS/90.1652).

The Committee established the material studied, in ampoules coded 88/548, as the International Standard for Antithrombin III Concentrate and, on the basis of the results of the collaborative study, assigned an activity of 5.2 International Units of Antithrombin III Concentrate to the contents of each ampoule.

The Committee agreed that there was a continued need for the International Reference Preparation of Antithrombin III, Plasma, as a reference material for the calibration of plasma standards.

Thrombin

The Committee was informed that, in view of the need to replace the International Reference Preparation of Thrombin with material of higher purity, the National Institute for Biological Standards and Control, Potters Bar, had obtained a quantity of purified human alpha-thrombin, and a collaborative study had been completed. It was also informed that the results of the collaborative study were being analysed and that stability studies were in progress.
Plasma fibrinogen

The Committee noted that, in accordance with the request made in its thirty-sixth report (WHO Technical Report Series, No. 745, 1987, p. 23), the National Institute for Biological Standards and Control, Potters Bar, had obtained candidate materials to serve as an international standard for erythropoietin derived from recombinant DNA (rDNA), and that the collaborative study in 26 laboratories in 11 countries referred to in its fortieth report (WHO Technical Report Series, No. 800, 1990, p. 21) had been completed (BS/90.1650).

The Committee established one of the materials studied, in ampoules coded 87/684, as the International Standard for Erythropoietin, rDNA-Derived, and, on the basis of the results of the collaborative study and with the agreement of the participants, assigned an activity of 86 International Units of Erythropoietin, rDNA-Derived, to the contents of each ampoule.

The Committee noted the differences shown in this study between human urinary erythropoietin and rDNA-derived erythropoietin synthesized in different cell lines, and recommended that WHO keep under consideration the possibility of establishing separate standards for naturally occurring erythropoietin and for rDNA-derived erythropoietin produced in different cell lines.

The Committee further noted that the three other preparations of rDNA-derived erythropoietin included in the collaborative study might be useful as international reference materials in the future.

Endocrinological and related substances

Recombinant-DNA-derived erythropoietin

The Committee noted that, in accordance with the request made in its thirty-sixth report (WHO Technical Report Series, No. 745, 1987, p. 23), the National Institute for Biological Standards and Control, Potters Bar, had obtained candidate materials to serve as an international standard for erythropoietin derived from recombinant DNA (rDNA), and that the collaborative study in 26 laboratories in 11 countries referred to in its fortieth report (WHO Technical Report Series, No. 800, 1990, p. 21) had been completed (BS/90.1650).

The Committee established one of the materials studied, in ampoules coded 87/684, as the International Standard for Erythropoietin, rDNA-Derived, and, on the basis of the results of the collaborative study and with the agreement of the participants, assigned an activity of 86 International Units of Erythropoietin, rDNA-Derived, to the contents of each ampoule.

The Committee noted the differences shown in this study between human urinary erythropoietin and rDNA-derived erythropoietin synthesized in different cell lines, and recommended that WHO keep under consideration the possibility of establishing separate standards for naturally occurring erythropoietin and for rDNA-derived erythropoietin produced in different cell lines.

The Committee further noted that the three other preparations of rDNA-derived erythropoietin included in the collaborative study might be useful as international reference materials in the future.

Porcine inhibin

The Committee noted that the collaborative study of the proposed international reference material for porcine inhibin referred to in its thirty-ninth report (WHO Technical Report Series, No. 786, 1988, p. 27) had been completed (BS/90.1648). It also noted that a preparation of porcine inhibin partially purified from ovarian follicular fluid had been included in the study, together with other preparations of human, bovine
and ovine origin, and that ten laboratories in eight countries had participated, using a range of *in vitro* bioassays. On the basis of the results, the Committee established the material, in ampoules coded 86/690, as the International Standard for Inhibin, Porcine, assigning a potency of 2000 International Units of Inhibin, Porcine, to the contents of each ampoule.

**Human inhibin**

The Committee was informed that there was a need for an international standard for recombinant human inhibin and that a candidate material had been acquired. It therefore requested the National Institute for Biological Standards and Control, Potters Bar, to arrange a collaborative study.

**Anti-thyroid microsome antibodies**

The Committee was informed that there was a need for an international reference material for anti-thyroid microsome antibodies. It noted that a material ampouled in 1966 and coded 66/387 (BS/78.1189) had been used for many years, a unitage of 1000 units being assigned to the contents of each ampoule. The Committee therefore requested the National Institute for Biological Standards and Control, Potters Bar, to provide documentation regarding the use of this material and its characteristics, including stability data.

**Insulin-like growth factor-1**

The Committee was informed that, in view of the findings referred to in its thirty-ninth report (WHO Technical Report Series, No. 786, 1989, p. 23), the National Institute for Biological Standards and Control, Potters Bar, had obtained a further 200 mg of insulin-like growth factor-1 to serve as a candidate international reference material for both bioassays and immunoassays. It was also informed that, after preliminary stability studies, the material had been distributed into ampoules and that a collaborative study would be arranged.

**Human calcitonin**

The Committee was informed that there was a need to replace the International Reference Preparation of Calcitonin, Human, prepared in 1970, given that purer products were now available for use in therapy and that the stocks were almost exhausted. It therefore requested the National Institute for Biological Standards and Control, Potters Bar, to obtain candidate preparations for distribution into ampoules and to arrange a collaborative study.

**Porcine calcitonin**

The Committee was informed that there was a need to replace the International Reference Preparation of Calcitonin, Porcine, prepared in
1970, given that purer products were now available for use in therapy and that stocks were almost exhausted. It therefore requested the National Institute for Biological Standards and Control, Potters Bar, to obtain candidate preparations for distribution into ampoules and to arrange a collaborative study.

Cytokines

Granulocyte/macrophage colony-stimulating factor
The Committee was informed that the collaborative study of the proposed international reference material for granulocyte/macrophage colony-stimulating factor, referred to in its fortieth report (WHO Technical Report Series, No. 800, 1990, p. 24), was in progress in 28 laboratories in ten countries using a range of in vitro bioassays and immunoassays.

Granulocyte colony-stimulating factor
The Committee was informed that the collaborative study of the proposed international reference material for granulocyte colony-stimulating factor, referred to in its fortieth report (WHO Technical Report Series, No. 800, 1990, p. 24), was in progress in 28 laboratories in ten countries using a range of in vitro bioassays and immunoassays.

Tumour necrosis factor, alpha
The Committee was informed that the collaborative study of the proposed international reference material for tumour necrosis factor, alpha, referred to in its fortieth report (WHO Technical Report Series, No. 800, 1990, p. 25), was in progress in 22 laboratories in eight countries using a range of in vitro bioassays and immunoassays.

Interleukin-1 alpha and beta
The Committee noted that the final report of the collaborative study referred to in its fortieth report (WHO Technical Report Series, No. 800, 1990, pp. 22-23) was now available (BS/90.1649).

Requirements for biological substances

Requirements for Haemophilus type b conjugate vaccines
The Committee noted that the WHO Secretariat had prepared draft requirements for Haemophilus influenzae type b conjugate vaccines

\(^{1}\) For a summary list of all the requirements for biological substances and other sets of recommendations, see Annex 6.
(BS/90.1634). After making some amendments, it adopted the Requirements for *Haemophilus* Type b Conjugate Vaccines and agreed that they should be annexed to this report (Annex 1).

**Requirements for influenza vaccine (inactivated) (revised 1990)**

The Committee noted that the WHO Secretariat had prepared revised requirements for influenza vaccine (BS/90.1635). After making some amendments, it adopted the Requirements for Influenza Vaccine (Inactivated) and agreed that they should be annexed to this report (Annex 2).

**Pharmaceutical and biological products prepared by recombinant DNA technology**

The Committee was informed that the comments it made at its fortieth meeting (WHO Technical Report Series, No. 800, 1990, p. 9) on the draft guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology (BS/89.1609 rev. 1) had been extensively reviewed by members of the WHO Expert Advisory Panel on the International Pharmacopoeia and Pharmaceutical Preparations. The Committee, while not formally adopting the guidelines, agreed that the revised document was valuable and that it should be annexed to this report (Annex 3).

**Requirements for antimicrobial susceptibility tests**

I. Agar diffusion tests using antimicrobial susceptibility discs (addendum 1990)

The Committee noted that, since the publication of the 1989 addendum to the Requirements for Antimicrobial Susceptibility Tests incorporating new codes for antimicrobial substances (WHO Technical Report Series, No. 800, 1990, Annex 3), the WHO Secretariat had received further requests for the allocation of codes for new antimicrobial substances, and that a draft addendum to the Requirements had been prepared. The Committee adopted this further addendum with some minor modifications and agreed that it should be annexed to this report (Annex 4).

The Committee agreed, following discussions, that its meetings were not an appropriate forum for the adoption of disc codes or for the revision of the Requirements. It therefore requested the Secretariat to explore alternative mechanisms for carrying out these activities.

**Guidelines and definitions for the drafting of Requirements for Biological Substances**

The Committee noted that the WHO Secretariat had prepared a draft document on the definitions and expressions used in the various Requirements for Biological Substances (BS/90.1637). It strongly supported this initiative and suggested that the WHO Secretariat take steps
to make it known to national control authorities as well as international organizations currently involved in negotiations aimed at harmonizing requirements. The Committee emphasized its own and WHO's long history of experience in this area and recommended that WHO should take the lead in this important area of biological standardization. It recommended that the document should be revised, on the basis of comments already received, and distributed to a broad group of individuals for additional comment. The Committee further suggested that the WHO Secretariat convene an informal consultation to discuss outstanding issues and to assist in preparing a revised document. In particular, it requested the WHO Secretariat to examine the suitability of using the term “potency” when the activity of the products concerned was related to the presence of infectious particles.

**Guidelines on quality assurance for biologicals**

The Committee was informed that a meeting had been held in Ottawa (11-15 June 1990) to prepare revised guidelines for national authorities and manufacturers on quality assurance for biological products, as requested in its fortieth report (WHO Technical Report Series, No. 800, 1990, p. 12). It reviewed the draft guidelines and made a number of comments. The Committee was also informed that a revised draft could be prepared by the WHO Secretariat and circulated to interested parties for comment. It expressed its appreciation to the WHO Secretariat for undertaking the preparation of this document, and agreed that it would be valuable to both manufacturers and national authorities.
CORRIGENDUM

Page 26, Table A.3, column D, line 10:
Delete  1 µg/kg
Insert  0.1 µg/kg
Annex 1

Requirements for *Haemophilus* type b conjugate vaccines (Requirements for Biological Substances No. 46)

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</tr>
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<td>Model certificate for the release of <em>Haemophilus</em> type b conjugate vaccines</td>
<td>37</td>
</tr>
</tbody>
</table>

**Introduction**

*Haemophilus influenzae* causes several infectious human diseases, the commonest and most serious being meningitis. Other bacteremic infections caused by this pathogen include pneumonia, epiglottitis, cellulitis, septic arthritis, osteomyelitis and pericarditis. Nearly all cases of meningitis and most cases of other bacteremic diseases caused by *H. influenzae* are due to type b organisms, one of the six capsular polysaccharide types of this species (1). Diseases caused by noncapsulated strains generally do not result in bacteraemia. These Requirements will therefore deal only with *Haemophilus influenzae* type b (*Haemophilus* type b) vaccines, derived from the type b polysaccharide, a linear copolymer composed of units of 3-β-D-ribose-f(1→1)-ribitol-5-PO₄⁻ (sometimes referred to as PRP) (2).
A number of the epidemiological characteristics of *H. influenzae* type b disease clearly show its public health importance. The annual incidence of disease caused by this bacterium among children in the first 5 years of life is in the range 0.02-0.5% (3–7). More than half the cases occur in young children less than 2 years of age and the highest incidence is in the first year. Nevertheless, this bacterium is the leading cause of bacterial meningitis in many countries. The mortality of treated *H. influenzae* type b meningitis ranges from approximately 5% in developed countries to 50% or greater in many developing countries. In the former, 25–40% of children surviving *H. influenzae* type b meningitis have permanent neurological sequelae, the most serious of which are hearing loss and mental retardation (8, 9). The bacterium is contagious and can cause outbreaks of meningitis where susceptible children are crowded together, e.g., in day-care centres (10, 11). An additional concern has been the development of strains resistant to antibiotics (12), and especially to ampicillin. Public health control of meningitis and other bacteraemic infections caused by *H. influenzae* type b will require active immunization of young infants.

The low incidence of *H. influenzae* type b diseases in older children and adults correlates with the presence of bactericidal antibodies, directed mainly to the *H. influenzae* type b capsular polysaccharide, in their serum (3). Consistent with this, the first vaccine against *H. influenzae* type b disease was made from the type b capsular polysaccharide. In 1977, *Haemophilus* type b polysaccharide vaccine was shown in Finland to be protective in older children (13); the vaccine was, however, ineffective in inducing antibodies or providing protection in children less than 18 months old (14–17). On the basis of these findings, the vaccine was licensed in the United States in 1985 for use in children over the age of 24 months. Its lack of efficacy in the age group at greatest risk stimulated the development of improved *Haemophilus* type b vaccines.

The two immunological properties of the *Haemophilus* type b polysaccharide that limit its use in infants and young children are its age-related immunogenicity and its failure to elicit a T-cell-dependent booster response (14–16, 18). However, the immunogenicity can be enhanced by linking the polysaccharide covalently to a protein (19–24). Several different *Haemophilus* type b polysaccharide conjugates stimulate T-cell-dependent antibody synthesis in infants and young children with both a booster response and, by comparison with nonconjugated vaccine, an increased proportion of immunoglobulin (IgG) antibody (22, 25–29).

The first *Haemophilus* type b conjugate vaccine was licensed in the United States in 1987 (30). Since then, more than 6 million doses have been given to children 18 months of age and older. In this age group, the *Haemophilus* type b conjugate vaccine elicits the production of more than 1.0 μg of antibody per ml of serum in at least 75% of those vaccinated. Post-licensure surveillance in the United States indicates that the conjugate vaccine elicits high levels of protection in this age group.
*Haemophilus* type b conjugate vaccines have also been evaluated in infants in other countries and shown to possess enhanced immunogenicity, by comparison with nonconjugated vaccines, with T-cell-dependent characteristics. In Finland, a conjugate vaccine given at 3, 4 and 6 months of age afforded significant protection from bacteraemic disease (26, 37), whereas in native Americans in Alaska the same conjugate vaccine given at 2, 4 and 6 months of age did not provide such protection (32). Further efficacy studies of several *Haemophilus* conjugate vaccines are in progress. The demonstration that *Haemophilus* type b conjugate vaccines are capable of inducing protective immunity in young infants has important public health implications.

**General considerations**

The production and control of *Haemophilus* type b conjugate vaccines are more complex than those of other capsular polysaccharide vaccines, such as meningococcal polysaccharide vaccines (33) or pneumococcal vaccines. Meningococcal vaccines consist of defined chemical entities and are expected to have comparable potencies, regardless of manufacturer. This, however, does not yet apply to *Haemophilus* type b conjugate vaccines, since both the configuration of the polysaccharide and the carrier proteins may be different (25). The common features of *Haemophilus* type b conjugate vaccines are that they contain antigenic determinants capable of stimulating the production of serum IgG antibodies to *H. influenzae* type b polysaccharide, and are capable of inducing a booster response in previously immunized young children (22, 24-30). Because of the different production methods used, the *Haemophilus* type b conjugate vaccines produced by different manufacturers differ in their chemical composition, so that certain control tests must be product-specific.

For the same reason, requirements for *Haemophilus* type b conjugate vaccines must take into account the differences in vaccine composition. National control authorities will therefore need to consider the control methods, specifications and degree of consistency of production achieved before agreeing to the use of such vaccines. Consistency of production and the demonstration that the product does not differ from vaccine lots shown to be safe and adequately immunogenic and protective in clinical studies are important components of the vaccine evaluation and licensing processes.

Currently, at least six manufacturers are producing or developing *Haemophilus* type b conjugate vaccines. Four such vaccines have been evaluated clinically and have either been licensed or are likely to be licensed by national control authorities for use in the near future. Extensive experience has been gained with the use of some of these *Haemophilus* type b conjugate vaccines in Finland and the United States. The composition of these vaccines is described in Table A1 and is considered in this Annex. The immunogenicity in humans of both the polysaccharide
Table A1
Formulation of four currently available Haemophilus type b conjugate vaccines*

<table>
<thead>
<tr>
<th>Nature of Haemophilus material</th>
<th>Quantity per single human dose (µg)</th>
<th>Nature of carrier protein</th>
<th>Quantity per single human dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Polysaccharide (size-reduced)</td>
<td>25</td>
<td>Diphtheria toxoid</td>
<td>18</td>
</tr>
<tr>
<td>B Polysaccharide (low relative molecular mass)</td>
<td>10</td>
<td>Diphtheria CRM 197 protein</td>
<td>25</td>
</tr>
<tr>
<td>C Polysaccharide (size-reduced)</td>
<td>15</td>
<td>Outer membrane protein complex of Neisseria meningitidis Group B</td>
<td>250</td>
</tr>
<tr>
<td>D Polysaccharide</td>
<td>10</td>
<td>Tetanus toxoid</td>
<td>20</td>
</tr>
</tbody>
</table>

* For guidance purposes only. See also section A.3.5.

and protein components of Haemophilus type b conjugate vaccines must be assessed before any such vaccine can be licensed; guidance on methods of evaluating immunogenicity is given in Appendix 1.

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

Should individual countries wish to adopt these Requirements as the basis of their national regulations on Haemophilus type b conjugate vaccines, it is recommended that modifications be made only on condition that the modified requirements ensure that the vaccine is at least as safe and potent as that prepared in accordance with the requirements formulated below. The World Health Organization should then be informed of the action taken.

Part A. Manufacturing requirements

A.1 Definitions

A.1.1 Proper name

The proper name of the vaccine shall be “Haemophilus type b conjugate vaccine” translated into the language of the country of use. The use of this name shall be limited to vaccines that satisfy the requirements formulated below.
A.1.2 **Descriptive definition**

*Haemophilus* type b conjugate vaccine is a preparation of polysaccharide from *H. influenzae* type b covalently bound to a carrier. The preparation shall satisfy the requirements formulated below.

A.1.3 **International reference materials**

The preparation of a WHO international reference material for assessing the antibody response to *Haemophilus* type b conjugate vaccine is under consideration.

In the absence of international reference materials for *Haemophilus* type b conjugate vaccines, manufacturers should set aside as reference material part of the vaccine tested in the clinical trial on the basis of which the licence was granted.

A.1.4 **Terminology**

*Master seed lot:* A bacterial suspension of *H. influenzae* type b organisms derived from a strain that has been processed as a single lot and is of uniform composition. It is used for preparing working seed lots. Master seed lots shall be maintained in the freeze-dried form or frozen at or below −45 °C.

*Working seed lot:* *H. influenzae* type b organisms derived from the master seed lot by growing the organisms and maintaining them in aliquots in the freeze-dried form or frozen state at or below −45 °C. The working seed lot is used, if applicable after a fixed number of passages, for inoculating production medium.

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

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

*Processed polysaccharide:* Purified polysaccharide that has been modified by chemical reaction in preparation for conjugation to the carrier.

*Carrier:* The protein to which processed polysaccharide is bound for the purpose of eliciting a T-cell-dependent immune response to the *Haemophilus* type b polysaccharide.

*Bulk conjugate:* A conjugate prepared from a lot or pool of lots of polysaccharide covalently bound to the carrier. It is the parent material from which the final bulk is prepared.

*Final bulk:* The homogeneous preparation present in a single container from which the final containers are filled either directly or through one or more intermediate containers.
Final lot: A number of sealed, final containers that are equivalent with respect to the risk of contamination during filling and, if appropriate, freeze-drying. A final lot must therefore have been filled from a single container and freeze-dried in one continuous working session.

A.2 General manufacturing requirements

The general requirements for manufacturing establishments, contained in the revised Requirements for Biological Substances No.1 (General Requirements for Manufacturing Establishments and Control Laboratories) (34) shall apply to establishments manufacturing *Haemophilus* type b conjugate vaccines with the addition of the following:

Details of standard operating procedures for the preparation and testing of *Haemophilus* type b conjugate vaccines adopted by a manufacturer together with evidence of appropriate validation of each production step shall be submitted for approval to the national control authority. Proposals for modifications, if any, of the manufacturing/control methods shall also be submitted for approval to the national control authority before they are implemented.

Personnel employed in the production and control facilities shall be adequately trained and protected against accidental infection with *H. influenzae* type b.

A.3 Production control

A.3.1 Control of source materials

A.3.1.1 Strains of *Haemophilus influenzae* type b

The strain of *H. influenzae* type b to be used in preparing *Haemophilus* type b conjugate vaccine shall be identified by a record of its history, including the source from which it was obtained and the tests made to determine the characteristics of the strain. The strain shall have been shown to be capable of producing type b polysaccharide.

A.3.1.2 Seed lot system

The production of *Haemophilus* type b polysaccharide shall be based on a working seed lot system. Cultures derived from the working seed shall have the same characteristics as cultures of the strain from which the master seed lot was derived. Seed lots shall be in conformity with the requirements of section A.3.2.1.

A.3.1.3 Culture media for production of *Haemophilus* type b polysaccharide

The *H. influenzae* type b working seed lot shall be inoculated in a liquid medium that does not contain blood-group substances or polysaccharides of high relative molecular mass.
A.3.1.4 Carrier proteins
Some carrier proteins that have been used to prepare *Haemophilus* type b conjugate vaccines are listed in Table A1 (page 18). The requirements to be satisfied by a carrier are that its composition must be characterized, and that it must be shown to be safe and nontoxic. If toxoids or variants of a toxin such as the diphtheria CRM 197 protein are used, appropriate tests must be carried out to ensure that these proteins are nontoxic. If diphtheria or tetanus toxoids are used as carriers, they must satisfy the requirements laid down in sections A.1, A.2 and A.3.1-A.3.4 of the Requirements for Biological Substances Nos. 8 and 10 (35) that apply to diphtheria and tetanus toxoid vaccines.

A.3.2 Single harvests
Consistency of growth of *H. influenzae* type b microorganisms shall be demonstrated by monitoring the growth rate, pH and yield of polysaccharide.

A.3.2.1 Control of bacterial purity
Samples of the culture taken before killing shall be tested for contamination by microscopic examination of Gram-stained smears and by inoculation on to appropriate plating media. Several microscopic fields shall be examined at high magnification so that at least 10000 organisms are inspected. If any contaminant is found, the culture or any product derived from it shall be discarded.

A.3.3 Purified polysaccharide
All steps in the purification procedure shall be carried out in clean containers made of material compatible with the solvents in use during the particular step of the procedure. Chemicals of an appropriate purity shall be employed.

Each lot of *Haemophilus* type b polysaccharide shall be tested for purity. All chemical analyses shall be based on the dry weight of the polysaccharide in its salt form.

The partially purified *Haemophilus* type b polysaccharides shall be stored frozen at or below −20 °C.

A.3.3.1 Moisture content
The moisture content of the purified polysaccharide shall be determined by suitable methods approved by the national control authority.

A.3.3.2 Ribose content
The ribose content shall be not less than 32% of the dry weight, as estimated by the Bial reaction for pentose (36), using D-ribose as a standard.

For the calcium and sodium salts of ribose, the ribose content can be calculated as 40.5% and 40.2% of the salt content, respectively.
A.3.3.3 Phosphorus content
The phosphorus content shall be between 6.8% and 9.0% of the dry weight, as determined by the method of Chen et al. (37).

The theoretical phosphorus content is 8.4%.

A.3.3.4 Protein content
Each lot of purified polysaccharide shall contain less than 1% by weight of protein, as determined by the method of Lowry et al., using bovine plasma albumin as a reference (38). At least 1 mg of polysaccharide shall be assayed.

Other methods of protein determination may be used if approved by the national control authority.

A.3.3.5 Nucleic acid content
Each lot of purified polysaccharide shall contain less than 1% by weight of nucleic acid as determined by spectroscopy, on the assumption that the internal transmission density (absorbance) of a 10 g/l nucleic acid solution contained in a cell 1 cm wide at 260 nm is 200 (38).

A.3.3.6 Endotoxin content
The endotoxin content of the purified polysaccharide shall be less than 10 International Units of Endotoxin per µg when measured by a Limulus amoebocyte lysate test. Alternatively, the Haemophilus type b polysaccharide shall pass a pyrogen test in rabbits at 1.0 µg of purified polysaccharide per kg of rabbit weight.

A.3.3.7 Molecular size
The molecular size of each lot of purified polysaccharide shall be estimated by gel filtration.

Sepharose CL-4B or CL-2B gels with a 0.2 mol/l buffer (38) are suitable for this purpose.

The distribution constant \(K_D\) shall be determined by measuring the molecular distribution of the polysaccharide at the main peak of the elution curve. The \(K_D\) value must be shown to be consistent for a given product.

A.3.3.8 Identity test
A test shall be performed on the purified polysaccharide to verify its identity.

The test method shall be either a serological method or \(^{13}\text{C}\) nuclear magnetic resonance spectroscopy (2).

A.3.4 Processed polysaccharide
Most of the processed polysaccharide preparations are partially depolymerized either before or during the chemical modification.
A.3.4.1 Chemical modification
Methods found to be satisfactory include the following:

1. The polysaccharide is reacted with cyanogen bromide to introduce groups reactive with "spacer molecules" or with the carrier protein. Excess reactants are removed from the polysaccharide by ultrafiltration.

2. Size-reduced polysaccharides are generated by periodate oxidation of the purified polysaccharide to generate free aldehyde groups. The resulting low molecular weight polysaccharide containing 15–30 repeat units is purified.

3. The polysaccharide is reacted with carbonyldimidazole and butanediamine to form a reactive intermediate with a terminal amino group. This group is acylated to form the final derivatized polysaccharide.

4. Adipic acid dihydrazide is covalently bound to the polysaccharide through cyanogen bromide activation of the polysaccharide.

The processed polysaccharide shall be assessed for the number of functional groups introduced for use in the conjugation reaction.

A.3.4.2 Molecular size distribution
The degree of size reduction of the polysaccharide will depend on the manufacturing process. A gel filtration method and the size distribution of the size-reduced polysaccharide shall be specified for each type of conjugate vaccine, as these may affect the reproducibility of the conjugation process.

A.3.5 Carrier protein
Different proteins have been used as carriers in Haemophilus type b conjugate vaccines. Test methods to confirm the identity of such proteins, to check that they are free from impurities and safe, and to determine their concentration and serological properties, shall be approved by the national control authority.

Proteins and purification methods that have been used include:

1. Diphtheria and tetanus toxoids. These must satisfy the relevant requirements published by WHO.

2. Diphtheria CRM 197 protein, a variant of diphtheria toxin of minimal toxicity, isolated from cultures of Corynebacterium diphtheriae C7 (B197) (20). Protein of purity greater than 90% is prepared by column chromatographic methods.

3. Outer membrane protein complex of Neisseria meningitidis group B extracted from washed bacterial cells with buffer containing detergent. The cell debris is removed and the membrane complex concentrated and washed with the buffer containing detergent to remove impurities.
### Table A2
**Methods currently used for conjugation of *Haemophilus influenzae* type b polysaccharide and control of conjugates**

<table>
<thead>
<tr>
<th>Method</th>
<th>Procedure</th>
<th>Assay for conjugation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reductive amination</td>
<td>Combine carrier protein and aldehyde form of polysaccharide in presence of reducing agent</td>
<td>Formation of unique amino acid and gel filtration</td>
</tr>
<tr>
<td>Carbodiimide-mediated coupling</td>
<td>Combine reactants in presence of carbodiimide</td>
<td>Gel filtration</td>
</tr>
<tr>
<td>Cyanogen-bromide activation of polysaccharide</td>
<td>Addition of carrier protein to cyanogen-bromide-activated polysaccharide</td>
<td>Gel filtration and assay for bound polysaccharide</td>
</tr>
<tr>
<td>Thioether bonding</td>
<td>Combine haloacyl polysaccharide with protein thiol</td>
<td>Formation of unique amino acid and gel filtration</td>
</tr>
</tbody>
</table>

* For guidance purposes only.

#### A.3.5.1 Processing of carrier protein

In some conjugation procedures, reactive functional groups or “spacers” may be introduced into the carrier protein before conjugation to the polysaccharide. As a measure of consistency, the degree of “substitution” of the protein needs to be monitored at this stage.

Protein activation methods that have been used include:

1. The introduction into diphtheria toxoid of a specified concentration of "spacer groups" reactive with activated polysaccharide.
2. The addition of thiol groups to the outer membrane protein complex of *Neisseria meningitidis* group B.

#### A.3.6 Bulk conjugate

A number of conjugation methods are currently in use (see Table A2); all involve multistep processes. Both the method and the control procedures used to ensure the reproducibility, stability, and safety of the conjugate should be established once the immunogenicity of a particular *Haemophilus* type b conjugate vaccine has been demonstrated.

Residual unreacted functional groups potentially capable of reacting *in vivo* may be present following the conjugation process. The manufacturing process shall be validated to show that it does not produce bulk vaccines containing such groups; any remaining groups should be made unreactive during manufacture by means of “capping agents”.

After the conjugate has been purified, the tests described below shall be performed in order to assess consistency of manufacture.
A.3.6.1 Residual reagents
The conjugate purification procedures shall remove the reagents used for conjugation and capping. The removal procedure shall be validated. Each batch shall be assayed for these reagents.

A.3.6.2 Unbound polysaccharide
The immunologically active polysaccharide component of the vaccine is the covalently bound Haemophilus type b polysaccharide.

Each batch of conjugate shall be tested for unbound polysaccharide in order to ensure that the amount present in the purified bulk is within the limits laid down by the national control authority.

Methods that have been used to assay unbound polysaccharide include gel filtration, ultrafiltration and hydrophobic chromatography.

Vaccines demonstrating immunogenicity in clinical studies currently contain amounts of unbound polysaccharide ranging from less than 10% to 37%.

A.3.6.3 Polysaccharide content
The content of H. influenzae type b polysaccharide shall be chemically determined by means of an appropriate validated assay.

A.3.6.4 Protein content
The protein content of the conjugate shall be chemically determined by means of an appropriate validated assay.

A.3.6.5 Polysaccharide to protein ratio
The polysaccharide to protein ratio of the conjugate shall be calculated. For each conjugate, the ratio shall be within the range approved for that particular conjugate by the national control authority. The ratios for four existing conjugates are shown in Table A3.

A.3.6.6 Molecular size
The molecular size of the polysaccharide is an important parameter in establishing consistency of production and in studying physicochemical stability during storage.

The molecular size of the polysaccharide shall be determined for each bulk, using a gel matrix appropriate to the expected size of the conjugate (38).

The distribution constants of four Haemophilus type b conjugate vaccines currently available are shown in Table A3.

A.3.6.7 Absence of blood-group substances
Purified bulk conjugates shall be shown to be free from detectable blood-group substances.

A.3.6.8 Sterility
The bulk purified conjugate shall be tested for bacterial and mycotic
<table>
<thead>
<tr>
<th>Stage of production</th>
<th>Test</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
</tr>
<tr>
<td><strong>Haemophilus</strong></td>
<td>Ribose content</td>
<td>&gt;32%</td>
</tr>
<tr>
<td>polysaccharide</td>
<td>Nucleic acid content</td>
<td>&lt;1.0%</td>
</tr>
<tr>
<td></td>
<td>Protein content</td>
<td>&lt;1.0%</td>
</tr>
<tr>
<td></td>
<td>Pyrogen (i.v. test)¹</td>
<td>1 µg/kg</td>
</tr>
<tr>
<td><strong>Carrier protein</strong></td>
<td>Purity</td>
<td>Not specified</td>
</tr>
<tr>
<td><strong>Limulus</strong> amoebocyte lysate</td>
<td>&gt;1500 Lf/mg N¹</td>
<td>Not specified</td>
</tr>
<tr>
<td></td>
<td>Pyrogen (i.v. test)¹</td>
<td>Not specified</td>
</tr>
<tr>
<td></td>
<td>Nucleic acid content</td>
<td>Not specified</td>
</tr>
<tr>
<td></td>
<td>&lt;1.0%</td>
<td>&lt;1.5%</td>
</tr>
<tr>
<td><strong>Bulk conjugate</strong></td>
<td>Polysaccharide/protein ratio</td>
<td>1.25–1.75</td>
</tr>
<tr>
<td></td>
<td>Distribution constant ($K_0$)</td>
<td>0.30–0.70</td>
</tr>
<tr>
<td></td>
<td>(gel filtration with CL-4B Sepharose gel)</td>
<td>0.05–0.10</td>
</tr>
<tr>
<td></td>
<td>95%² &lt;0.75</td>
<td>85%² &lt;0.25</td>
</tr>
<tr>
<td></td>
<td>Free polysaccharide</td>
<td>60%³ &lt;0.60</td>
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<tr>
<td></td>
<td>&lt;37%</td>
<td>&lt;15%</td>
</tr>
<tr>
<td></td>
<td>&lt; 5%</td>
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<td></td>
<td>Free protein</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td></td>
<td>&lt; 1%</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td><strong>Final vaccine</strong></td>
<td>Polysaccharide dose (±20%)</td>
<td>25 µg</td>
</tr>
<tr>
<td></td>
<td>Pyrogen (i.v. test)¹</td>
<td>10 µg</td>
</tr>
<tr>
<td></td>
<td>Pyrogen (i.m. test)¹</td>
<td>15 µg</td>
</tr>
<tr>
<td></td>
<td>1 µg/kg</td>
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<tr>
<td></td>
<td>Not specified</td>
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</tr>
<tr>
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<td>10 µg/µl/kg</td>
</tr>
<tr>
<td></td>
<td>Not specified</td>
<td>Not specified</td>
</tr>
</tbody>
</table>

¹ For guidance purposes only.
² Weight of polysaccharide, carrier protein or final vaccine per kg of rabbit weight at which the test must be passed; i.v. = intravenous, i.m. = intramuscular.
³ Limit of flocculation units per mg of protein nitrogen.
⁴ Percentage of conjugate whose $K_0$ should lie below the specified value.
sterility in accordance with the requirements of Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (39) or by a method approved by the national control authority. If a preservative has been added to the product, appropriate measures shall be taken to prevent it from interfering with the test.

A.3.6.9 Stability
Manufacturers shall characterize their conjugate in order to validate the maximum storage period of bulk conjugates compatible with the validity period indicated for the final product.

The polysaccharide component of conjugate vaccines is subject to gradual hydrolysis at a rate which may vary with the type of conjugate.

A.3.7 Final bulk
A.3.7.1 Sterility
Each final bulk shall be tested for bacterial and mycotic sterility as indicated in section A.3.6.8.

A.3.8 Filling and containers
The requirements concerning filling and containers given in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (34) shall apply.

Single-dose or multiple-dose containers may be used. Vaccine in the latter shall contain a suitable antimicrobial preservative.

A.3.9 Control tests on final product
A.3.9.1 Identity
An identity test, which shall be a serological test, using antibodies specific for the purified polysaccharide, shall be performed on at least one labelled container from each final lot.

A.3.9.2 Sterility
The contents of final containers and, if applicable, diluents for freeze-dried final products shall be tested for bacterial and mycotic sterility as indicated in section A.3.6.8.

A.3.9.3 Haemophilus type b polysaccharide content
The polysaccharide content of vaccines in final containers shall be determined and shall be within 20% of the stated dose. The national control authorities shall approve the test procedure.

The conjugate vaccines produced by different manufacturers differ in formulation (see Table A1). A quantitative assay for the H.influenzae type b polysaccharide in the final container may be product-specific; colorimetric or serological methods may be used.
A.3.9.4 Potency
There is as yet no direct method of measuring the potency of *Haemophilus* type b conjugate vaccines which can give results guaranteeing that they will elicit protective immunity in the target population. However, if the results of the tests for purity, molecular size, composition and dosage confirm consistency of production and conformity with the specifications of the vaccine used in clinical trials, this is some indication, though not definite proof, of suitable immunogenicity. To demonstrate immunogenicity, therefore, a sample of each final lot of vaccine shall be tested by inoculation into mice, and the level of *H. influenzae* type b polysaccharide antibodies stimulated by the vaccine determined. The test method shall be approved by the national control authority. The reference vaccine shown to have given protective immunity in infants shall be included as a control (see section A.1.3). The mean antibody response to the test vaccine shall not be less than that to the reference vaccine.

The immunogenicity and T-cell-dependent properties of *Haemophilus* type b conjugate vaccines can be evaluated in mice (27). However, the results of such evaluations have not yet been correlated with the immunological properties of the vaccines in human infants.

A.3.9.5 Moisture content
If the vaccine is freeze-dried, the average moisture content shall be determined by methods accepted by the national control authority. Values shall be within limits that have been shown to be suitable.

A.3.9.6 Pyrogen content
The vaccine in the final container shall be tested for pyrogenic activity by intravenous injection into rabbits. The national control authority shall determine the dose of *Haemophilus* type b polysaccharide conjugate that, when administered to rabbits, is expected to pass the test.

Existing *H. influenzae* type b conjugate vaccines pass the test when injected into rabbits in amounts ranging from 0.025 to 1.0 μg per kg of body weight (see Table A3).

Evidence may be presented to the national control authority to justify substitution of a *Limulus* amoebocyte lysate test for the rabbit thermal induction test.

A.3.9.7 Adjuvant content
If an adjuvant is present in the diluent or vaccine, the content shall be determined by a method approved by the national control authority.

If aluminium or calcium compounds are used as adjuvants, the concentration of aluminium shall not exceed 1.25 mg and that of calcium 1.3 mg per single human dose.

A.3.9.8 Preservative content
If a preservative has been added to the vaccine, the content of preservative
in each final lot shall be determined. The method and the concentration shall be approved by the national control authority.

A.3.9.9 Innocuity
A representative sample from every final lot shall be tested for unexpected toxicity. Only obviously healthy guinea-pigs weighing 250-350 g and mice weighing 17-22 g shall be used. The animals shall not have been used previously for any test purpose. The duration of the test shall be 7 days. Each animal shall be observed every working day. The test shall consist of the intraperitoneal injection of not less than one recommended human dose in a volume of not more than 1 ml into each of five mice and the intraperitoneal injection of not less than five human doses into each of two guinea-pigs. The innocuity test is satisfactory if all animals satisfy all of the following requirements:

- they survive the test period;
- they do not exhibit any unexpected local or general response to the product; and
- their weight at the end of the test period is not less than that at the time of injection.

A.3.9.10 Inspection of final containers
Each container in each final lot shall be inspected visually, and those showing abnormalities such as improper sealing, lack of integrity and, if applicable, clumping or the presence of particles shall be discarded.

A.4 Records
The requirements given in Part A, section 6, of the revised Requirements for Biological Substances No.1 (General Requirements for Manufacturing Establishments and Control Laboratories) shall apply.

Written records shall be kept of all tests, irrespective of their results. The records shall be of a type approved by the national control authority.

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

A.6 Labelling
The requirements given in part A, section 8, of the revised Requirements for Biological Substances No.1 (General Requirements for Manufacturing Establishments and Control Laboratories) shall apply with the addition of the following:
The label on the carton or the leaflet accompanying the container shall indicate:

- the amounts of *Haemophilus* type b polysaccharide and protein contained in each single human dose;
- the temperature recommended during transport.

### A.7 Distribution and transport

The requirements on release for distribution and shipping given in Part A, section 9, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (34) shall apply.

### A.8 Stability test, storage and validity period

#### A.8.1 Stability test

The polysaccharide component of conjugate vaccines is subject to gradual hydrolysis at a rate which may vary with the type of conjugate.

Tests shall be conducted before licensing to determine to what extent the stability of the product has been maintained throughout the proposed validity period; final containers from at least three final lots derived from different bulk conjugates shall be tested at the end of that period to assess stability during storage. The unbound polysaccharide and unbound protein content shall be determined. The vaccine shall meet the requirements for final product (see Part A, sections 3.9.1-3.9.10) up to the expiry date.

When any changes are made in the production procedure that may affect the stability of the product, the vaccine produced by the new method shall be shown to be stable.

The statements concerning storage temperature and expiry date appearing on the label, as required in Part A, section 6, shall be based on experimental evidence and shall be submitted for approval to the national control authority.

#### A.8.2 Storage conditions

Storage of liquid vaccine at a temperature of 2–8 °C has been found to be satisfactory.

Freeze-dried vaccine should be stored at 2–8 °C.

#### A.8.3 Validity period

The validity period shall be approved by the national control authority. It shall take into consideration the date on the stability of the bulk purified conjugate as well as the results of the stability tests referred to in section A.8.1.
Part B. Requirements for national control authorities

B.1 General

The general requirements for control laboratories contained in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (34) shall apply.

The national control authority shall:

- specify the clinical testing requirements;
- specify the potency requirement;
- approve the method of manufacture;
- approve the methods of purification and conjugation;
- approve the concentration of preservative and, if applicable, of adjuvant;
- approve the test for stability; and
- provide the national reference materials.

B.2 Official release and certification

A vaccine lot shall be released only if it satisfies Part A of the present Requirements.

A statement signed by the appropriate official of the national control authority shall be provided at the request of the manufacturing establishment and shall certify that the lot of vaccine in question satisfies all national requirements as well as Part A of the present Requirements. The certificate shall state the number under which the lot was released by the national controller, and the number appearing on the labels of the containers. Importers of Haemophilus type b conjugate vaccines shall be given a copy of the official national release document.

The purpose of the certificate is to facilitate the exchange of vaccines between countries. An example of a suitable certificate is given in Appendix 2.

Authors

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References


20. Anderson, P. Antibody responses to *Haemophilus influenzae* type b and


Appendix 1

**Evaluation of immunogenicity of Haemophilus type b conjugate vaccines in humans**

Different lots of *Haemophilus* type b conjugate vaccine from each manufacturer should be evaluated for immunogenicity in the target age group before licensing.

**Response to polysaccharide**

The results of the radioimmunoassay (RIA) of antibodies to the polysaccharide (1-3) have been shown to be the best available correlate of the clinical effectiveness of *Haemophilus* type b vaccines. One method of assessing the immunogenicity is to assay the serum antibody response to the *Haemophilus* type b conjugate vaccine using a suitable RIA procedure\(^1\) in which the *Haemophilus* type b reference human serum is included. This applies to serum samples taken before and 2-3 months after each injection. The percentage of vaccinees with a serum antibody concentration equal to, or greater than, 0.15 µg/ml and 1.0 µg/ml should be reported, as well as the geometric mean antibody concentration and the distribution of values. The duration of the protective antibody response should also be assessed. It is important to show that the conjugate vaccine stimulates a statistically significant serum IgG response.

The functional activity of the conjugate-induced antibodies should be assessed by measuring the serum bactericidal activity against *H. influenzae* type b (1, 4). Enzyme-linked immunosorbent assays (ELISA) have also been used to measure total *Haemophilus* type b antibody content, isotype and subclass composition.

**Response to carrier protein**

It is essential that serum antibodies to the carrier protein be measured in recipients of *Haemophilus* type b conjugates to ensure that the conjugate vaccine does not interfere with protective immunity to that protein. In addition, the measurement provides information about the potential of the *Haemophilus* type b conjugate to serve as a dual immunogen for both the *H. influenzae* type b polysaccharide and the carrier protein. To date, proteins such as diphtheria and tetanus toxoids and an outer membrane protein complex of *Neisseria meningitidis* Group B have been used in the preparation of *Haemophilus* type b conjugates. The assay for the antibodies to carrier proteins from pathogenic bacteria elicited by *Haemophilus* type b conjugate vaccines should be a bioassay or an established correlate.

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\(^1\) Information on a suitable RIA procedure is available on request from Biologicals, World Health Organization, Avenue Appia, 1211 Geneva 27, Switzerland.
If the carrier protein is diphtheria or tetanus toxoid or diphtheria CRM 197 protein, a diphtheria toxin variant, the manufacturer should provide evidence that, when the *Haemophilus* type b conjugate is injected at the same time as adsorbed toxoid vaccine, the recipients produce at least the same amount of specific antitoxin as control patients (i.e., recipients of the toxoid vaccine in the recommended formulation but without the *Haemophilus* type b conjugate).

If the carrier protein is the outer membrane protein complex of *N. meningitidis* Group B, the manufacturer should demonstrate that the sera of vaccinees contain functional antibodies to *N. meningitidis* Group B at a level comparable to, or higher than, those in an unvaccinated control group.

References


Appendix 2

Model certificate for the release of *Haemophilus* type b conjugate vaccines

The following lots of *Haemophilus* type b conjugate vaccine produced by ____________ in ____________, whose numbers appear on the labels of the final containers, meet all national requirements, Part A of the Requirements for Biological Substances No. 46 (Requirements for *Haemophilus* Type b Conjugate Vaccines [if applicable, revised 19______, addenda 19______]), and the Requirements for Biological Substances No.1 (General Requirements for Manufacturing Establishments and Control Laboratories [revised 1965]).

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

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

The number of this certificate is ________________________________

The Director of the National Control Laboratory (or Authority as appropriate):

Name (typed) ________________________________

Signature ________________________________

Date ________________________________

---

1 To be provided by the national control authority of the country where the vaccines have been manufactured, on request by the manufacturer.
2 Name of manufacturer.
3 Name of country.
4 If any national requirement is (are) not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national control authority.
5 With the exception of the provisions on shipping, which the national control authority may not be in a position to control.
7 Or his or her representative.
Annex 2
Requirements for influenza vaccine (inactivated) (Requirements for Biological Substances No. 17, revised 1990)

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Introduction

Influenza is important as a cause of severe morbidity and mortality that disrupts work in schools, the public services and elsewhere. During major epidemics many people require medical treatment or hospitalization. Excess mortality often accompanies influenza epidemics, the vast majority affected being the elderly. Since they constitute the most rapidly increasing sector of the populations of many countries — projections show that their numbers are likely to double by early in the next century — the epidemiology of influenza can be expected to change accordingly, especially in the developed countries.

The only means of influenza prophylaxis generally available at present is vaccination.

In 1967, a group of experts formulated requirements for inactivated influenza vaccine and these were published as an annex to the twentieth report of the Expert Committee on Biological Standardization (1). During the following five years, technical developments in the purification of the virus suspensions from which vaccines were made, as well as in the measurement of the virus content, were such that the potency of whole virus vaccines could be expressed in international units. Accordingly, an addendum to the requirements was annexed to the twenty-fifth report of the Expert Committee on Biological Standardization (2, pp. 15-17).

In its twenty-ninth report (3), the Committee recognized that technical developments had completely altered the method of measurement of the haemagglutinin content of the vaccines and that the International Reference Preparation of Influenza Virus Haemagglutinin (Type A) established in 1967 was no longer appropriate for controlling the haemagglutinin content of inactivated influenza vaccines since it no longer represented the haemagglutinin of the prevalent strains. Accordingly, the International Reference Preparation was withdrawn, and the Committee recommended that the requirements for inactivated influenza vaccine should be revised. Revised requirements were approved by the WHO Expert Committee on Biological Standardization in 1978 and published in 1979 (4).

Manufacturers have gained much experience in the last decade and great progress has been made in the use of continuous cell lines (5) at certain stages in the production of biologicals. In the light of the foregoing and the recommendations and suggestions made at WHO informal consultations on influenza vaccines held in 1986 (6) and 1989, a new revision of the Requirements for Inactivated Influenza Vaccine has been prepared that provides for the use of continuous cell lines for the isolation of the candidate viruses.
General considerations

Inactivated influenza vaccines have been in widespread use for about 47 years. The efficacy of immunization has varied according to circumstances, but protection rates of 75-90% have been reported. Differences in protective efficacy may result from continuing antigenic variation in the prevalent epidemic strains.

Because of this variation, the composition of inactivated influenza virus vaccine, unlike that of most viral vaccines, must be kept constantly under review. Accordingly, WHO annually publishes recommendations concerning the strains to be included in the vaccine.

In recent years, the amino-acid sequence and three-dimensional structure of the most important viral antigen, the haemagglutinin, have been determined. Changes in the structure of the haemagglutinin molecule, which result in changes in antigenicity as new epidemic strains appear, involve surface residues in the region of the molecule furthest from the viral envelope. The information available does not permit prediction of future variations because the mechanism of selection of antigenic variants, which occurs during antigenic drift, is not known and multiple evolutionary pathways appear possible. Antigenic shift (i.e., the appearance of epidemic strains with a new haemagglutinin subtype) is also unpredictable.

In addition to antigenic drift and shift, evidence has recently been obtained of another type of variation among influenza viruses, namely the preferential growth of certain virus subpopulations in the different host cells in which the virus is cultivated. Influenza viruses grown in embryonated eggs often exhibit minor antigenic and biological differences as compared with those isolated and maintained in MDCK (canine kidney) cells. Sequence analysis of the haemagglutinin gene of such variants has shown that, typically, virus grown in mammalian cells differs from virus from the same source cultivated in eggs only by the substitution of a single amino acid in the haemagglutinin molecule.

Influenza vaccines usually contain one or more influenza A viruses. However, when a new subtype of influenza A virus bearing new haemagglutinin (and neuraminidase) antigen(s) appears, it is likely that vaccine containing the antigen(s) of the influenza A subtype(s) formerly prevalent will be ineffective, so that a vaccine containing the new pandemic virus will be required. In addition, during interpandemic periods, influenza A viruses undergo frequent and progressive antigenic drift in their haemagglutinin and neuraminidase antigens. Vaccines containing formerly prevalent viruses are expected to be less protective against virus variants showing antigenic drift than against the homologous virus.

Influenza B strains do not vary in subtype. However, they do undergo antigenic variation though much less frequently than the A strains. The influenza B component of polyvalent vaccines therefore requires less
frequent change. For this reason, influenza vaccines usually contain only one influenza B virus.

As a result of developments in technology, the WHO Expert Committee on Biological Standardization recommended in its twenty-ninth report (3) that the potency of influenza vaccines should be expressed in µg of haemagglutinin per ml (or dose), as determined by suitable immunodiffusion methods. In order to standardize these methods, reference antigen (calibrated in µg of the haemagglutinin per ml) and specific anti-haemagglutinin serum, suitable for use in the assay of the haemagglutinin content of each component of inactivated vaccines, are prepared and distributed by the National Institute for Biological Standards and Control, Potters Bar, and the Center for Biologics Evaluation and Research, Bethesda. A new reference antigen and antiserum will be prepared each time it is necessary to introduce a new virus strain into the vaccines.

The formulation of a recommendation for a fixed quantity of antigen of the A and B components of inactivated influenza vaccines is inappropriate because of the antigenic variability of the viruses and the fact that, for new variants, differing amounts of antigen will be required to induce antibody levels consistent with immunity. The antibody response depends on the age of the vaccinated person, the presence or absence of prior immunological experience with viruses possessing haemagglutinin (and neuraminidase) antigens related to that of the vaccine strain, and the number of doses and type of vaccine given (whole virus, disrupted virus antigen, purified surface antigen).

In emergencies, e.g., when an influenza epidemic is expected, the abnormal demand for vaccine may create difficulties in satisfying these requirements. No attempt has been made to indicate how they may then be modified, since under these circumstances each national health authority must exercise its own judgement.

Each of the following sections constitutes a recommendation. Those parts of each section printed in type of normal size have been written in the form of requirements, so that, if a health administration so desires, they may be used as they stand as definitive national requirements. Those parts of each section printed in small type are comments and recommendations for guidance. In order to facilitate the international distribution of vaccine made in accordance with these requirements, a summary protocol for the recording of the results of the tests is given in Appendix 1.

Individual countries may wish to adopt these Requirements as the basis of their national regulations on inactivated influenza vaccines. If national requirements differ from these Requirements, it is recommended that such differences be shown to ensure that the vaccine is at least as safe and potent as that prepared in accordance with the requirements formulated below. It is desirable that the World Health Organization be informed of any such differences.
Part A. Manufacturing requirements

A.1 Definitions

A.1.1 International name

The international name shall be “Vaccinum Influenzae (Inactivatum)” or “Vaccinum Influenzae ex Virorum Fragmentis Praeparatum” or “Vaccinum Influenzae (ex corticis antigenis praeparatum)”. 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 specified below.

A.1.2 Descriptive definition

Influenza vaccine is a sterile, aqueous suspension of a strain or strains of influenza virus, type A or B, or a mixture of these two types, which have been grown individually in embryonated hens' eggs. Three types of influenza vaccine are available, as follows: (i) Vaccinum Influenzae (Inactivatum) is a suspension of whole virus particles inactivated by a suitable method; (ii) Vaccinum Influenzae ex Virorum Fragmentis Praeparatum is a sterile aqueous suspension treated so that the virus particles have been partially or completely disrupted by physicochemical means (split vaccine); and (iii) Vaccinum Influenzae (ex corticis antigenis praeparatum) is a suspension treated so that the preparation consists predominantly of haemagglutinin and neuraminidase antigens (subunit vaccine).

The preparation shall satisfy all the requirements formulated below.

A.1.3 Choice of vaccine strain

The World Health Organization reviews the world epidemiological situation annually and if necessary recommends new strains in accordance with the available epidemiological evidence.

Such strains, or those antigenically related to them, should be used in accordance with the regulations in force in the country concerned.

It is now common practice to use recombinant strains giving high yields of the appropriate surface antigens.

The passage history of the parent and recombinant virus strains shall be approved by the national control authority.

A.1.4 Reference materials for haemagglutinin

WHO influenza reference haemagglutinin antigens are preparations antigenically representative of the virus strains likely to be included in
current vaccines. They are distributed on demand when a new virus appears and the likelihood of its spread throughout the world makes its inclusion in a vaccine desirable.

The preparations contain a calibrated quantity of haemagglutinin antigen of influenza virus measured in μg/ml. The calibrations are performed at the National Institute for Biological Standards and Control, Potters Bar, and the Center for Biologies Evaluation and Research, Bethesda, using single radial immunodiffusion tests (7) with purified virus of known haemagglutinin antigen concentration. The reference preparations are used to calibrate the haemagglutinin content of national reference preparations for use in the manufacture and laboratory control of inactivated influenza vaccines by an in vitro immunodiffusion test.

These reference haemagglutinin antigens, together with the specific antihaemagglutinin sera, may be obtained from the National Institute for Biological Standards and Control, Potters Bar, and the Center for Biologies Evaluation and Research, Bethesda, for the purpose of such tests.

A.1.5 Terminology

Master seed lot: A quantity of virus, derived from a WHO-recommended strain, that has been processed at one time to assure a uniform composition and fully characterized. It is used for the preparation of working seed lots. The master seed lot and its passage level are approved by the national authority.

Working seed lot: A quantity of virus of uniform composition and fully characterized that is derived from a master seed lot by a number of passages that does not exceed the maximum approved by the national control authority. The working seed lot is used for the production of vaccines.

Single harvest: A quantity of virus suspension derived from a group of embryonated eggs that were inoculated with the same virus strain, incubated together and harvested together in one session.

Monovalent virus pool: A pool of a number of single harvests of a single virus strain processed at the same time.

Final bulk: The finished vaccine prepared from one or more monovalent pools present in the container from which the final containers are filled. It may contain one or more virus strains.

Final lot: A collection of sealed, final containers that are homogeneous with respect to the risk of contamination during filling. A final lot must therefore have been filled in one working session from a single final bulk.
A.2 **General manufacturing requirements**

The general requirements for manufacturing establishments contained in the revised Requirements for Biological Substances No.1 (General Requirements for Manufacturing Establishments and Control Laboratories) (8, p.11) shall apply to establishments manufacturing inactivated influenza vaccine, with the addition of the following:

The areas where processing of inactivated influenza vaccine takes place shall be separate from those where work with live influenza virus is performed.

A.3 **Production control**

A.3.1 **Control of source materials**

Strains of influenza virus used in the production of inactivated influenza vaccine shall be identified by historical records, which shall include information on the origin of the strains and their subsequent manipulation.

When cell cultures are permitted for the isolation of the virus strain, the number of virus passages shall be approved by the national control authority. Only strains that have been approved by the national control authority shall be used. It is now common practice to use recombinant strains giving high yields of the appropriate surface antigens. However, it has been noted that antigenic changes may occur during the development of high-yielding recombinants, and the absence of such changes should be shown by tests using monoclonal antibodies to the haemagglutinin or by raising antisera in animals and carrying out haemagglutination-inhibition tests. Where recombinant strains are used, the parent high-yield strain and the method of preparing the recombinant should be approved by the national control authority.

Reagents for use in the standardization of inactivated vaccine may be obtained from the National Institute for Biological Standards and Control, Potters Bar, or from the Center for Biologics Evaluation and Research, Bethesda.

Reference strains for antigenic analysis may be obtained from the WHO Collaborating Centers for Reference and Research on Influenza, Centers for Disease Control, Atlanta, and the National Institute for Biological Standards and Control, Potters Bar.

A.3.1.1 **Seed lot system**

The production of vaccine shall be based on the seed lot system. Each seed lot shall be identified as influenza virus of the appropriate strain by methods acceptable to the national control authority (section A.1.3).

The maximum number of passages between a master seed lot and a working seed lot shall be approved by the national control authority. The vaccine shall be not more than one passage from the working seed lot.

Each manufacturer shall identify the haemagglutinin and neuraminidase
antigens of the vaccine virus strains by suitable serological tests capable of detecting biologically significant variation, as well as cross-contamination during manipulation.

A.3.1.2 Tests on seed lots
The seed virus shall be shown to be free from demonstrable adventitious viable microorganisms by appropriate tests in accordance with the requirements of Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (2, pp. 49-52).

When possible, the seed virus should be prepared using specific pathogen-free eggs, tested to demonstrate the absence of avian leukemia viruses and adenoviruses (5).

The seed lot should be stored at a temperature lower than –60 °C, unless it is in the lyophilized form, in which case it should be stored at a temperature lower than –20 °C.

A.3.1.3 Virus propagation
Influenza virus used in the production of inactivated influenza vaccine shall be propagated in embryonated hens’ eggs. The eggs shall be obtained from flocks that appear to be in good health and have been approved by the national control authority.

A.3.2 Production precautions
The general production precautions formulated in Part A, section 3, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories), (8, p.15) shall apply to the manufacture of inactivated influenza vaccines, with the addition of the following:

- only allantoic and amniotic fluids shall be harvested;
- β-lactam antibiotics shall not be used at any stage in the manufacture of the vaccine.

Minimal concentrations of other suitable antibiotics may be used.

If vaccines are produced by the splitting of the virus by chemical means, the splitting conditions and the concentration of the chemicals used shall be approved by the national control authority.

A.3.3 Production of monovalent virus pools
A.3.3.1 Single harvests
The virus of each strain shall be grown in the allantoic cavity of embryonated hens’ eggs derived from healthy flocks. After incubation at a controlled temperature, the allantoic and amniotic fluids shall be harvested together. A number of single harvests of the same strain of virus may be combined to give a monovalent virus pool.
A.3.3.2 Inactivation of monovalent virus pools

Time of inactivation. Monovalent virus pools shall be inactivated as soon as possible after their preparation. However, if delay is unavoidable, they should be held at a temperature of 5 °C ± 3 °C for not longer than five days before inactivation.

Before monovalent virus pools are inactivated, samples shall be taken and tested for bacterial and fungal contamination.

Inactivation procedure. The virus in the monovalent virus pools shall be inactivated by a method that has been demonstrated to be consistently effective in the hands of the manufacturer and has been approved by the national control authority. The inactivation process shall also have been shown, to the satisfaction of the national control authority, to be capable of inactivating avian leukemia viruses and mycoplasmas. If the virus pool is stored after inactivation, it shall be held at a temperature of 5 °C ± 3 °C. The inactivation procedure should cause minimum alteration of the haemagglutinin and neuraminidase.

If formalin (40% formaldehyde) or β-propiolactone (2-oxetanone) is used, the concentration by volume should not exceed 0.1% at any time during inactivation. Other suitable inactivating agents can also be used.

A.3.3.3 Concentration and purification

The monovalent material shall be concentrated and purified by high-speed centrifugation or other suitable methods approved by the national control authority, either before or after the inactivation procedure.

The aim is to separate the virus from other constituents in the allantoic and amniotic fluids as efficiently as possible. It is advisable to concentrate and purify the virus under optimum conditions to preserve its antigenic properties.

A.3.4 Control of monovalent virus pools

A.3.4.1 Effective inactivation

The monovalent virus pool shall be shown not to contain viable influenza virus when tested by inoculation of embryonated hens’ eggs, by a method approved by the national control authority.

A suitable method consists of inoculating 0.2 ml of undiluted monovalent pool and 1 : 10 and 1 : 100 dilutions of the monovalent pool into the allantoic cavities of groups of fertile eggs (ten eggs in each group), and incubating the eggs at 33–37 °C for three days. At least eight of the ten eggs should survive at each dosage level. A volume of 0.5 ml of allantoic fluid is harvested from each surviving egg. The fluid harvested from each group is pooled and 0.2 ml of each of the three pools is inoculated, undiluted, into a further group of ten fertile eggs. Haemagglutinin activity should not be detected in these new groups of eggs.

In some countries, the requirement that 80% of the eggs should survive during incubation may be impossible to satisfy. The national control authority should then specify the requirement to be satisfied.
A.3.4.2 Haemagglutinin content
The content of haemagglutinin in the monovalent virus pool shall be
determined by a suitable technique, such as single radial immunodiffusion
or immunoelectrophoresis. In the test, a WHO influenza reference
haemagglutinin antigen or a national preparation calibrated against it shall
be used for purposes of comparison (see section A.3.1).

A.3.4.3 Presence of neuraminidase
Vaccine should be prepared under conditions that allow retention of
significant levels of neuraminidase activity.

In some countries, a test is included for the presence of neuraminidase. The
ratio of haemagglutinin to neuraminidase should be constant for the particular
virus strain and method of vaccine production used, but the neuraminidases of
different strains vary markedly in their stability during processing.

A.3.4.4 Virus disruption
Monovalent pools in which the virus has been split by chemical means
shall be shown by procedures approved by the national control authority
to consist predominantly of disrupted virus particles. This test need be
performed on only one sample of monovalent pool for each vaccine
provided that the test result is satisfactory.

A.3.4.5 Surface antigens
The purity of monovalent pools intended for the preparation of subunit
vaccine shall be determined by polyacrylamide gel electrophoresis or by
other suitable techniques approved by the national control authority.
Essentially, only haemagglutinin and neuraminidase antigens should be
present. This test need be performed on only one monovalent pool for
each vaccine provided that the test result is satisfactory.

A.3.4.6 Identity
Antigenic specificity shall be confirmed by either:

– an immunodiffusion or haemagglutinin-inhibition technique using
  appropriate specific immune sera; or

– injection of vaccine into mice, chickens or other suitable animals and
demonstration of the production of antibodies to the haemagglutinin of
the influenza virus used to produce the vaccine.

With split and subunit vaccines, the identity test may be performed before
virus disruption.

A.3.5 Control of final bulk
Final bulks are prepared by mixing and diluting monovalent pools of the
relevant strains.

In the preparation of the final bulk, only preservatives or other substances,
including diluents, approved by the national control authority shall be
added. Such substances shall have been shown by appropriate tests not to
impair the safety or effectiveness of the product in the concentrations used, and shall not be added before samples have been taken for any tests that would be affected by their presence.

A.3.5.1 Test for content of haemagglutinin antigen
The haemagglutinin concentration in the final bulk shall be determined as described in section A.3.4.2, unless such a test is performed on each final lot.

A.3.5.2 Sterility tests
Each bulk shall be tested for sterility by a method approved by the national control authority.

Many countries have regulations governing sterility testing. Where these do not exist, the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (2) shall be satisfied. If a preservative has been added to the vaccine, appropriate measures shall be taken to prevent it from interfering with the sterility test.

A.3.5.3 Total protein
The total protein content shall be not more than six times the total haemagglutinin content of the vaccine, as determined in the test for haemagglutinin content, but in any case not more than 100 µg of protein per virus strain per human dose and not more than a total of 300 µg of protein per human dose.

A.3.5.4 Ovalbumin
The ovalbumin content shall be not more than 5 µg per human dose. The amount of ovalbumin shall be determined by a suitable technique using a suitable reference preparation of ovalbumin.

Values of less than 1 µg of ovalbumin per human dose are attainable.

A.3.5.5 Tests for chemicals used in production
The concentration of each detergent, 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. For preservatives, both the method of testing and the concentration shall be approved by the national control authority.

Tests for chemicals used only before the final bulk is prepared may be performed on monovalent pools.

A.4 Filling and containers
The requirements concerning filling and containers given in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Labora-
tories) (δ, p.16) shall apply. Single- and multiple-dose containers may be used. If the latter are used, a suitable preservative, approved by the national control authority, shall be incorporated.

A.5 Control tests on final lot

A.5.1 Identity test

An identity test shall be performed by a method approved by the national control authority on at least one labelled container from each final lot; the container shall be removed from its final pack immediately before testing.

The identity of the haemagglutinins in the vaccine shall be determined by an immunological technique, such as immunodiffusion or haemagglutinin inhibition, using the appropriate specific immune serum.

In some countries, a test to identify the specific neuraminidase antigen is also included.

A.5.2 Sterility test

Final containers shall be tested for sterility as described in section A.3.5.2.

A.5.3 Haemagglutinin content

The test for haemagglutinin antigen concentration is performed as described in section A.3.4.2.

The vaccine shall contain in each human dose 7-20 μg of haemagglutinin of each strain used in the preparation and not less than 80% and not more than 120% of the number of μg of haemagglutinin stated on the label.

A.5.4 Innocuity tests

Each final lot shall be tested for abnormal toxicity by appropriate tests by means of parenteral injections in mice and guinea-pigs. The test procedures shall be approved by the national control authority.

A.5.5 Endotoxin

In some countries, a test for endotoxin is included, e.g., the L. monosaccarolyticus lysate test. The permissible level of endotoxin is determined by the national control authority.

A.5.6 Inspection of final containers

Each container in each final lot shall be inspected, and those showing abnormalities shall be discarded.

A.6 Records

The requirements given in Part A, section 6, of the revised Requirements for Biological Substances No.1 (General Requirements for Manufacturing Establishments and Control Laboratories) (δ, p.17) shall apply.
A.7 **Samples**

The requirements given in Part A, section 7, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (8, p.18) shall apply.

A.8 **Labelling**

The requirements given in Part A, section 7, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (8, p.18) shall apply, with the addition of the following:

The label on the container shall state:

- that the vaccine has been prepared from virus propagated in embryonated hens' eggs;
- the strain or strains of influenza virus present in the preparation;
- the haemagglutinin content in μg per virus strain, expressed as μg of haemagglutinin per dose;
- the number of doses, if the product is issued in a multiple-dose container.

The label on the carton enclosing one or more final containers or the leaflet accompanying the container shall include the following additional information:

- the method used for inactivating the virus;
- the name and maximum quantity of any antibiotic present in the vaccine;
- the name and concentration of any preservative added.

A.9 **Distribution and transport**

The requirements given in Part A, section 9, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (8, p.18) shall apply.

A.10 **Storage and expiry date**

The requirements given in Part A, section 10, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (8, p.19) shall apply.

A.10.1 **Storage conditions**

Inactivated influenza vaccine shall be stored at a temperature of 5 °C ± 3 °C.
A.10.2 Expiry date

The expiry date shall be fixed with the approval of the national control authority.

In general, the expiry date should not exceed one year from the date of issue by the manufacturer since one year’s viral strains may not be appropriate the next year.

Part B. National control requirements

B.1 General

The general requirements for control laboratories, contained in Part B of the revised Requirements for Biological Substances No.1 (General Requirements for Manufacturing Establishments and Control Laboratories) (8, p.19) shall apply.

The national control authority shall give directions to manufacturers concerning the influenza virus strains to be used, the haemagglutinin content, whether or not neuraminidase is present, and the recommended human dose.

B.2 Release and certification

A vaccine lot shall be released only if it satisfies Part A of these Requirements.

A statement signed by the appropriate official of the national control authority shall be provided at the request of the manufacturing establishment and shall certify that the lot of vaccine in question satisfies all national requirements as well as Part A of these Requirements. The certificate shall state the number under which the lot was released by the national controller, and the number appearing on the labels of containers. Importers of influenza vaccine (inactivated) shall be given a copy of the official national release document.

The purpose of the certificate is to facilitate the exchange of inactivated influenza vaccine between countries.

An example of a suitable certificate is given in Appendix 2.

B.3 Clinical evaluation of influenza vaccines

In the case of a new manufacturer or of a significant change in the manufacturing process, the national control authority shall assess the safety and immunogenicity of the vaccine by arranging for studies in human volunteers of some of the lots of vaccine that have satisfied the above-mentioned requirements. Such studies shall include the assessment of the immune responses and adverse reactions in various age groups, and the appropriateness of the various strains present in the vaccine.
Authors

The first draft of these revised Requirements was prepared by Dr Y. Ghendon, Microbiology and Immunology Support Services, Dr V. Grachev, Biologicals, and Dr D. Magrath, Chief, Biologicals, World Health Organization, Geneva, Switzerland.

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References

Appendix 1  
**Summary protocol for influenza vaccine (inactivated) (master/working seed lot Type A or Type B)**

The model summary protocol that follows is provided as general guidance to manufacturers. It is not intended to constrain them in the presentation of data relevant to the complete review of the quality-control tests performed on the vaccine. It is important to note that satisfactory test results do not necessarily imply that the vaccine is safe and effective, since many other factors must be taken into account, including the characteristics of the manufacturing facility.

Name and address of manufacturer  
Laboratory reference no. of lot  
Date when the processing was completed

**Information on manufacture**

Virus used to inoculate eggs for the manufacture of the lot:

(a) strain and subspecies  
(b) passage level  
(c) source and reference no.  
(d) remarks  
Results of sterility test  
Conditions of storage

**Monovalent virus pool Type A or Type B**

Name and address of manufacturer  
Laboratory reference no. of virus pool  
Virus used to inoculate eggs:  

(a) master seed strain and source  
(b) passage level of master seed  
(c) working seed lot, reference no. and source  
Date of inoculation  
Date of harvesting allantoic and amniotic fluids  
Storage conditions before inactivation

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1 If there are more than four virus pools in the monovalent pool, the relevant data should be given on a separate sheet.
Date of inactivation

Time of inactivation

Method of inactivation

Concentration of inactivating agent

Storage conditions after inactivation

Concentration/purification procedure

Antibiotics used during preparation, if any

**Tests on monovalent pool**

*Test for absence of viable influenza virus*

No. of eggs inoculated

Incubation time and temperature

Date of test

Results

*Determination of haemagglutinin content*

Method

Date of determination

Results

*Tests for presence of neuraminidase (if performed)*

Method

Date of test

Results

*Virus disruption (for Vaccinum Influenzae ex Virorum Fragmentis Praeparatum)*

Method

Date

Results

*Surface antigen (for Vaccinum Influenzae ex corticis antigenii praeparatum)*

Method

Date

Results

*Identity tests*

Method
Date of test ________________________________________________
Results ________________________________________________

Final bulk
Name and address of manufacturer ____________________________
Identification of final bulk __________________________________
Identification of monovalent virus pool used to prepare final bulk ______
Date of manufacture ____________________________

Control of final bulk
Preservative(s) added and concentration ________________________
Any other substances added and concentration __________________

Determination of haemagglutinin content
Method ________________________________________________
Date of determination __________________________________
Results ________________________________________________

Sterility
Date of test ________________________________________________
Results ________________________________________________

Total protein content
Method ________________________________________________
Date of test ________________________________________________
Results ________________________________________________

Ovalbumin content
Method ________________________________________________
Date of test ________________________________________________
Results ________________________________________________

Tests for chemicals used
Date of tests ________________________________________________
Results ________________________________________________

Final lot
Identity test
Method ________________________________________________
Date of test ________________________________
Results _________________________________

Sterility
Method _________________________________
Date of test ______________________________
Results _________________________________

Determination of haemagglutinin content
Method _________________________________
Date of determination ____________________
Results _________________________________

Innocuity
No. and species of animals ____________________________
Doses injected _____________________________
Period of observation _______________________
Date of test ______________________________
Results _________________________________

Endotoxin content
Method _________________________________
Date of test ______________________________
Results _________________________________

Inspection of final container
Results _________________________________

Other tests _______________________________

Additional comments (if any) __________________________

A sample of a completed final container label and package insert shall be attached.
Certification by producer

Name of head of production of the final vaccine ___________________________

Certification by head of the quality assurance department taking overall responsibility for production and control of the final vaccine:

I certify that lot no... of influenza vaccine (inactivated), whose number appears on the label of the final container, meets all national requirements¹ and satisfies Part A of the Requirements for Biological Substances No.17, revised 1990.

Signature __________________________________________________________

Name (typed) _______________________________________________________

Date _______________________________________________________________

Certification by the national controller

If the vaccine is to be exported, provide a copy of the certificate from the national control authority as described in section B.2, a label of a final container, and a leaflet of instructions to users.

¹ If any national requirement(s) is (are) not met, specify which one(s) and indicate why release of the lot has nevertheless been authorized.
Appendix 2

Model certificate for the release of influenza vaccine (inactivated)\(^1\)

The following lots of influenza vaccine (inactivated) produced by _____\(^2\) in ________\(^3\), whose numbers appear on the labels of the final containers, meet all national requirements,\(^4\) Part A of the Requirements for Biological Substances No.17 (Requirements for Influenza Vaccine (Inactivated) [revised 1990; if applicable, addenda 19 ___])\(^5\) and the Requirements for Biological Substances No.1 (General Requirements for Manufacturing Establishments and Control Laboratories [revised 1965]).\(^6\)

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

The number of this certificate is _______________________________________

The Director of the National Control Laboratory (or Authority as appropriate):\(^7\)

Name (typed) ___________________________________________________________

Signature _____________________________________________________________

Date _________________________________________________________________

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\(^1\) To be provided by the national control authority of the country where the vaccines have been manufactured, on request by the manufacturer.

\(^2\) Name of manufacturer.

\(^3\) Country.

\(^4\) If any national requirement(s) is (are) not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national control authority.

\(^5\) With the exception of the provisions on shipping, which the national control authority may not be in a position to control.


\(^7\) Or his or her representative.
Annex 3

Guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology

1. Introduction
2. General considerations
3. Scope of guidelines
4. Control of source materials
5. Control of production
6. Characterization of bulk product
7. Routine control of final dosage form
8. Reference materials
9. Preclinical safety evaluation

References
Appendix
Explanations of terms

1. Introduction

These guidelines are concerned with the quality assurance of pharmaceutical and biological products made using recombinant DNA (rDNA) techniques and intended for use in humans.

Individual countries may wish to use this document to develop their own national guidelines or requirements for rDNA-derived products. It is not intended to apply to the control of genetically modified live organisms designed to be used directly in humans, e.g., live vaccines.

The purpose of the document is to indicate:

- appropriate methods for the manufacture and testing of rDNA-derived products; and
- information specific to rDNA products that should be included in submissions by manufacturers to national control authorities in support of applications for the authorization of clinical trials and marketing.

It is recognized that rDNA technology is a rapidly evolving field and that it is important that a flexible approach to the control of these products be adopted so that requirements can be modified in the light of experience of production and use, and the further development of new technologies. The
guidelines presented here therefore supersede those published in 1983 (1), and the intention is to provide an updated and scientifically sound basis for the manufacture and control of medicinal products produced by new biotechnologies.

2. **General considerations**

Advances in molecular genetics and nucleic acid chemistry now enable genes coding for natural biologically active proteins to be identified, analysed in fine detail, transferred from one organism to another and expressed under controlled conditions so as to synthesize efficiently the polypeptides for which they code. A gene is characterized by a specific nucleotide sequence in each strand of the double-stranded DNA molecule. When the strands are separated, each forms a template for the synthesis of a complementary copy, thus providing a mechanism for the faithful reproductions of genes while at the same time conserving the linear sequence of the four mononucleotide building blocks. The process of decoding this information and synthesizing the gene product takes place in the following two stages: (i) transcription of the DNA coding strand in the form of messenger RNA (mRNA) and; (ii) translation of the information carried by the mRNA molecule into a polypeptide. Genes coding for modified products possessing enhanced biological activity and/or fewer undesirable characteristics, as well as for entirely novel substances, can now be constructed.

A naturally occurring gene or a synthetically derived nucleotide sequence that codes for a specific product can be propagated by inserting the DNA into a suitable vector. For this purpose, highly specific restriction endonuclease enzymes (which cleave the vector DNA at predetermined sites) and ligases (which join the gene insert to the vector) are used, after which the vector is introduced into a suitable host organism. Individual clones that carry the desired gene can then be selected and grown in mass culture so as to ensure the efficient expression of the desired gene product. The factors affecting the expression of foreign genes introduced into a new host are, however, complex, and the efficient, controlled and faithful expression of stable, cloned DNA sequences is an important objective of current research.

Many vectors in use at present are bacterial plasmids and much gene cloning has been carried out in prokaryotes. However, other vector–host cell systems involving eukaryotes, including yeasts or continuously growing (transformed) cell lines of mammalian or insect origin, have been developed and are, in some cases, already used for production. The use of animal cells as hosts is considered by some to offer distinct advantages as compared with bacterial systems. They can, for example, effect modifications, such as the addition of carbohydrate groups, which may take place on mammalian proteins. Correct processing is also more likely and secretion of the product into the culture medium avoids the need to
disrupt the cells and thus reduces potential contamination with host-cell proteins. On the other hand, the use of animal cells as hosts does raise specific safety issues (see below).

Certain factors may compromise the quality, safety and efficacy of rDNA-derived products and these need special attention, as indicated in the following paragraphs.

Products from naturally occurring genes expressed in foreign hosts may differ structurally, biologically or immunologically from their natural counterparts. Such differences can arise either at the genetic, post-transcriptional or post-translational level, or during production and/or purification.

In addition, rDNA-derived products may contain potentially hazardous contaminants not normally present in their equivalents prepared by conventional means and which the purification process must be capable of eliminating. Examples include endotoxins in products expressed in bacterial cells and contaminating cellular DNA and viruses in those derived from animal cells. Contamination with nucleic acid from transformed mammalian cells is a particular concern because of the possible presence of potentially oncogenic DNA. The choice of manufacturing procedure will, of course, influence the nature and range of possible contaminants.

The “scaling up” of laboratory techniques into processes suitable for large-scale production may significantly affect the quality of the product and thus have major implications for control and testing. Unintended variability in the culture during production may lead to changes that favour the expression of other genes in the host/vector system or that cause alterations in the polypeptide product. Such variations might result in decreased yield of the product and/or quantitative and qualitative differences in the impurities present. Similar considerations apply to the use of continuous culture production. Consequently, procedures to ensure the consistency both of production conditions and of the final product are essential.

3. **Scope of guidelines**

The guidelines cover the following three main areas:

1. Control of starting materials, including baseline data both on the host cell and on the source, nature and sequence of the gene used in production.
2. Control of the manufacturing process.
3. Control of the final product.

In this respect, rDNA products are considered to be similar to biologicals produced by traditional methods, such as bacterial and viral vaccines, where adequate control of the starting materials and manufacturing
procedure is just as necessary as that of the product. The guidelines therefore place considerable emphasis on "in-process" controls for ensuring the safety and effectiveness of the product, as well as on the comprehensive characterization of the final product itself. The validation of certain aspects of the manufacturing process, such as the ability of the purification procedure to remove unwanted materials, e.g., DNA, is also considered to be essential.

Requirements relating to establishments in which biological products are manufactured (e.g., the revised Requirements for Biological Substances No.1, (2) apply to rDNA-derived products, as do the general requirements for the quality control of biological products. Appropriate attention therefore needs to be given to the quality of all reagents used in production, including components of fermentation media. If animal-derived additives are used (e.g., calf serum), they should be shown to be free from adventitious agents. It is undesirable to use in production any agent known to provoke sensitivity reactions in certain individuals, such as penicillin or other β-lactam antibiotics. Many of the general requirements for the quality control of biological products, such as tests for potency, abnormal toxicity, pyrogenicity, stability and sterility, also apply to products made by rDNA techniques.

While the guidelines set out below should be considered as generally applicable, individual products may present particular quality-control problems. The production and quality control of each product must therefore be given careful individual consideration, any special features being taken fully into account. Furthermore, the guidelines for a product must reflect its intended clinical use. Thus, a preparation that is to be administered repeatedly over a protracted period of time, or in large doses, is likely to need careful testing for traces of antigenic contaminants. Different criteria might justifiably apply, however, to a product to be used only once but in a life-threatening condition.

When the term "bulk product" is used in these guidelines, it refers to the substance in question following purification but before final formulation.

4. Control of source materials

4.1 Expression vector and host cell

A description of the host cell, its source and history, and of the expression vector used in production should be given. This should include details of the origin and identity of the gene being cloned as well as the construction, genetics and structure of the expression vector. An explanation of the source and function of component parts of the vector, such as the origins of replication, promoters or antibiotic-resistance markers, should be provided, as should a restriction-enzyme digestion map indicating at least those sites used in construction.

Details of the method by which the vector is introduced into the host cell
and the state of the vector within the cell, i.e., whether integrated or extrachromosomal, and copy number, should be provided. The genetic stability of the host-vector combination should be documented.

4.2 Sequence of cloned gene

The nucleotide sequence of the gene insert and of the flanking control regions of the expression vector should be indicated. All relevant expressed sequences should be clearly delineated.

4.3 Expression

Measures used to promote and control the expression of the cloned gene in the host cell during production should be described in detail.

5. Control of production

5.1 Manufacturer’s working cell bank

The production of a rDNA product should be based on a seed lot system involving a manufacturer's working cell bank derived from the master seed lot. A host cell containing the expression vector should be cloned and used to establish a master seed lot. During the establishment of the seed, no other cell lines should be handled simultaneously in the same laboratory suite or by the same persons.

Full information should be provided on the origin, form, storage and life expectancy at the anticipated rate of use of seed material. Evidence for the stability of the host-vector expression system in the seed stock under storage and recovery conditions should also be provided. New seed lots should be fully characterized and acceptance criteria established.

Where higher eukaryotic cells are used for production, distinguishing cell markers, such as specific isoenzymes or immunological features, are useful in establishing the identity of the seed. Information on the tumorigenicity of continuous cell lines should be obtained and reported. Where microbial cultures are used, specific phenotypic features that can form a basis for identification should be described.

The DNA sequence of the cloned gene should normally be confirmed at the stage of the master seed lot. However, in certain cases, e.g., where multiple copies of the gene are inserted into the genome of a continuous cell line, it may be inappropriate to sequence the cloned gene at this stage. In such circumstances, Southern blot analysis of the total cellular DNA, Northern blot analysis of transcripts that contain the product sequence, or sequence analysis of product-related mRNA may be informative, and particular attention should be paid to the characterization of the final product.

Evidence that the seed lot is free from infective bacterial, mycoplasmal, fungal, viral and, where appropriate, potentially oncogenic adventitious
agents should be provided. Special attention should be given to viruses that commonly contaminate the animal species from which cell lines are derived. Seed lots should preferably be free from all adventitious agents. However, certain cell lines contain endogenous viruses, e.g., retroviruses. Tests capable of detecting such organisms should be carried out under a variety of conditions known to cause their induction, and the results reported. Specific contaminants identified as endogenous agents in the master seed lot, or as part of the vector, should be shown to be inactivated and/or removed by the purification procedures used in production.

5.2 Production at finite passage

Procedures and materials used both for cell growth and for the induction of the product should be described in detail. For each production run, data on the extent and nature of any microbial contamination of the culture vessels immediately before harvesting should be provided. Acceptable limits for such contamination should be set and the sensitivity of the methods used to detect it indicated.

Data on the consistency of fermentation conditions and culture growth, and on the maintenance of product yield should be presented. Criteria for the rejection of culture lots should be established. The maximum number of cell doublings or passage levels to be permitted during production should be specified, based on information on the stability of the host-cell/vector system on serial subculture up to and beyond the level used in production.

Host-cell/vector characteristics at the end of production cycles should be monitored, for which purpose detailed information on plasmid copy number and degree of retention of the expression vector within the host cell may be of value, as may restriction-enzyme mapping of the vector containing the gene insert. The nucleotide sequence of the insert encoding the rDNA product should be determined, where appropriate (see section 5.1), at least once after full-scale culture for each master seed lot. If the vector is present in multiple copies integrated into the host-cell genome, confirming the rDNA sequence directly may be difficult. In such cases, the isolation and determination of the nucleotide sequence of the product-related mRNA, Northern blot analysis of product-related transcripts or Southern blot analysis of total DNA should be considered.

5.3 Continuous culture production

As recommended in section 5.2, all procedures and materials used for cell culture and induction of the product should be described in detail. In addition, particular consideration should be given to the procedures used in production control. Monitoring is necessary throughout the life of the culture, although the frequency and type of monitoring required depend on the nature of both the production system and the product.

The molecular integrity of the gene being expressed and the phenotypic
and genotypic characteristics of the host cell after long-term cultivation should be established. Evidence should also be produced to show that variations in yield do not exceed the specified limits. The acceptance of harvests for further processing should be clearly linked to the monitoring schedule in use, and a clear definition of a “batch” of product for further processing will be required. Criteria for the rejection of harvests or termination of the culture should also be established. Regular tests for microbial contamination should be performed as appropriate to the harvesting strategy.

The maximum period of continuous culture should be specified, based on information on the stability of the system and consistency of the product during and after this period. In long-term continuous culture, the cell line and product should be fully re-evaluated at intervals determined by information on the stability of the host-vector system and the characteristics of the product.

5.4 Purification

The methods used for harvesting, extraction and purification should be described in detail. Special attention should be given to the elimination of viruses, nucleic acid, and undesirable antigenic materials.

In procedures involving affinity chromatography using biological substances, such as monoclonal antibodies, appropriate measures should be taken to ensure that these substances, or any other potential contaminants arising from their use, such as adventitious viruses, do not compromise the safety of the final product.

The ability of the purification procedure to remove unwanted product-related or host-cell-derived proteins, nucleic acid, carbohydrate, viruses or other impurities, including media-derived components and undesirable chemicals introduced by the purification process itself, should be investigated thoroughly, as should the reproducibility of the process. Data from validation studies on the purification procedures may be required to demonstrate clearance of DNA or viruses, both at each purification step and overall. In such pilot-scale studies, tests should be carried out with a carefully selected group of viruses exhibiting a range of physicochemical characteristics representative of potential contaminants, or with radiolabelled DNA, deliberately added to the crude preparation (“spiking”). The results will indicate the extent to which these contaminants can theoretically be removed during purification. Any virus-inactivation process used should be shown to be effective and not to compromise the quality of the product.

6. Characterization of bulk product

The identity, purity, potency and stability of the bulk product should be established. The type of testing necessary and the degree of purity
expected will depend on several factors, including the nature and intended use of the product, the method of production and purification, and experience with the production of several batches of the product.

6.1 Characterization of purified active substance

Rigorous characterization of the active substance by chemical, physical and biological methods will be essential. Particular attention should be given to using a wide range of analytical techniques exploiting different physicochemical properties of the molecule (size, charge, isoelectric point, amino-acid composition and hydrophobicity). It may also be necessary to include suitable tests to establish that the product has the desired conformation and state of aggregation. Techniques suitable for such purposes include polyacrylamide gel electrophoresis; isoelectric focusing; size-exclusion, reversed-phase, ion-exchange, hydrophobic-interaction or affinity chromatography; peptide mapping; amino-acid analysis; light scattering; and ultraviolet spectroscopy. Circular dichroism and other spectroscopic techniques can also provide valuable information.

Where relevant and possible, the properties of the product should be compared with those of the naturally occurring molecule.

The product should be shown to possess the expected biological activity; this should be of the expected magnitude and the potency of the product in appropriate units should be determined. In addition, the determination of the specific activity (units of activity/weight of product) of highly purified material is of particular value.

Sufficient sequence information to characterize the product should be obtained. The degree of sequence verification required will depend on the scope of other characterization tests. For some purposes, partial sequence determination and peptide mapping may suffice; for others, full sequence determination may be necessary. Attention should be paid to the possible presence of N-terminal methionine, signal or leader sequences and other possible N- and C-terminal modifications (such as acetylation, amidation or partial degradation by exopeptidases). Other post-translational modifications, such as glycosylation, should be identified and adequately characterized. Special consideration should be given to the possibility that such modifications are likely to differ from those found in a natural counterpart and may influence the biological, pharmacological and immunological properties of the product.

6.2 Purity

Data should be provided on the contaminants present in the product, including estimates of their maximum levels. The degree of contamination considered acceptable and criteria for the rejection of a production batch should be specified.

It is important that the techniques used to demonstrate purity be based on as wide a range of physicochemical properties as possible. Attention
should be given to tests for viral and nucleic acid contamination and for other unwanted materials of host or product origin, as well as materials that may have been added during the production or purification processes. Limits should be specified for all impurities detected, and these should be identified and characterized as appropriate.

Substances that are to be administered repeatedly or in large doses should be assayed for trace antigenic constituents and product-related impurities, such as aggregates or degradation products likely to contaminate the final product, and strict upper limits specified. Tests such as immunoblotting, radioimmunoassays and enzyme-linked immunosorbent assays using high-affinity antibodies raised against the product, host-cell lysates, appropriate subcellular fractions and culture medium constituents can be used to detect contaminating antigens. Because the detection of antigens will be limited by the specificity and sensitivity of the antisera used, these immunoassays will complement, but not replace, other techniques, such as staining of gels used in sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Patients given large or repeated doses of a product during clinical trials should be monitored for the production of antibodies both to contaminating antigens and to the product.

7. **Routine control of final dosage form**

It will be apparent that not all the tests described above need to be carried out on each batch of final dosage form. Some tests are required only to establish the validity or acceptability of a procedure, while others might be performed on a limited series of batches in order to establish consistency of production. Thus, a comprehensive analysis of the initial production batches should be undertaken to establish consistency with regard to identity, purity and potency; after the stability of the final dosage form has been established, a more limited series of tests may be appropriate, as outlined below.

7.1 **Consistency**

An acceptable number, e.g., five, successive batches of final dosage form should be characterized as fully as possible to determine consistency of composition. Any differences between one batch and another should be noted. The data obtained from such studies should be used as the basis for the product specification.

7.2 **Identity**

Each batch of final dosage form should be subjected to a selection of the tests used to characterize the purified active substance in order to confirm product identity. The specific tests that adequately characterize any particular product on a lot-to-lot basis, however, depend on both the nature of the product and the method of production. Depending on the
scope of other identification tests, sequence verification of a number of amino acids at the N- and C-termini, or the use of other methods, such as peptide mapping, will be necessary.

7.3 Purity

The purity of each batch of final dosage form should be determined and be within specified limits. The analysis should include sensitive and reliable assays for DNA of host-cell origin (e.g., hybridization analysis of immobilized contaminating DNA, using appropriate probes) for each batch of product prepared from continuous lines of mammalian cells (transformed cell lines); strict upper limits should be specified for the DNA content of the product. Theoretical concerns regarding transforming DNA derived from the cell substrates can be minimized by the general reduction in contaminating nucleic acid (3). DNA analyses should also be performed on each batch of product obtained from other eukaryotic cells, and limits specified for DNA content, until such time as further information on safety is obtained. Wherever appropriate from the point of view of the quality and safety of the product, tests for DNA of prokaryotic expression systems should be carried out.

For products to be administered for an extended period of time or in high doses, the residual cellular proteins should also be determined by an assay of appropriate sensitivity and strict upper limits specified.

7.4 Potency

The potency of each batch of the final dosage form should be established using, wherever possible, an appropriate national or international reference material calibrated in units of biological activity. In the absence of such preparations, an approved in-house reference preparation may be used for assay standardization.

When sufficient correlation studies between physicochemical or in vitro bioassays and in vivo biological assays have been carried out showing that estimates based on in vitro tests are sufficiently precise and accurate, the requirement for an in vivo bioassay may be relaxed.

8. Reference materials

The studies described in section 6 together with those in section 7 will contribute to a definitive specification for the product.

A suitable batch of the product, preferably one that has been clinically evaluated, should be fully characterized in terms of its chemical composition, purity and biological activity, including, where possible, full amino-acid sequencing, and retained for use as a chemical and biological reference material. Where appropriate, these properties should be compared with those of a highly purified preparation of the naturally occurring molecule.
9. **Preclinical safety evaluation**

The general aim of preclinical safety evaluation is to determine whether new medicinal products have the potential to cause unexpected and undesirable effects. However, classical safety or toxicological testing, as recommended for chemical drugs, may be of only limited relevance for rDNA-derived products. These pose particular problems in relation to toxicity testing in animals, and their safety evaluation will have to take a large number of factors into account. Thus, certain proteins, e.g. interferons, are highly species-specific, so that the human protein is much more pharmacologically active in humans than in any other animal species. Furthermore, the amino-acid sequences of human proteins will often differ significantly from those of their natural counterparts in other species, as will the carbohydrate groups. Thus human proteins frequently produce immunological responses in foreign hosts which may ultimately modify their biological effects and may result in toxicity due to immune complex formation. Such toxicity would, of course, have little bearing on the safety of the product in the intended human host.

For these and other reasons, it is likely that a flexible approach will be necessary for the preclinical safety evaluation of rDNA-derived products. Although there can be no doubt that some safety testing will be required for most products, the range of tests that need to be carried out should be decided on a case-by-case basis, in consultation with the national control authority. A wide range of pharmacological, biochemical, immunological, toxicological and histopathological investigative techniques should be used, where appropriate, in the assessment of a product’s effect, over an appropriate range of doses and during both acute and chronic exposure. However, the points made above concerning species-specificity and antibody formation should always be taken into consideration. Where studies are expected to last more than four weeks, the use of test species known to be low responders from the point of view of antibody production against the test substance should be considered.

**References**

Appendix

Explanations of terms

*Bulk harvest*: A homogeneous pool of individual harvests or lysates processed in a single manufacturing run.

*Bulk product*: The product following purification, but before final formulation. It is obtained from a bulk harvest, and is kept in a single container and used in the preparation of the final dosage form.

*Continuous culture production*: A system in which the number of passages or population doublings after production has been started is not restricted. Strict criteria for terminating production must be specified by the manufacturer.

*Final dosage form*: The finished formulated product; it may be freeze-dried and contain excipients, which should have been shown not to affect stability adversely.

*Manufacturer's working cell bank*: A homogeneous suspension of the seed material derived from the master seed bank(s) at a finite passage level, dispensed in aliquots into individual containers for storage. All containers are treated identically and, once removed from storage, are not returned to the seed stock.

*Master seed*: A homogeneous suspension of the original cells, already transformed by the expression vector containing the desired gene, dispensed in aliquots into individual containers for storage. All containers are treated identically during storage and once removed from it are not returned to the seed stock.

*Plasmid*: An autonomously replicating, circular, extrachromosomal DNA element. It usually carries a few genes, some of which confer resistance to various antibiotics; such resistance is often used to discriminate between organisms that contain the plasmid and those that do not.

*Production at finite passage*: A cultivation method involving a limited number of passages or population doublings which must not be exceeded during production.

*Vector*: A piece of DNA that can direct its own replication within a host cell and to which other DNA molecules can be attached and thus amplified. Many vectors are bacterial plasmids, but in other instances a vector may be integrated into the host-cell chromosome following its introduction into the cell and is maintained in this form during the growth and multiplication of the host organism.
Annex 4

Requirements for antimicrobial susceptibility tests: I. Agar diffusion tests using antimicrobial susceptibility discs (Requirements for Biological Substances No. 26, addendum 1990)

As part of these Requirements (revised 1981\(^1\)), the Expert Committee on Biological Standardization at its thirty-third meeting\(^2\) adopted a revised list of the codes used to identify antimicrobials contained in susceptibility test discs. The list of codes was again revised at the thirty-sixth,\(^3\) thirty-eighth\(^4\) and fortieth\(^5\) meetings of the Expert Committee to incorporate additions, deletions and changes in nomenclature. During the period since the last revisions, further requests have been received by the WHO Secretariat for the allocation of codes for new antimicrobial substances. The new entries that have been agreed should be incorporated by making the following further additions to the list of codes given in Part A, section 1.6 of the Requirements:\(^2\)

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Code</th>
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<td>butoconazole</td>
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<td>CTZ</td>
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<td>cefcatacol</td>
<td>CTL</td>
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<td>cefpiramide</td>
<td>CPM</td>
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<td>cefprozil</td>
<td>CPR</td>
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<tr>
<td>clarithromycin</td>
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<tr>
<td>fleroxacin</td>
<td>FLE</td>
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<tr>
<td>sulconazole</td>
<td>SUC</td>
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<tr>
<td>tosufloxacin</td>
<td>TFX</td>
</tr>
</tbody>
</table>

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Annex 5

**Biological substances: international standards and reference reagents**

A list of international biological standards, international biological reference preparations, and international biological reference reagents is issued as a separate publication. 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

**Antibiotics**

<table>
<thead>
<tr>
<th>Substance</th>
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<tbody>
<tr>
<td>Teicoplanin</td>
<td>51 550 IU/ampoule</td>
<td>First International Standard 1990</td>
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**Endocrinological and related substances**

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<tr>
<td>Erythropoietin, rDNA-derived</td>
<td>86 IU/ampoule</td>
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<tr>
<td>Inhibin, porcine</td>
<td>2000 IU/ampoule</td>
<td>First International Standard 1990</td>
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**Antibodies**

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<tbody>
<tr>
<td>Anti-measles serum</td>
<td>5 IU/ampoule</td>
<td>Second International Standard 1990</td>
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</table>

**Blood products**

<table>
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<th>Substance</th>
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<tbody>
<tr>
<td>Antithrombin III concentrate</td>
<td>5.2 IU/ampoule</td>
<td>First International Standard 1990</td>
</tr>
</tbody>
</table>

The above 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.

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Anti-D (anti-Rh\(_d\))
complete blood-typing serum
(chemically modified)

128 IU/ampoule
First International Standard 1990

This substance is held and distributed by the International Laboratory for Biological Standards, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Plesmanlaan 125, Amsterdam, Netherlands.
Annex 6

Requirements for biological substances and other sets of recommendations

The specification of requirements to be fulfilled by preparations of biological substances is necessary in order to ensure that these products are safe, reliable, and potent prophylactic or therapeutic agents. International recommendations on requirements are intended to facilitate the exchange of biological substances between different countries and to provide guidance to workers responsible for the production of these substances as well as to others who may have to decide upon appropriate methods of assay and control.

Recommended requirements and sets of recommendations concerned with biological substances are formulated by international groups of experts and are published in the Technical Report Series of the World Health Organization, as listed here.

Requirements

1. General Requirements for Manufacturing Establishments and Control Laboratories
   Revised 1965, TRS 323 (1966)

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)

7. Requirements for Poliomyelitis Vaccine, Oral
   Revised 1989, TRS 800 (1990)

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1 Abbreviated here as TRS.
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

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)

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

23. Requirements for Meningococcal Polysaccharide Vaccine
    Adopted 1975, TRS 594 (1976)
    Addendum 1976, TRS 610 (1977)
    Addendum 1977, TRS 626 (1978)
24. Requirements for Rubella Vaccine (Live)
   Adopted 1976, TRS 610 (1977)

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
   Revised 1988, TRS 786 (1989)

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

29. Requirements for Rabies Vaccine for Veterinary Use

30. Requirements for Thromboplastins and Plasma used to Control Oral Anticoagulant Therapy
    Revised 1982, TRS 687 (1983)

31. Requirements for Hepatitis B Vaccine prepared from Plasma
    Revised 1987, TRS 771 (1988)

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)
    Adopted 1984, TRS 725 (1985)

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)

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)

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

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

46. Requirements for Haemophilus Type b Conjugate Vaccines

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)

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)

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

Laboratories approved by WHO for the production of yellow fever vaccine, revised 1987
TRS 771 (1988)
Amendment, TRS 814 (1991)

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)
Note

Amendment to the list of laboratories approved by WHO for the production of yellow fever vaccine

The previous list, published as Annex 10 in WHO Technical Report Series, No. 771, 1988, should be amended as follows:

Delete
Pasteur Institute
Paris
France

Insert
Pasteur Mérieux Sera and Vaccines
Lyon
France
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<td>The biology of malaria parasites</td>
<td>229</td>
<td>32.–</td>
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<tr>
<td>744</td>
<td>Hospitals and health for all</td>
<td>82</td>
<td>12.–</td>
<td>Report of a WHO Expert Committee on the Role of Hospitals at the First Referral Level (149 pages)</td>
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<td>745</td>
<td>WHO Expert Committee on Biological Standardization</td>
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<tr>
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