WHO EXPERT COMMITTEE ON
BIOLOGICAL STANDARDIZATION

Twenty-fourth Report

WORLD HEALTH ORGANIZATION

GENEVA

1972
© World Health Organization 1972

Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. Nevertheless governmental agencies or learned and professional societies may reproduce data or excerpts or illustrations from them without requesting an authorization from the World Health Organization.

For rights of reproduction or translation of WHO publications in toto, application should be made to the Office of Publications and Translation, World Health Organization, Geneva, Switzerland. The World Health Organization welcomes such applications.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Director-General of the World Health Organization concerning the legal status of any country or territory or of its authorities, or concerning the delimitation of its frontiers.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

PRINTED IN SWITZERLAND
CONTENTS

GENERAL .................................................. 7

PHARMACOLOGICAL SUBSTANCES

Antibiotics
1. Doxycycline .............................................. 9
2. Gramicidin S ............................................ 9
3. Lymercycline ............................................ 9
4. Minoxycline ............................................. 10
5. Virginiamycin .......................................... 10
6. Ceclidin ............................................... 10
7. Tetacycline ............................................ 10
8. Spectinomycin ......................................... 11

Hormones, vitamins and miscellaneous substances
9. Glucagon .................................................. 11
10. Vitamin D ............................................... 11
11. Vitamin B12 ........................................... 12
12. Sulfaspram, noregplam, oxophenarsine ............ 12
13. Mel B (melarsoprol), dimercaprol, MSb .......... 12

IMMUNOLOGICAL SUBSTANCES

Antigens
14. Cholera vaccine ........................................ 13
15. Clostridium welchii (perfringens) types B and D vaccines ........................................ 13
16. Diptheria toxoid (plain) ............................... 14
17. Influenza virus haemagglutinin (type A) .......... 14
18. Pertussis vaccine ....................................... 14
19. Rabies vaccine .......................................... 15
20. Typhoid vaccine ........................................ 15

Antibodies
21. Long-acting thyroid stimulator (LATS) .............. 15
22. Anti-Rho (anti-D) complete blood-typing serum .......... 15
23. Anti-hr (anti-c) incomplete blood-typing serum ........ 16
24. Anti-Rh (anti-D) immunoglobulin .................... 16
25. Anti-Clostridium chauvoei serum .................... 17
26. Diphtheria antitoxin for flocculation test ........................................ 17
27. Gas-gangrene antitoxin (histolyticus) ................................................. 17
28. Anti-Salmonella pullorum sera .............................................................. 18
29. Anti-syphilitic human serum ................................................................. 18

INTERNATIONAL REQUIREMENTS FOR BIOLOGICAL SUBSTANCES

30. Requirements for poliomyelitis vaccine (oral) ...................................... 18
31. Requirements for cholera vaccine ......................................................... 19
32. Other requirements for biological substances ....................................... 19

ANNEXES

Annex 1. Requirements for poliomyelitis vaccine (oral) ......................... 20
Annex 2. Requirements for biological substances and other sets of recommendations 59

INDEX ........................................................................................................ 63
WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Geneva, 3-9 November 1971

Members:

Dr I. Archetti, Chief, Virus Department, Istituto Superiore di Sanità, Rome, Italy
Dr H. H. Cohen, Director, Rijks Instituut voor de Volksgezondheid, Bilthoven, Netherlands
Dr J. Desbordes, Director, Microbiology Section, Laboratoire des Actions de Santé, Paris, France (Vice-Chairman)
Dr N. K. Dutta, Director, Haffkine Institute, Bombay, India
Dr L. Higy-Mandić, Chief, Department of Biological Standardization, Institute of Immunology, Zagreb, Yugoslavia
Dr D. W. Howes, Chief Virologist, Viral Products Section, National Biological Standards Laboratory, Parkville, Victoria, Australia (Rapporteur)
Mr J. W. Lightbown, Division of Biological Standards, National Institute for Medical Research, London, England
Dr H. Mirehamsy, Associate Director, Razi State Institute, Teheran, Iran
Dr R. Murray, Director, Division of Biologics Standards, National Institutes of Health, Bethesda, Md., USA (Chairman)
Dr F. P. Nagler, Chief, Virus Laboratories, Department of National Health and Welfare, Ottawa, Ont., Canada
Dr J. Spaun, Deputy Director, Department of Biological Standardization, Statens Seruminstitut, Copenhagen, Denmark

Secretariat:

Dr D. R. Bangham, Director, Division of Biological Standards, National Institute for Medical Research, London, England (Consultant)
Dr P. Krag, Director, Department of Biological Standardization, Statens Seruminstitut, Copenhagen, Denmark (Consultant)
Dr A. S. Outshoorn, Chief Medical Officer, Biological Standardization, WHO, Geneva, Switzerland (Secretary)
Dr W. W. Wright, Director, Division of Drug Biology, Food and Drug Administration, Department of Health, Education and Welfare, Washington, D. C., USA (Consultant)

* Unable to attend:

Dr S. G. Dzagurov, Director, Tarasević State Institute for the Control of Medical Biological Preparations, Moscow, USSR
Professor D. G. Evans, Director, Lister Institute of Preventive Medicine, London, England
Professor H. O. Schild, Pharmacology Department, University College, London, England
WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Twenty-fourth Report

The WHO Expert Committee on Biological Standardization met in Geneva from 3 to 9 November 1971. Dr V. Fattorusso, Director, Division of Pharmacology and Toxicology, opened the meeting on behalf of the Director-General. He welcomed the members of the Committee and emphasized that its work was of great value for the control of particular biological substances. He was sure that the decisions taken and the recommendations made would be fully in keeping with the high standard and traditions maintained over the years.

GENERAL

International Units for Biological Products

Many biological products used in medicine cannot be adequately characterized by chemical and physical means alone. However, the activity of a biological substance may be evaluated by a biological assay, preferably by comparison with a characterized sample of the substance called a biological standard. International biological standards are preparations intended to serve throughout the world as sources of defined biological activity quantitatively expressed as international units. International units provide a convenient means of expressing in uniform terms the results of biological assays obtained by laboratories in different parts of the world. The wide use of these standards and the corresponding international units in calibrating national standards reflects the value placed on them by national regulatory agencies responsible for the control of biological products. National standards are thus linked to the relevant international standards.

The Third World Health Assembly, in 1950, adopted a resolution\(^1\) recommending that Member States of the Organization recognize officially the then current international standards and units, which were listed in the resolution. It also recommended that the standards and units be introduced into national pharmacopoeias and indicated that, where national authorities did not have their own pharmacopoeias, the potency of biological products be expressed in international units. The Eighteenth World Health Assembly,

in 1965, adopted a similar resolution and listed the international standards and units current at that time. This resolution additionally recommended that international standards and units (or their equivalents) be cited in national pharmacopoeias and be recognized in the national regulations that relate to the control of biological products.

Attention should be drawn at this point to another category of reference substances established by the WHO Expert Committee on Biological Standardization and known as international reference preparations. International reference preparations are, in many instances, similar in nature and function to international standards but have not been established as international standards on account of various considerations, which may be technical, scientific, or procedural. Their official status for national control of biological products is different from that of international standards since they have not been included in resolutions of the World Health Assembly. Consequently, acceptance by Member States throughout the world of any international unit that might have been defined for these preparations is not implied. Nevertheless, national control authorities, recognizing the value and utility of these international units, often use them in their control measures.

Essentially, therefore, an international standard is for the specific purpose of providing an international unit to serve in the control of potency of the corresponding biological products and its acceptance as such by Member States is implied by virtue of the resolutions referred to above.

Thus, when an international standard is established by the WHO Expert Committee on Biological Standardization there may be important implications for national authorities. Implementation of the recommendations of the World Health Assembly on international biological standards would mean that national control authorities would themselves adopt the international unit (or its national equivalent) and use it in their control of the corresponding biological products.

However, when chemical and physical methods become available that are adequate for the characterization of a particular biological product, there is no longer a need for the international biological standard or reference preparation, which may then be discontinued. When an international standard (or international reference preparation) is discontinued the international unit automatically ceases to exist and national authorities may have to make changes in their specifications and methods for the control of the relevant biological products. It is desirable therefore that national control authorities should be given due notice of the intended discontinuation of any international unit so that any changes that may be required can be effected.

---

1 Resolution WHA18.7 (Off. Rec. Wild Hlth Org., 1965, 143, 5).
PHARMACOLOGICAL SUBSTANCES

ANTIBIOTICS

1. Doxycycline

The Committee noted\(^1\) that as requested in its twenty-second report\(^2\) the National Institute for Medical Research, London, had obtained a preparation of doxycycline suitable to serve as an international reference preparation and that a collaborative assay was in progress. The Committee authorized that National Institute for Medical Research to establish the material as the International Reference Preparation of Doxycycline on the basis of the results of the collaborative assay and to define the international unit with the agreement of the participants.

2. Gramicidin S

The Committee noted\(^3\) that the stocks of the International Reference Preparation of Gramicidin S available for distribution had been exhausted. Since pure samples of this antibiotic, adequately characterized by chemical and physical means, were now available, the Committee agreed that an international reference preparation defining the international unit of biological activity was no longer necessary.

The Committee requested the WHO Secretariat to consider the need for and the possibility of providing this antibiotic as an international chemical reference substance.

3. Lymecycline

The Committee noted\(^4\) the results of the collaborative assay of the proposed second international reference preparation of Lymecycline. The Committee was informed that in accordance with the authorization given in its twenty-third report\(^5\) the National Institute for Medical Research, London, had established the second International Reference Preparation of Lymecycline in replacement of the first international reference preparation and, with the agreement of the participants, had defined the International Unit for Lymecycline as the activity contained in 0.0010548 mg of the International Reference Preparation of Lymecycline.

---

\(^1\) Unpublished working document WHO/BS/71.1050.
\(^3\) Unpublished working document WHO/BS/71.1049.
\(^4\) Unpublished working document WHO/BS/71.1048.
4. Minocycline

The Committee noted\(^1\) that as requested in its twenty-third report\(^2\) the National Institute for Medical Research, London, had obtained a preparation of minocycline suitable to serve as an international reference preparation. Since this material was part of the same batch as the only known national standard for minocycline a limited collaborative assay was being arranged.

5. Virginiamycin

The Committee noted\(^3\) that as requested in its twenty-third report\(^4\) the Central Veterinary Laboratory, Weybridge, in collaboration with the WHO Secretariat, had collected further information on the use and control of virginiamycin. Since this substance is a mixture of variable composition, containing components that act synergistically, it is unlikely that a single preparation of any particular composition would be suitable as an international reference preparation.

The Committee therefore requested the WHO Secretariat to consider arrangements for defining specifications for this antibiotic, as used in both human and veterinary medicine, that would indicate the nature of the reference preparation or preparations needed.

6. Clindamycin

The Committee noted\(^5\) that in accordance with the request in its twenty-third report\(^6\) the National Institute for Medical Research, London, had obtained a preparation of clindamycin suitable to serve as an international reference preparation. Since this material was part of the same batch as the only known national standard for clindamycin, a limited collaborative assay had been performed. The Committee therefore established this preparation as the International Reference Preparation of Clindamycin and, with the agreement of the participants, defined the International Unit for Clindamycin as the activity contained in 0.0011947 mg of the International Reference Preparation of Clindamycin.

7. Tetracycline

The Committee was informed that in furtherance of the request in its twenty-second report\(^6\) revision of the international specifications for purity

---

\(^1\) Unpublished working document WHO/BS/71.1051.
\(^3\) Unpublished working document WHO/BS/71.1045.
\(^5\) Unpublished working document WHO/BS/ 71.1052.
of tetracycline was being considered\textsuperscript{1} and that this would take account of procedures available for controlling the proportions of toxic degradation products.

8. Spectinomycin

The Committee noted\textsuperscript{2} that there may be a need for international reference material for spectinomycin, and requested the National Institute for Medical Research, London, in conjunction with the WHO Secretariat, to determine whether there was a need for an international reference preparation and, if so, to obtain suitable material.

HORMONES, VITAMINS AND MISCELLANEOUS SUBSTANCES

9. Glucagon

The Committee noted\textsuperscript{3} that, as requested in its twenty-third report,\textsuperscript{4} the National Institute for Medical Research, London, had ascertained that the preparation of porcine glucagon referred to in that report was suitable to serve as an international standard, and part of the material was available for this purpose. Since the biological activity of glucagon cannot be adequately determined by chemical and physical means, the Committee agreed that there was a need for an international standard for the control of glucagon preparations. The Committee therefore requested the National Institute for Medical Research to obtain the material offered and to proceed with the collaborative assay requested.

10. Vitamin D

The Committee noted\textsuperscript{5} a proposal that the existing International Standard for Vitamin D, which was a preparation of colecalciferol, should be discontinued because chemical and physical methods were adequate for characterization of preparations of vitamin D. Since, however, biological assays of vitamin D would still continue to be used, there would be difficulties for national control authorities if the international standard and unit were discontinued immediately. The Committee therefore agreed that before this international standard be discontinued steps should first be

\textsuperscript{1} \textit{WHO Hlth Org. techn. Rep. Ser.}, 1972, No. 487.
\textsuperscript{2} Unpublished working document WHO/BS/71.1047.
\textsuperscript{3} Unpublished working document WHO/BS/71.1042.
\textsuperscript{5} Unpublished working document WHO/BS/71.1030.
taken to ensure the availability of appropriate reference material for the
control of vitamin D preparations and requested the WHO Secretariat to
consider the measures needed, possibly including the provision of a suitable
international chemical reference substance that could also be used for biol-
logical assays.

The Committee was aware that the international units assigned to the
international standards for vitamin A and for provitamin A, discontinued
in 1954 and 1956 respectively, are still widely quoted. In view of the possi-
bility of a similar situation developing with respect to vitamin D when the
international standard is discontinued, the possibility of arranging for
appropriate specifications for vitamin A and vitamin D preparations with-
out the use of international units should also be explored.

11. Vitamin B12

The Committee noted\(^1\) a proposal to discontinue the International
Reference Preparation of Vitamin B\(_{12}\) because adequate chemical and
physical methods were now available for the characterization of prepara-
tions of vitamin B\(_{12}\) that were intended for use in clinical medicine or might
serve as references in assays. The Committee agreed with this proposal and
discontinued this international reference preparation. Since, however, bio-
logical assays would still be used there might be difficulties for some national
control authorities if reference material were not available. The Committee
therefore agreed that remaining stocks should be made available to labora-
tories that might need this material.

The Committee requested the WHO Secretariat to consider providing
a chemical reference substance that could also be used for biological assays.

12. Sulfarsphenamine, Neoarsphenamine, Oxophenarsine

The Committee noted\(^2\) a proposal to discontinue the International
Reference Preparations of Sulfarsphenamine, Neoarsphenamine, and Oxo-
phenarsine because they are not widely used for biological tests. The
Committee requested the WHO Secretariat to ascertain the use of these
compounds in clinical medicine and the need for reference materials.

13. Mel B (Melarsoprol), Dimercaprol, MSb

The Committee noted\(^2\) a proposal to discontinue the existing Inter-
national Reference Preparations of Mel B (Melarsoprol) and of Dimercapro-
lar because adequate chemical and physical methods are now available for

\(^1\) Unpublished working document WHO/BS/71.1031.
\(^2\) Unpublished working document WHO/BS/71.1036.
characterization of these substances. Since, however, these compounds are widely used in the treatment of trypanosomiasis, the Committee requested the WHO Secretariat to investigate the need for reference materials, which may be necessary for the control of these two substances before the international reference preparations are discontinued.

The Committee also noted a proposal to discontinue the International Reference Preparation of MSb on the grounds that there was no demand for it and the compound was apparently no longer used. Since little was known about the current use of MSb, the Committee requested the WHO Secretariat to collect further information on this question.

IMMUNOLOGICAL SUBSTANCES

ANTIGENS

14. Cholera Vaccine

The Committee noted the results of the collaborative assay, referred to in its twenty-first and twenty-second reports, of the preparations obtained for the replacement of the International Reference Preparations of Cholera Vaccine (Ogawa) and of Cholera Vaccine (Inaba). The results of the tests of antigenicity of the proposed replacements relative to the existing international reference preparations showed a wide variation between laboratories as a result of which there may be limitations at present to the control of cholera vaccine. The Committee agreed that notwithstanding this variation the preparations could serve as international reference preparations for determination of antigenicity. The Committee therefore established the preparations studied as the second International Reference Preparation of Cholera Vaccine (Ogawa) and the second International Reference Preparation of Cholera Vaccine (Inaba) in replacement of the first international reference preparations.

15. Clostridium welchii (Perfringens) Types B and D Vaccines

The Committee noted that preliminary studies of the preparations of Clostridium welchii (perfringens) types B and D vaccines, referred to in its

---

2 Unpublished working document WHO/BS/71.1036.
5 See section 31, p. 19.
6 Unpublished working document WHO/BS/71.1044.
twenty-third report,1 showed that these preparations were suitable to serve as international standards and that the Central Veterinary Laboratory, Weybridge, would arrange the collaborative assays requested.

16. Diphtheria Toxoid (Plain)

The Committee noted 2 the results of studies of the preparation of diphtheria toxoid (plain), referred to in its twenty-second report,3 that might serve as the replacement for the International Standard for Diphtheria Toxoid (Plain). These results showed that valid assays could not be made of the proposed replacement in relation to the existing international standard. This may be attributable to differences in purity and composition between the preparations because of changes in methods of manufacture developed since the international standard was established in 1951; the more recent preparations have assay slopes that are different from those of the earlier type of preparation. The Committee was informed that information obtained during these studies indicated that there was little evidence of the use in man of purified diphtheria toxoid as a non-adsorbed product and that it would be advisable to reassess the continued need for this international standard. The Committee therefore requested the Statens Serum-institut, Copenhagen, to provide further information to enable a decision to be made.

17. Influenza Virus Haemagglutinin (Type A)

The Committee noted 4 that studies made in a national laboratory of the International Reference Preparation of Influenza Virus Haemagglutinin (Type A), a freeze-dried preparation of the inactivated virus, had confirmed the presence of some common contaminating bacteria. The Statens Serum-institut, Copenhagen, was carrying out further tests on the preparation. The results thus far available showed that the micro-organisms present were not of significance to the intended use of the preparation and there was no evidence of any change in its haemagglutinating activity. It had also been successfully used in the extensive studies previously reported 5 of a number of influenza virus vaccines. However, the Committee agreed that, once the preparation had been reconstituted, it was desirable for it to be used immediately.

18. Pertussis Vaccine

The Committee was informed that in regard to the replacement of the International Standard for Pertussis Vaccine, requested in its seventeenth

---

report, arrangements would shortly be made for the collaborative assay that had been postponed until the results were available of the research studies referred to in its twenty-third report.  

19. Rabies Vaccine

The Committee noted that the continuing studies, requested in its eighteenth report, of the stability of the International Reference Preparation of Rabies Vaccine had not so far revealed any undue instability of the preparation.

20. Typhoid Vaccine

The Committee was informed of further controlled field studies and laboratory tests of an acetone-inactivated typhoid vaccine of the kind used in earlier field trials and similar to the International Reference Preparation of Typhoid Vaccine (Acetone-Inactivitated).

In view of the continuing problems in the development of satisfactory laboratory tests for evaluating typhoid vaccine, the Committee again emphasized the value of performing such laboratory tests in parallel with controlled field trials of typhoid vaccines.

ANTIBODIES

21. Long-Acting Thyroid Stimulator (LATS)

The Committee noted that studies of the preparation of long-acting thyroid stimulator (LATS), referred to in its eighteenth and nineteenth reports, had shown that owing to difficulties in available methods of assay the substance had not found wide use for clinical diagnosis. The Committee therefore agreed that there was no need to establish international reference material.

22. Anti-Rh0 (Anti-D) Complete Blood-Typing Serum

The Committee noted the further stability studies referred to in its nineteenth report of the freeze-dried preparation of anti-Rh0 (anti-D)

---

4 Unpublished working document WHO/BS/71.1040.
8 Unpublished working document WHO/BS/71.1037.
complete blood-typing serum. The Committee was informed that there was only limited interest in an international standard for this substance and more information would be necessary in order to assess the need for one.

23. Anti-hr' (Anti-c) Incomplete Blood-Typing Serum

The Committee noted that studies of the freeze-dried preparation of anti-hr' (anti-c) incomplete blood-typing serum referred to in its nineteenth report showed that the preparation had satisfactory stability. The Committee also noted that a collaborative assay was being planned.

24. Anti-Rh_0 (Anti-D) Immunoglobulin

The Committee noted that, as requested in its nineteenth report, the National Institute for Medical Research, London, in collaboration with the International Blood Group Reference Laboratory, London, had collected information on methods of assay of anti-Rh_0 (anti-D) human IgG immunoglobulin preparations. A WHO Scientific Group on the Prevention of Rh Sensitization had also considered this subject. The information available showed that various methods of assay were being studied, including one by radioimmunoassay used in the International Reference Centre for the Use of Anti-D (Rh_0) in the Prevention of Rh Sensitization, London. There was little definite evidence, however, of general agreement on the value of any particular methods for defining the activity of preparations of this immunoglobulin and for prescribing dosage.

The Committee also noted that the suggestion made in its nineteenth report that portions of materials used in clinical trials in various countries should be set aside to serve as possible standards could not be followed since in most instances only small quantities were made and these were used immediately. A single freeze-dried preparation of immunoglobulin had, however, been made from pooled human sera obtained from several countries and containing anti-D antibodies, and some studies had already been made on it.

The Committee also noted that the National Institute for Medical Research, the International Blood Group Reference Laboratory, and the International Reference Centre for the Use of Anti-D (Rh_0) in the Prevention of Rh Sensitization, in collaboration with the WHO Secretariat, would arrange further collaborative studies with a view to determining the suita-

---

3 Unpublished working document WHO/BS/71.1038.
5 Unpublished working document WHO/BS/71.1053.
bility of the assay methods and the best use of the available preparation as a standard in such assays.

25. Anti-Clostridium chauvoei Serum

The Committee reconsidered the need for an international reference preparation of anti-Clostridium chauvoei serum, a subject that had previously been considered at its seventeenth meeting,1 and noted2 that there was now little use of this serum in the prevention and treatment of Clostridium chauvoei infections. The Committee agreed that there was no need for an international reference preparation.

26. Diphtheria Antitoxin for Flocculation Test

The Committee noted3 that the collaborative studies, referred to in its twenty-second and twenty-third reports,4 of the proposed replacement for the fourth International Reference Preparation of Diphtheria Antitoxin for Flocculation Test had now been made. The Committee was informed of the results of these studies, which were made using in vitro tests of flocculating activity in relation to the first International Standard for Diphtheria Antitoxin. The Committee agreed that such a comparison should form the basis for the specification of the flocculating activity of the replacement, since the first international standard had been in existence throughout all the previous replacements of the international reference preparation.

Since stocks of the existing international reference preparation were nearly exhausted, the Committee authorized the Statens Serum Institut, Copenhagen, to establish the preparation studied as the fifth International Reference Preparation of Diphtheria Antitoxin for Flocculation Test and to specify its flocculating activity from the results obtained.

27. Gas-Gangrene Antitoxin (Histolyticus)

The Committee noted5 that the preparation of gas-gangrene antitoxin (histolyticus), referred to in its twenty-third report,6 for replacement of the second International Standard for Gas-gangrene Antitoxin (Histolyticus) had been obtained and that a collaborative assay had been made. The Committee was informed that the results confirmed that the potency per

---

unit weight of the preparation was the same as that of the international standard, a result that was expected since both preparations were taken from the same bulk material. The definition of the international unit on the basis of this equivalence was agreeable to the participants. Since stocks of the international standard were nearly exhausted, the Committee authorized the Statens Seruminstitut, Copenhagen, to establish the preparation studied as the third International Standard for Gas-gangrene Antitoxin (Histolyticus). The international unit would be the activity contained in 0.2 mg of the preparation.

28. Anti-Salmonella pullorum Sera

The Committee noted\(^1\) that suitable sera had been obtained of the two types of anti-Salmonella pullorum serum referred to in its twenty-second report\(^2\) and that collaborative studies were in progress.

29. Anti-Syphilitic Human Serum

The Committee noted\(^3\) that the Statens Seruminstitut, Copenhagen, had made studies of the International Standard for Syphilitic Human Serum in comparison with a number of sera that had been included in the collaborative assay made in 1956 for the establishment of this international standard and that had been stored under similar conditions. These studies, which were made with complement fixation tests, had shown that the international standard and fifteen of the sixteen sera studied had retained their relative potency after a period of fifteen years. The Committee also noted\(^4\) that further studies using a flocculation test (VDRL) would be made.

INTERNATIONAL REQUIREMENTS FOR BIOLOGICAL SUBSTANCES

30. Requirements for Poliomyelitis Vaccine (Oral)

The Committee studied the revised requirements for poliomyelitis vaccine (oral) that had been prepared by the WHO Secretariat\(^4\) in collaboration with a number of experts. The original requirements were published in 1962 and the first revision in 1965. After making certain modifications, the Committee agreed that the present text of these requirements, which

---

\(^1\) Unpublished working document WHO/BS/71.1045.
\(^3\) Unpublished working document WHO/BS/71.1039.
now included requirements for vaccine prepared using monkey kidney cells or human diploid cells, was satisfactory and that they would be useful for the control of poliomyelitis vaccine (oral) produced in different countries.

The Committee adopted these requirements and agreed that they should be annexed to the present report (see Annex 1).

31. Requirements for Cholera Vaccine

The Committee agreed that since replacement of the International Reference Preparations of Cholera Vaccine (Ogawa) and of Cholera Vaccine (Inaba) had now been made (see section 14, p. 13), the provisions in the revised Requirements for Cholera Vaccine 1 relating to limits of antigenicity as determined in the active mouse protection test (Part A, section 5.4.1 of the requirements) were no longer applicable and the Committee therefore requested the WHO Secretariat to arrange for suitable modifications to be made to these requirements.

32. Other Requirements for Biological Substances

In view of the recent developments in methods of sterility testing of biological products and the improvements in control measures that were now feasible, the Committee agreed that a revision of the Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) 2 should be undertaken.

ACKNOWLEDGEMENTS

The Committee wishes to record its thanks to the following members of the WHO Secretariat for their special contributions to its deliberations: Dr W. C. Cockburn, Chief, Virus Diseases; Dr B. Cvjetanović, Chief, Bacterial Diseases; Dr D. S. Rowe, Director, WHO International Reference Centre for Immunoglobulins; Dr G. Torrigiani, Immunology; Mr K. O. Wallen, Chief, Pharmaceuticals; Dr Y. Watanabe, Bacterial Diseases.

---

Annex 1

REQUIREMENTS FOR POLIOMYELITIS VACCINE (ORAL)

(Requirements for Biological Substances No. 7)

Revised 1971 *

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
</tr>
<tr>
<td>General considerations</td>
</tr>
<tr>
<td>Part A. Manufacturing requirements</td>
</tr>
<tr>
<td>1. Definitions</td>
</tr>
<tr>
<td>2. General manufacturing requirements</td>
</tr>
<tr>
<td>3. Production control</td>
</tr>
<tr>
<td>4. Filling and containers</td>
</tr>
<tr>
<td>5. Control tests on final product</td>
</tr>
<tr>
<td>6. Records</td>
</tr>
<tr>
<td>7. Samples</td>
</tr>
<tr>
<td>8. Labelling</td>
</tr>
<tr>
<td>9. Distribution and shipping</td>
</tr>
<tr>
<td>10. Storage and expiry date</td>
</tr>
<tr>
<td>Part B. National control requirements</td>
</tr>
<tr>
<td>1. General</td>
</tr>
<tr>
<td>2. Release and certification</td>
</tr>
<tr>
<td>Part C. Requirements for poliomyelitis vaccine (oral) prepared using human diploid cells</td>
</tr>
</tbody>
</table>

* Requirements for Poliomyelitis Vaccine (Oral) (Requirements for Biological Substances No. 7) were first published in *Wild Hlth Org. techn. Rep. Ser.*, 1962, No. 237. The original draft of these requirements was prepared by a Study Group on Requirements for Biological Substances, which met in Geneva from 7-12 November 1960. The membership of this Study Group is shown in Appendix 1, page 56. A revised version of the requirements, incorporating additions and amendments made by a WHO Expert Group on Requirements for Biological Substances, which met in Geneva from 16-22 March 1965, was published in *Wild Hlth Org. techn. Rep. Ser.*, 1966, No. 323. The membership of this Expert Group is shown in Appendix 2, page 56.

The draft version of the present revised requirements was prepared by the following members of the WHO Secretariat: Dr W. C. Cockburn, Chief Medical Officer, Virus Diseases, WHO, Geneva, Switzerland; Dr S. G. Dzagurov, Director, Tarasevich State Institute for the Control of Medical Biological Preparations, Moscow, USSR (Consultant); Dr L. Hayflick, Department of Medical Microbiology, Stanford University School of Medicine, Palo Alto, Calif., USA (Consultant); Professor W. Hennessen, Behringwerke AG, Marburg/Lahn, Federal Republic of Germany (Consultant); Dr R. L. Kirschstein, Division of Biologies Standards, National Institutes of Health, Bethesda, Md., USA (Consultant); Dr R. Murray, Director, Division of Biologies Standards, National Institutes of Health, Bethesda, Md., USA (Consultant); Dr F. P. Nagler, Chief, Virus Laboratories, Department of National Health and Welfare, Ottawa, Ont., Canada (Consultant); Dr A. S. Oughton, Chief Medical Officer, Biological Standardization, WHO, Geneva, Switzerland; Dr F. T. Perkins, Director, Division of Immunological Products Control, National Institute for Medical Research (Hampstead Laboratories), London, England (Consultant); Dr S. S. Vrancheva, Medical Officer, Biological Standardization, WHO, Geneva, Switzerland.

— 20 —
Introduction

Since the Requirements for Poliomyelitis Vaccine (Oral) (Requirements for Biological Substances No. 7) were first formulated and later revised more experience has been gained in the control of this vaccine and the twenty-third meeting of the WHO Expert Committee on Biological Standardization\(^1\) agreed that there was a need for a further revision. In addition, the development of the use of human diploid cells instead of the earlier use of monkey kidney-cells for growing polioviruses for vaccine production made it necessary to give consideration to recent technical advances. Since publication of a set of amendments was not considered a satisfactory way of revising the requirements, a complete new document has been prepared. Some of it is identical with the requirements originally formulated or with those in the previous revision, but alterations have been made where they were considered necessary and in addition certain new requirements, including those for human diploid cell poliomyelitis vaccine, have been introduced.

In revising these requirements account has been taken of the opinions of consultants, the regulations and requirements for the manufacture and control of this vaccine that have been formulated in a number of countries, as well as information from both published and unpublished reports. In addition, opinions and data have been received from a number of experts.

Grateful acknowledgement is made to the experts and institutions listed below for their comments and advice and for supplying additional data relevant to these requirements:

Dr I. Archetti, Istituto Superiore di Sanità, Rome, Italy
Dr A. J. Beale, Head, Biological Division, The Wellcome Research Laboratories, Beckenham, Kent, England
Professor O. Bonin, Paul Ehrlich Institute, Frankfurt am Main, Federal Republic of Germany
Dr J. Bond, WHO Regional Office for the Americas, Washington, D.C., USA
Dr B. D. Byčenko, Deputy Director, Tarasevič State Institute for the Control of Medical Biological Preparations, Moscow, USSR
Dr H. Cohen, Director, Rijks Instituut voor de Volksgezondheid, Bilthoven, Netherlands
Professor M. P. Čumakov, Director, Institute of Poliomyelitis and Virus Encephalitis, USSR Academy of Medical Sciences, Moscow, USSR
Mr V. F. Davey, Deputy Director (Technical), Commonwealth Serum Laboratories, Parkville, Victoria, Australia
Dr W. R. Dowdle, Center for Disease Control, Atlanta, Ga., USA
Professor G. Edsall, Superintendent, State Laboratory Institute, Department of Public Health, Boston, Mass., USA
Professor D. G. Evans, London School of Hygiene and Tropical Medicine, London, England

Dr C. B. Gerichter, Director, Division of Laboratories, Ministry of Health, Jerusalem, Israel
Dr J. J. Graydon, Senior Consultant, Commonwealth Serum Laboratories, Parkville, Victoria, Australia
Mr A. Hampson, Commonwealth Serum Laboratories, Parkville, Victoria, Australia
Dr J. W. F. Hampton, National Biological Standards Laboratory, Viral Products Section, Parkville, Victoria, Australia
Professor G. Heymann, Paul Ehrlich Institute, Frankfurt am Main, Federal Republic of Germany
Dr M. R. Hilleman, Director, Virus and Cell Biology Research, Merck Institute for Therapeutic Research, West Point, Pennsylvania, USA
Dr Shigeo Honjo, Chief, Second Division of Experimental Animals, Department of Veterinary Science, National Institute of Health, Tokyo, Japan
Dr D. W. Howes, National Biological Standards Laboratory, Viral Products Section, Parkville, Victoria, Australia
Dr C. Huygelen, Director, Biologics Department, Recherche et industrie thérapeutiques, Genval, Belgium
Professor D. Ikić, Director, Institute of Immunology, Zagreb, Yugoslavia
Dr I. Z. E. Imam, Director General, Public Health Laboratories, Virus Research Centre, Agouza, Cairo, Egypt
Professor M. Kantoch, Head, Department of Virology, State Institute of Hygiene, Warsaw, Poland
Dr F. Kogoj, Vice-President, Yugoslav Academy of Sciences and Arts, Zagreb, Yugoslavia
Dr P. Krag, Director, International Laboratory for Biological Standards, Statens Serum-institut, Copenhagen, Denmark
Dr A. Lafontaine, Ministry of Health and Family Affairs, Institute of Hygiene and Epidemiology, Brussels, Belgium
Dr D. R. E. MacLeod, Assistant Director, Connaught Medical Research Laboratories, Willowdale, Ont., Canada
Dr A. D. Macrae, Consultant Virologist, Regional Virus Laboratory, City Hospital, Edinburgh, Scotland
Dr H. Malherbe, The Poliomyelitis Research Foundation, Johannesburg, South Africa
Dr H. Mirchamsy, Associate Director, Razi State Institute, Teheran, Iran
Dr P. S. Moorhead, Associate Professor, University of Pennsylvania, The School of Medicine, Department of Medical Genetics, Philadelphia, Pa., USA
Dr Keizo Nakamura, National Institute of Health, Japan, Tokyo
Dr R. Netter, Director, Virology Section, Laboratoire des Actions de Santé, Paris, France
Dr S. A. Plotkin, The Wistar Institute, Philadelphia, Pa., USA
Dr M. Pontecorvo, Director, Biological Division, Istituto Sieroterapico e Vaccinogeno Toscano, Siena, Italy
Dr A. J. Rhodes, Associate Medical Director, Laboratory Services Branch, Ontario Department of Health, Ont., Canada
Dr J. de Rudder, Director of Manufacture, Pasteur Institute, Paris, France
Dr G. C. Schild, World Influenza Centre, National Institute for Medical Research, Mill Hill, London, England
Dr J. B. Shrivastav, Director General of Health Services, Delhi, India
General Considerations

The strains of poliovirus used in the production of poliomyelitis vaccine (oral) must have been shown to yield vaccines that are immunogenic and free from harmful effects when administered orally to susceptible children and adults. At the present time the Sabin strains are mainly employed for vaccine production but the Koprowski strains are used in some countries. In view of the great practical problems involved in ensuring that new strains would be suitable for the manufacture of vaccine used on a wide scale, it is unlikely that any new candidate strains will be accepted in the foreseeable future. No provision has been made in these requirements for vaccines made from new strains. To conserve stocks of seed material of the strains that are now in use, national control authorities should provide reference virus preparations for each of the 3 virus types to determine the sensitivity of the cell cultures for virus titration for the control of consistency of vaccine quality. Such reference preparations need not necessarily be the seed virus from which the vaccine is prepared and the use of appropriate preparations would enable adequate tests to be carried out.

The use of the seed lot system is universal practice for making this vaccine but the identity of the seed prepared from the Sabin strains requires clarification since there is confusion as to the definition of the seed from

---

1 The following are the strains at present in use:

<table>
<thead>
<tr>
<th>Sabin</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSc 2 ab</td>
<td>P 712 CH 2 ab</td>
<td>Leon 12a 1 b</td>
<td></td>
</tr>
<tr>
<td>CHAT</td>
<td>W-II</td>
<td>WM-3</td>
<td></td>
</tr>
</tbody>
</table>
which the original vaccine was prepared that was shown to be immunogenic and free from harmful effects in man. A memorandum published in the *Bulletin of the World Health Organization* ¹ gives a detailed list of the various passage levels of viruses of the Sabin strains now in use for poliomyelitis vaccine production. The information on type 3 virus is especially important as it is now evident that this type is less stable, on subcultivation in cell cultures, than either of the other two attenuated virus types. The number of passages allowed in order to make satisfactory vaccine should therefore be strictly limited. National control authorities should decide on the detailed procedures applicable to the preparation and use of seed lots.

The health of monkeys used for biomedical purposes as well as safeguards for personnel working with monkeys have been considered by WHO as well as by other organizations. Suitable recommendations have been discussed by a WHO Scientific Group ² and many of these recommendations have been incorporated in the present requirements in relation to monkeys used for the production and testing of vaccine.

Requirements for tests for the presence of extraneous agents are brought up to date in the light of experience gained in some countries (these agents include several simian viruses, e.g., SV40, herpes B, foamy virus, Marburg virus, and monkeypox virus). The test for B virus in rabbits has been revised. The volumes tested and the use of subcultivation in order to allow detection of contaminating viruses, which may be present in low concentrations, have been stipulated.

In some countries newer techniques have been developed for tests for bacteria and fungi and other microbial contaminants of biological products and the national control authorities have introduced appropriate requirements embodying these developments. When future revisions are made to the requirements for sterility tests, they should be applied where appropriate in the present requirements.

The question of formulating requirements for the virus titre for each of the poliomyelitis virus types in the final product poses a problem. Several factors would have to be taken into consideration including the particular combination of virus types, the recommended dosage schedule for primary and reinforcing vaccination, and the existence in some population groups of

---

¹ *Bull. World Health Organ.*, 1969, 40, 925-946. According to the system of strain notation used in the case of Sabin strains the original seed virus is designated as SO and the original vaccine as SO + 1 for each type. The authors of the memorandum considered that it was necessary to make vaccines, particularly type 3 vaccines, from seed lots at the lowest passage level from the original virus and that a satisfactory low passage seed lot should be made available. Seed lots should be not more than 3 passages (for types 1 and 2) and 2 passages (for type 3) from the original seed virus.

a type of intestinal flora that may affect the ability of the vaccine virus to multiply in the gut. No specific recommendations for virus titre, therefore, have been included in these requirements. National control authorities should determine the needs applicable to particular circumstances. In doing so advice should be obtained from experts in vaccine production and control as well as from epidemiologists and clinicians.

In some countries a manufacturing establishment may limit vaccine production to filling final containers with vaccine obtained in the bulk form from another manufacturing establishment. In such cases, conformity with all the requirements applicable to the final vaccine (Part A, sections 5 et seq.) must be the responsibility of the manufacturer of the final product.\(^1\) It is desirable also that the label carry the names of both manufacturing establishments. In other countries, with the approval of the national control authority, certain tests in production and control that have not been made in the manufacturing establishment may be made in an independent laboratory. In such cases the manufacturer must nevertheless assume the responsibility for the safety and efficacy of the product.

Experience in the production of poliomyelitis vaccine by growing the virus on monkey kidney-cell cultures has shown that many such tissues are contaminated with one or more extraneous viruses and have to be rejected. Thus, in recent years the trend in the production of newer live viral vaccines has been to use primary cell cultures from animals derived from closed colonies. Few, if any, extraneous agents have been isolated from these cell cultures. Other studies have led to the development of diploid cell strains from other mammalian sources, primarily human. The ultimate goal must lie in the establishment of a cell bank comprising cells whose growth cycle is capable of indefinite arrest by refrigeration until such time as they have been shown, by exhaustive tests, to be free from all known contaminants. Thereafter an aliquot of such cells may be used for virus vaccine production with greater confidence that the harvest of virus from such a substrate will be free from contaminants.

One human diploid cell strain\(^2\) derived from embryo lung tissue has been in widespread use and 4 different live virus vaccines that have been

---

\(^1\) A guide to the processing and testing procedures that may be used in such cases is available on request from the World Health Organization, 1211 Geneva 27, Switzerland.

\(^2\) The isolation of human diploid cells has been described (see Hayflick, L. & Moorhead, P., Exp. Cell Res., 1961, 25, 585. The human diploid cell strain (WI-38) has been isolated and frozen at the eighth population doubling by Hayflick (see Hayflick, L., Exp. Cell Res., 1965, 37, 614) and there has now been a large accumulation of experience in the use of these cells. Information may be obtained from Professor L. Hayflick, Stanford University, Palo Alto, Calif., USA or from Dr F. T. Perkins, National Institute for Medical Research, Hampstead Laboratories, Holly Hill, London, N.W.3, England. From the latter information may also be obtained on another human diploid cell strain (MRC-5) (see Jacobs, J. P., Jones, C. M. & Baille, J. P., Nature (Lond.), 1971, 227, 168) which has been extensively studied.
produced using cultures of this cell strain have been licensed by certain national control authorities.

In a number of countries poliomyelitis vaccine (oral) produced in such human diploid cell cultures has been administered orally to persons in whom satisfactory immunity developed, without untoward effects. Since, however, poliomyelitis vaccine (oral) prepared using human diploid cells has many advantages but also presents special problems, it is desirable that the manufacture of such vaccine be undertaken only with the approval of the national authority of the country of origin of the vaccine. Further, when such vaccine is imported for use from another country, it must be the responsibility of the national control authority of the importing country to verify conformity with appropriate requirements in the country of origin of the product. Additional requirements for poliomyelitis vaccine (oral) prepared using human diploid cells have been formulated and are given in Part C of this document.

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

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

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

*In the following sections an asterisk preceding a section indicates that additional or alternative requirements applicable when the vaccine is made using human diploid cells will be found in Part C, page 47.*
Part A. Manufacturing Requirements

1. Definitions

1.1 International name and proper name

The international name shall be "Vaccinum poliomyelitidis perorale Typus I, II, III" (whichever type or types apply). The proper name shall be the equivalent of the international name in the language of the country of origin.

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

1.2 Descriptive definition

Vaccinum poliomyelitidis perorale is a preparation of live attenuated poliovirus types 1, 2, or 3 grown in in vitro cultures of suitable cells, containing any one type or any combination of the three types, prepared in a form suitable for oral administration, and satisfying all the requirements formulated in this document.

1.3 International standards and international reference preparations

Since no international reference preparations of live attenuated polioviruses are available, no requirements based on comparisons with such preparations can at present be formulated. National control authorities should provide reference preparations of each of the three live poliovirus types for use in tests of virus concentration (see Part A, sections 3.5.4 and 3.5.3) to determine the sensitivity of the cell cultures used. It is necessary also to provide an appropriate rct(40): reference strain for each type for the marker tests (see Part A, section 3.5.5.2) as well as appropriate reference preparations for the neurovirulence tests (see Part A, section 3.5.5.1).

The International Standards for Anti-poliovirus Sera Type 1, Type 2 and Type 3 (established in 1962) are dispensed in ampoules containing dried hyperimmune monovalent serum. Each ampoule of each type contains 10 IU. These preparations are in the custody of the International Laboratory for Biological Standards, Statens Seruminstitut, Copenhagen. Samples are distributed free of charge on request to national control laboratories. These international standards are intended for the calibration of national standards for use in the estimation of antibody responses to poliomyelitis vaccine in man and animals.

*1.4 Terminology

Original vaccine: A monovalent vaccine, prepared according to the author's specification from the original seed virus, and shown on oral ad-
ministration to man in extensive field trials to be immunogenic and free from harmful effects.

*Seed lot*: A quantity of virus derived from an original vaccine processed together and of uniform composition. Seed lots are not more passages removed from the original seed virus than a number approved by the national control authority.

*Single harvest*: A virus suspension of one virus type harvested from cell cultures prepared from the kidneys of one monkey.

*Bulk suspension*: A pool of a number of single harvests of the same virus type.

*Final bulk*: The finished biological preparation present in the container from which the final containers are filled. The final bulk may be prepared from one filtered bulk suspension, or from a blend of filtered bulk suspensions, or from a dilution thereof.

*Filling lot (final lot)*: A collection of sealed, final containers of liquid vaccine or dragees that are homogeneous with respect to the risk of contamination during filling or preparation of the finished vaccine. A filling lot must, therefore, have been filled or prepared in one working session.

*Tissue culture infective dose 50% (TCID₅₀)*: The quantity of a virus suspension that will infect 50% of cell cultures.

*Plaque-forming unit (PFU)*: The smallest quantity of a virus suspension that will produce a plaque in monolayer cell cultures.

*2. General manufacturing requirements*

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

Production areas shall be decontaminated before they are used for the manufacture of oral poliomyelitis vaccine.

The production of oral poliomyelitis vaccine shall be conducted by a separate staff, which shall consist of healthy persons, who shall be examined medically at regular intervals. Steps shall be taken to ensure that all such persons in the production areas and monkey quarters are immune against poliomyelitis and do not excrete poliovirus or other micro-organisms of significance to the safety of the vaccine. Personnel working in monkey quarters shall be examined also for tuberculosis as outlined in Part A,

section 2 of the Requirements for Biological Substances No. 11 (Requirements for Dried BCG Vaccine).\(^1\)

Visitors and persons not directly concerned with the production processes shall not be permitted to enter the production areas.

It is desirable to pay particular attention to the recommendations given in Part A, section 1 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)\(^2\) in regard to the training and experience of the person in charge of production and testing and of those assigned to various areas of responsibility in the manufacturing establishment as well as to registration of such personnel with the national control authority.

3. Production control

3.1 Control of source materials

3.1.1 Virus strains

Strains of poliovirus used in the production of oral poliomyelitis vaccine shall be identified by historical records, which should include information on the origin of the strains and on the methods used in their attenuation. Only strains that are approved by the national control authority shall be used.\(^3\)

*3.1.2 Monkeys used for obtaining kidney tissue and testing of virus

Monkeys of a species approved by the national control authority, in good health, and that have not previously been used for experimental purposes, shall be used as the source of kidney tissue for the production of virus.

The monkeys shall be kept in well-constructed animal rooms in cages spaced as far apart as possible with adequate ventilation. Adequate precautions shall be taken to prevent cross infection between cages. The monkeys shall be housed not more than 2 per cage and cage-mates shall not be interchanged. The monkeys shall be kept in the country of manufacture of the vaccine in quarantine groups\(^4\) for a period of at least 6 weeks before use. If at any time during the quarantine period the overall death rate of a shipment consisting of one or more groups reaches 5% (excluding deaths from accidents or causes specifically determined not to be due to infectious diseases) monkeys from that entire shipment shall continue in quarantine from such time for a further period of not less

---


\(^3\) See *General Considerations*, p. 23.

\(^4\) A quarantine group is a colony of selected, healthy monkeys kept in one room, with separate feeding and cleaning facilities, and having no contact with other monkeys during the quarantine period.
than 6 weeks. The groups shall be kept continually in isolation, as in quarantine, even after completion of the quarantine period, until the monkeys are used or discarded. After the last monkey of a group has been taken the room that housed the group shall be thoroughly cleaned and decontaminated before being used for a fresh group.

All actions of working personnel shall be based on the premise that there is a great potential hazard at all times in the quarantine area. Personnel shall be provided with protective clothing, including gloves, footwear, and masks or visors. Street clothes shall not be permitted in the animal rooms. Smoking and eating shall be forbidden to personnel while they are in the animal rooms.

Responsibility shall be assigned to a supervisor for reporting unusual illness among employees and ensuring that all injuries are properly treated. No worker who has cuts or abrasions on exposed areas of the body shall enter the animal area. Any unexplained febrile illness while off duty shall be considered as potentially related to the employee's occupation.

Monkeys from which kidneys are to be removed shall be thoroughly examined, particularly for evidence of tuberculosis and B virus infection. Monkeys prepared for removal of kidneys should be anaesthetized.

If a monkey shows any pathological lesion relevant to the use of its kidneys in the preparation of a seed lot or vaccine, this monkey shall not be used, and none of the remaining monkeys of the quarantine group concerned shall be used unless it is evident that their use will not impair the safety of the product.

All operations described in this section shall be conducted outside the areas where vaccine is made.

It is advisable that the monkeys used should be shown to be free from antibodies to SV40 virus.

*3.1.3 Seed lot system

The production of vaccine shall be based on the seed lot system. The seed lot used for the production of vaccine shall be that used in preparing original vaccine, or a seed lot prepared therefrom by a method and at a passage level from the original seed virus approved by the national control authority. Seed lots shall be prepared in monkey kidney-cell cultures under conditions satisfying the requirements of Part A, section 3.2, and shall meet the criteria of section 3.1.4 below. The monkeys used shall be those that conform to the requirements of Part A, section 3.1.2.

It is recommended that a large seed lot be set aside as the basic material to which the manufacturer can return for the preparation of batches of vaccine.

All seed lots shall be stored at a temperature below —60°C.
*3.1.4 Tests on seed lots
The seed lot used for the production of vaccine shall be free from detectable extraneous viruses and shall satisfy the requirements specified for single harvests and bulk suspensions given in Part A, sections 3.3, 3.4, 3.5 and 3.6. Each seed lot shall have been derived from material tested in parallel with an original seed virus or original vaccine, according to the requirements of Part A, section 3.5.5.

*3.2 Production precautions
The general production precautions as formulated in the requirements of Part A, section 3, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) \(^1\) shall apply to the manufacture of oral poliomyelitis vaccine, with the addition of the following:

*3.2.1 Monkeys used for the production of vaccine
Monkeys used for the production of vaccine shall be those that conform to the requirements of Part A, section 3.1.2.

*3.2.2 Monkey kidney-cell cultures for vaccine production
Cultures of monkey kidney cells that have not been propagated in series shall be prepared from kidneys that show no pathological signs. Virus for the preparation of vaccine shall be grown by aseptic methods in such cultures. If animal serum is used in the propagation of the cells, the maintenance medium after virus inoculation shall contain no added serum.

Suitable antibiotics in small concentrations may be used. If penicillin is used it may be included only in the medium for propagation of the cells and its concentration should not exceed the equivalent of 0.12 mg of sodium benzylpenicillin per ml. Non-toxic pH indicators may be added, such as phenol red in a concentration of 0.002%. Only substances that have been approved by the national control authority may be added.

Each group of cell cultures derived from a single monkey shall be prepared and tested as an individual group.

*3.2.3 Tests of cell cultures used for vaccine production
On the day of inoculation with seed lot virus, each cell culture shall be examined for degeneration caused by an infective agent. If this examination shows evidence of the presence in a cell culture of any adventitious agent, the whole group of cultures concerned shall not be used for vaccine production.

On the day of inoculation with the seed lot virus a sample of at least 20 ml of the pooled fluid removed from the cell cultures of the kidneys of each single monkey shall be divided into two equal portions. One portion of the pooled fluid shall be tested in monkey kidney-cell cultures prepared from the same species, but not the same animal, as that used for vaccine production. The other portion of the pooled fluid shall be tested in monkey kidney-cell cultures from another species, provided that the tests on the pooled fluids shall be done in cell cultures from at least one species known to be sensitive to SV40 virus. The pooled fluid shall be inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm\(^2\) per ml of pooled fluid. At least one bottle of each kind of cell culture shall remain uninoculated and shall serve as a control.

Animal serum may be used in the propagation of the cells, provided it does not contain SV40 antibody or other inhibitors, but the maintenance medium after inoculation of test material should contain no added serum except as described below.

The cultures shall be incubated at a temperature of 37°C and shall be observed for a total period of at least 4 weeks. During this observation period and after not less than 2 weeks' incubation, from each of these cultures at least one subculture of fluid shall be made in the same tissue culture system. The subculture shall also be observed for at least 2 weeks.

Serum may be added to the original culture at the time of subculturing provided that the serum does not contain SV40 antibody or other inhibitors.

A further sample of at least 10 ml of the pooled fluid shall be tested for the presence of B virus and other viruses in rabbit kidney-cell cultures. Serum used in the nutrient medium of these cultures shall have been shown to be free from inhibitors.\(^1\) The sample shall be inoculated into bottles of these cell cultures, in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm\(^2\) per ml of pooled fluid. At least one bottle of the cell cultures shall remain uninoculated and serve as a control.

The cultures shall be incubated at a temperature of 37°C and shall be observed for a period of at least 2 weeks.

It is suggested that, in addition to these tests, a further sample of the pooled fluid removed from the cell cultures on the day of inoculation with the seed lot virus be tested for the pres-

\(^1\) *Herpesvirus hominis* has been used as an indicator for freedom from B virus inhibitors on account of the danger of handling herpes B virus.
ence of adventitious agents by inoculation of 10 ml into human cell cultures sensitive to measles.

In some countries a sample of 2% of the pooled fluid is tested in each of the culture systems specified.

For the tests to be valid at least 80% of the culture vessels shall be available and suitable for evaluation at the end of the respective test periods.

If by these tests evidence is found of the presence of an adventitious agent, the single harvest from the whole group of cell cultures concerned shall not be used for vaccine production.

If the presence of B virus is demonstrated the manufacture of oral polio-myelitis vaccine shall be discontinued and the national control authority shall be informed. Manufacturing shall not be resumed until a thorough investigation has been completed and precautions have been taken against reappearance of the infection, and then only with the approval of the national control authority.

If these tests are not done immediately, the samples taken shall be kept at a temperature of —60°C, with the exception of the sample for the test for B virus, which may be held at 4°C, provided that the test is done within 7 days of storage.

*3.2.4 Tests of control cell cultures

On the day of inoculation with the seed lot virus, cultures prepared from 25% of the cell suspension obtained from the kidneys of each single monkey shall remain uninoculated, and shall serve as controls. These control cell cultures shall be incubated under the same conditions as the inoculated cultures for at least 2 weeks, and shall be examined during this period for evidence of cytopathic changes. For the tests to be valid, not more than 20% of the control cell cultures may be discarded for non-specific, accidental reasons. At the end of the observation period, the control cell cultures shall be examined for degeneration caused by an infective agent. If this examination or any of the tests required in this section shows evidence of the presence in a control culture of any adventitious agent, the poliovirus grown in the corresponding inoculated cultures from the same group shall not be used for vaccine production.

If the presence of B virus is demonstrated, the measures concerning vaccine production described in Part A, section 3.2.3 shall be taken.

*3.2.4.1 Tests for haemadsorbing viruses

At the time of harvest or not more than 4 days after the day of inoculation of the production cultures with seed lot virus, a sample of 4% of the control cell cultures shall be taken and shall be tested for haemadsorbing viruses. At the end of the observation period the remaining control cell cultures shall also be tested for these viruses. The tests shall be made by
the addition of appropriate red cells that have not been stored for more than 7 days.

These tests are usually made using guinea-pig red cells. A number of other types of red cell have also been used, including human (blood group IV O), monkey, and chicken or other avian, in addition to guinea-pig red cells.

In some countries cell cultures grown on coverslips are stained with haematoxylin and eosin as a check for these extraneous agents.

*3.2.4.2 Tests for other extraneous agents

At the time of harvest, or not more than 7 days after the day of inoculation of the production cultures with seed lot virus, a sample of at least 20 ml of the pooled fluid from each group of control cultures shall be taken and tested in the 2 kinds of monkey kidney-cell culture, as described in Part A, section 3.2.3. Another sample of at least 10 ml of the pooled fluid from each group of control cultures shall be taken and tested in rabbit kidney-cell cultures as described in Part A, section 3.2.3.

In several countries it is obligatory to make additional tests for the presence of adventitious agents on a further sample of the pooled fluid removed from the control cultures by inoculation of 10 ml into human cell cultures sensitive to measles.

In some countries a sample of 2% of pooled fluid is tested in each of the cell culture systems specified.

At the end of the observation period for the original control cell cultures, similar samples of the pooled fluid shall be taken and the tests referred to in this section in the 2 kinds of monkey kidney-cell culture and in the rabbit cell cultures shall be repeated.

*3.2.5 Temperature of incubation

After inoculation of the production bottles with virus, both inoculated and control cell cultures shall at no time be at a temperature outside the range of 33°C to 35°C for the relevant periods of incubation.

The temperature should not vary by more than ± 0.5°C.

3.3 Control of single harvests

*3.3.1 Single harvest

Virus suspension shall be harvested not later than 4 days after virus inoculation.

3.3.2 Sampling

Samples for testing single harvests shall be taken immediately on harvesting. If the tests for adventitious agents and in animals as described in
Part A, sections 3.3.4 and 3.3.6 respectively are not performed immediately, the samples for these tests shall be kept at a temperature below \(-60^\circ\text{C}\). Samples for tests for B virus as described in Part A, section 3.3.5, may be held at \(4^\circ\text{C}\) provided that the tests are done within 7 days of storage.

### 3.3.3 Tests for bacteria, fungi, and mycoplasma

A volume of 10 ml or at least 0.5% of each single harvest shall be tested for bacterial and mycotic sterility as described in Part A, section 5, of Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances).\(^1\)

Each single harvest shall be tested also for the presence of \textit{Mycobacterium tuberculosis} by appropriate culture methods and for mycoplasma by a method approved by the national control authority.

The tests for \textit{Mycobacterium tuberculosis} may be made as described in Part A, section 3.4.6. Tests for mycoplasma should be done using both solid and liquid media that have been shown to be capable of supporting the growth of sterol-requiring and non-sterol-requiring mycoplasmas, and using at least 10 ml of the single harvest for each group of tests.

\*### 3.3.4 Tests of neutralized single harvests in monkey kidney-cell cultures

A sample of at least 10 ml of each single harvest shall be neutralized by type-specific poliomyelitis antiserum prepared in animals other than monkeys. In preparing antisera for this purpose the immunizing antigens used shall be cultured in non-simian cells.

Care should be taken to ensure that the serum used is monospecific. This may be demonstrated by titration of the serum against homotypic and heterotypic virus of known virus titre using that dilution of the serum that is used for neutralization.

Half (corresponding to at least 5 ml of single harvest) of the neutralized suspension shall be tested in monkey kidney-cell cultures prepared from the same species, but not the same animal, as that used for vaccine production. The other half of the neutralized suspension shall be tested in monkey kidney-cell cultures from another species, provided that the tests on the neutralized suspension shall be done in cell cultures from at least one species known to be sensitive to SV40 virus.

The neutralized suspensions shall be inoculated into bottles of these cell cultures in such a way that the dilution of the suspension in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm\(^2\) per ml of neutralized suspension. At least one bottle of each kind

---

of cell culture shall remain uninoculated and shall serve as a control and shall be maintained by nutrient medium containing the same concentration of the specific serum used for neutralization.

Animal serum may be used in the propagation of the cells, provided it does not contain SV40 antibody or other inhibitors, but the maintenance medium after inoculation of test material should contain no added serum other than the poliovirus neutralizing serum, except as described below.

The cultures shall be incubated at a temperature of 37°C and shall be observed for a total period of at least 4 weeks. During this observation period and after not less than 2 weeks’ incubation, from each of these cultures at least one subculture of fluid shall be made in the same tissue culture system. The subcultures shall also be observed for at least 2 weeks.

Serum may be added to the original cultures at the time of subculturing provided that the serum does not contain SV40 antibody or other inhibitors.

In several countries it is obligatory to make additional tests on a further sample of the neutralized single harvests for adventitious agents by inoculation of 10 ml into human cell cultures sensitive to measles.

It is suggested that fluorescent antibody techniques may be useful for detecting SV40 virus and other viruses in the cells.

For the tests to be valid at least 80% of the culture vessels shall be available and suitable for evaluation at the end of the respective test periods.

If any cytopathic changes occur in any of the cultures the causes of these changes shall be investigated. If the cytopathic changes are shown to be due to unneutralized poliovirus the test shall be repeated. If there is evidence of the presence of SV40 virus or other adventitious agent attributable to the single harvest such single harvest shall not be used for vaccine production.

*3.3.5 Tests in rabbit kidney-cell cultures for B virus and other viruses

A sample of at least 20 ml of each single harvest shall be tested for the presence of B virus and other viruses in rabbit kidney-cell cultures. Serum used in the nutrient medium of these cultures shall have been shown to be free from inhibitors. The sample shall be inoculated into bottles of these cell cultures, in such a way that the dilution of the harvest suspension in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm² per ml of harvest suspension. At least one bottle of the cell cultures shall remain uninoculated and serve as a control.

---

1 See footnote on page 32.
The cultures shall be incubated at a temperature of 37°C and shall be observed for a period of at least 2 weeks.

For the test to be valid at least 80% of the culture vessels shall be available and suitable for evaluation at the end of the test period.

If there is evidence of B virus or any other virus attributable to the single harvest, such single harvest shall not be used for vaccine production.

If the presence of B virus is demonstrated the measures concerning vaccine production described in Part A, section 3.2.3, shall be taken.

3.3.6 Tests in animals

The single harvests shall be tested in animals, as described in Part A, section 3.4, unless these tests are performed on the bulk suspension (see Part A, section 3.4.1).

3.3.7 Preservatives and stabilizers

Preservatives or stabilizers that may be added to the single harvests or to the bulk suspension shall have been shown to the satisfaction of the national control authority not to impair the safety and effectiveness of the vaccine in the concentrations used.

All tests described in Part A, sections 3.3, 3.4, and 3.5 shall be done on samples taken before any preservatives or stabilizers are added.

3.4 Control of the bulk suspension before filtration

3.4.1 Bulk suspension

Each bulk suspension shall be tested in animals for adventitious agents, as described in the following sections, unless these tests have already been made on single harvests (see Part A, section 3.3.6).

3.4.2 Sampling

Samples for testing the bulk suspension before filtration shall be taken immediately after the bulk suspension has been prepared and, if not tested immediately, shall be kept at a temperature below −60°C until the tests for the presence of adventitious micro-organisms described in Part A, sections 3.4.4, 3.4.5, and 3.4.6 are performed. Samples for tests for B virus as described in Part A, section 3.4.3, may be held at 4°C provided that the test is done within 7 days of storage.

3.4.3 Tests in rabbits

A sample of the bulk suspension shall be tested for the presence of B virus and other viruses by injection into at least 10 healthy rabbits each weighing between 1.5 kg and 2.5 kg. The sample shall consist of at least 100 ml. Each rabbit shall receive not less than 10 ml nor more than 20 ml,
of which 1 ml is given intradermally at multiple sites, and the remainder subcutaneously. The rabbits shall be observed for at least 3 weeks for death or signs of illness.

In some countries the sample consists of at least 1% of the bulk suspension, provided that this is not less than 100 ml, up to a maximum of 500 ml, and the period of observation of the rabbits is 4-5 weeks.

All rabbits that die after the first 24 hours of the test shall be examined by autopsy, with the removal of the brain and organs for detailed examination to establish the cause of death; animals showing signs of illness shall be killed and subjected to a similar autopsy.

The bulk suspension passes the test if at least 80% of the inoculated rabbits remain healthy and survive the observation period and if none of the rabbits shows evidence of infection with B virus or with other adventitious agent or lesions of any kind attributable to the bulk suspension.

If the presence of B virus is demonstrated, the measures concerning vaccine production described in Part A, section 3.2.3, shall be taken.

3.4.4 Test in adult mice

A sample of the bulk suspension shall be tested for the presence of adventitious agents pathogenic to mice by intracerebral inoculation of 0.03 ml and intraperitoneal inoculation of at least 0.5 ml of the suspension into each of at least 20 adult mice, each weighing 15-20 g. The mice shall be observed for at least 3 weeks. All mice that die after the first 24 hours of the test or that show signs of illness shall be examined for evidence of viral infection; this shall be done macroscopically by direct observation as well as by subinoculation of appropriate tissue suspensions by the intracerebral and intraperitoneal routes into at least 5 additional mice, which shall be observed for 3 weeks.

The bulk suspension passes the test if at least 80% of the inoculated animals remain healthy and survive the observation period and none of the mice shows evidence of infection with any adventitious agent attributable to the bulk suspension.

3.4.5 Test in suckling mice

A sample of the bulk suspension shall be tested for the presence of adventitious agents pathogenic to mice by intracerebral inoculation of 0.01 ml and intraperitoneal inoculation of at least 0.1 ml of the suspension into each of at least 10 mice less than 24 hours old originating from more than one litter. The mice shall be observed daily for at least 14 days. All mice that die after the first 24 hours of the test or that show signs of illness shall be examined for evidence of viral infection; this shall be done macro-
scopically by direct observation and the tissues shall be examined micro-
scopically and by subinoculation of appropriate tissue suspensions by the
intracerebral and intraperitoneal routes into at least 5 additional suckling
mice, which shall be observed daily for 14 days.

In some countries, in addition, a blind passage is made of
a suspension of the pooled emulsified tissue (minus skin and
viscera) of all mice surviving the original 14-day test.

The bulk suspension passes the test if at least 80% of the mice originally
inoculated remain healthy and survive the observation period and if none of
the mice shows evidence of infection with any adventitious agent attribu-
table to the bulk suspension.

3.4.6 Test in guinea-pigs

A sample of the bulk suspension shall be tested for the presence of
Mycobacterium tuberculosis or other adventitious agents by intraperitoneal
inoculation of 5.0 ml into each of at least 5 guinea-pigs each weighing
350-500 g. The animals shall be observed for at least 42 days for death
or signs of disease. All guinea-pigs that die after the first 24 hours of the
test or that show signs of illness shall be examined macroscopically and
the tissues shall be examined both microscopically and culturally for evi-
dence of infection with Mycobacterium tuberculosis. Animals that survive
the observation period shall also be examined by autopsy macroscopically
for evidence of infection with Mycobacterium tuberculosis.

The bulk suspension passes the test if at least 80% of the inoculated
guinea-pigs remain healthy and survive the observation period, and if none of
the animals shows evidence of infection with Mycobacterium tuberculosis
or other adventitious agent attributable to the bulk suspension.

The test may be made using the pellet from 100 ml of centri-
fuged bulk suspension resuspended in 30 ml of the supernatant.
It is desirable to record the rectal temperature of the animals
daily during the first 3 weeks.

3.5 Control of bulk suspension after filtration

3.5.1 Filtration of bulk suspension

The bulk suspension shall be filtered through a filter that will retain
bacteria and other large micro-organisms.

3.5.2 Sampling

Samples of the filtered bulk suspension shall be taken immediately
after filtering and if not tested immediately, shall be kept at a temperature
below −20°C until the tests described in the following sections are made.
3.5.3 Identity test

The poliovirus type in the filtered bulk suspension shall be serologically identified. Care should be taken to ensure that the sera used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre.

3.5.4 Virus concentration

The determination of the amount of infective poliovirus per ml of filtered bulk suspension shall be made in cell cultures. This determination shall be made in terms of PFU per ml and/or in terms of TCID_{50} per ml in parallel with the determination of the virus concentration of a reference preparation containing the same poliovirus type of known virus titre (see Part A, section 1.3). The determination of the number of PFU per ml shall be based on a total count of at least 100 clearly defined plaques on at least 5 different cell sheets. The determination of the number of TCID_{50} per ml shall be based on the use of 10-fold dilution steps with 10 tubes per dilution, or on any other arrangement of dilutions and tubes yielding equal precision.

The detailed procedures for carrying out this test and for interpreting the results should be those approved by the national control authority.

The virus concentration as determined by this test should be the basis for the quantity of virus used in the tests in monkeys for neurovirulence (Part A, section 3.5.5.1) and for preparing the final bulk (Part A, section 3.6).

3.5.5 Tests for consistency of virus characteristics

The poliovirus in the filtered bulk suspension shall be tested in comparison with the seed lot or a reference virus preparation (see Part A, section 1.3) with regard to certain characteristics, as described in the following subsections.

The object of these tests is to ensure that the vaccine virus has not undergone changes during its multiplication in vaccine preparation.

From the results of these tests for successive batches of vaccine a critical assessment may be made of consistency of vaccine quality (see Part B, section 2).

3.5.5.1 Tests in monkeys for neurovirulence

Monkeys used for neurovirulence tests shall be those that conform to the relevant requirements in Part A, section 3.1.2. The pathogenicity of the filtered bulk suspension for Macaca or Cercopithecus monkeys shall be tested in comparison with that of the seed lot or a reference virus preparation for neurovirulence testing (see Part A, section 1.3) by inoculation
into the intrathalamic and the intralumbar regions of the central nervous system. The interval between the inoculation of the groups of monkeys with the reference material and with any filtered bulk suspension shall be not greater than 3 months. The number of monkeys inoculated by each route shall be that approved by the national control authority. A pre-injection serum sample obtained from each monkey shall be shown to contain no neutralizing antibody in a dilution of 1 : 4 when tested against no more than 1000 TCID$_{50}$ of each of the 3 types of poliovirus. For the test to be valid, the virus content of the inoculum shall be $10^{5.0}$ to $10^{6.4}$ TCID$_{50}$ per ml or $10^{5.0}$ to $10^{6.4}$ PFU/ml and the reference virus preparations shall have a similar virus content. The volume of virus suspension injected into each thalamus of a monkey shall be 0.5 ml and that injected into the lumbar spinal cord not less than 0.1 ml. All monkeys shall be observed for at least 18 days for symptoms suggestive of poliomyelitis or other virus infection. Those monkeys that die after the first 24 hours of the test as well as those that survive the observation period shall be autopsied and histological examinations made of at least the lumbar cord, the cervical cord, the lower medulla, the upper medulla and mesencephalon, and the motor cortex of each monkey.

If in a monkey there is evidence of faulty injection technique and the histopathological findings are negative in this test, this shall be recorded but that monkey shall not be included in the results. If, however, such a monkey shows positive histopathological changes then that monkey shall be included.

In some countries, reliance is placed mainly on the intrathalamic test. In this case, 30 monkeys are inoculated intrathalamically with undiluted vaccine but in addition 5 groups, each of 5 monkeys, are inoculated intraspinally with undiluted vaccine, vaccine diluted to $10^{-5}$, and vaccine diluted to $10^{-4}$ respectively. In other countries, reliance is placed mainly on the intraspinal test using the homotypic seed lot or a reference preparation of homotypic virus for comparison. In this case, intralumbar inoculations of vaccine or reference preparation, titre adjusted if necessary, and dilutions of $10^{-5}$, $10^{-4}$, $10^{-3}$ and $10^{-2}$ are made, using at least 5 monkeys for each dose level, but in addition intrathalamic inoculation is made with the undiluted preparation, titre adjusted if necessary, and a dilution of $10^{-1}$ using 10 monkeys for each dose level.

In either type a post-inoculation virus titre of the vaccine inoculated should be determined in order to check the virus content of the inoculum.

The filtered bulk suspension passes the test if at least 80% of the animals in each group remain healthy, survive the observation period and show evidence of valid inoculation trauma, and if none of the clinical and histopathological findings indicates a significant difference in pathogenicity between the vaccine virus and the reference material.
3.5.5.2 Tests in vitro

The virus in the filtered bulk suspension shall be tested\(^1\) for the property of reproducing at the temperatures of 36°C and 40°C for types 1 and 2 and 36°C and 40.3°C for type 3 (rct/40 marker) in comparison with the seed lot or a reference virus preparation for the marker tests (see Part A, section 1.3) and with appropriate rct/40- and rct/40+ strains of poliovirus of the same type. The incubation temperatures used in this test shall be controlled to within \(\pm 0.1^\circ\mathrm{C}\).

The filtered bulk suspension passes the test if, for both the virus in the bulk suspension and that in the appropriate reference material, the titre determined at 36°C is at least 100 000 times that determined at the higher temperature. Unless the titres obtained for all the reference viruses show expected values, the test shall be repeated.

In some countries it is obligatory that at the higher temperature the virus titre does not exceed 10 TCID\(_{50}\)/ml or 10 PFU/ml.

It is also desirable that the temperatures used in the test should include one in the region of 39.0°C to 39.5°C at which the titre of the reference material should be reduced by a factor between 1000 to 100 000 times its value at 36°C. In one laboratory a temperature of 39.2°C has been found suitable. The object is to obtain definite ratios for the reproductive capacities of bulk suspension and the reference material over a range of temperatures so that a more accurate comparison can be made.

National control authorities should provide reference virus preparations and an appropriate rct/40+ virus strain for this test.

It is recommended that the manufacturer should perform at least one other test for a genetic marker, since a genetic change could occur that may not be detected by the rct/40 marker test only. Other tests currently used are those based on the study of antigenic character of the strain, or tests for the sensitivity of reproduction to different concentrations of sodium bicarbonate (d-marker).

3.6 The final bulk

The operations necessary for preparing the final bulk shall be conducted in such a manner as to avoid contamination of the product.

The dilution and mixing procedures involved in preparing the final bulk should be those approved by the national control authority.

3.6.1 Added substances

Any substance that may be added to the product in preparing the final bulk should have been shown to the satisfaction of the

national control authority not to impair the safety and efficacy of the vaccine in the concentration used.

3.6.2 *Tests for bacteria and fungi*

The final bulk shall be tested for bacterial and mycotic sterility in accordance with the requirements given in Part A, section 5, of Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances).¹

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 Laboratories)² shall apply to vaccine filled in the liquid form. Vaccine incorporated into a solid medium (as in the case of dragees) shall be processed in accordance with the specifications for the production of tablets and capsules in pharmaceutical manufacture.³

Care should be taken to ensure that the material of which the container is made does not adversely affect the virus content of the vaccine under the recommended conditions of storage.

5. **Control tests on final product**

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

5.1 *Identity test*

The poliovirus type or types shall be serologically identified.

Care should be taken to ensure that the sera used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre.

5.2 *Tests for bacteria and fungi*

Liquid vaccine shall be tested for bacterial and mycotic sterility according to the requirements given in Part A, section 5, of the Requirements for Biological Substances No. 6 (Requirements for the Sterility of Biological Substances).¹

---

5.3 *Virus titration*

The determination of the poliovirus titre shall be made as described in Part A, section 3.5.4, of these requirements. In the case of vaccine containing more than one poliovirus type each type shall be titrated separately using appropriate monospecific serum for each of the other type or types that may be present.

The detailed procedures for carrying out this test, including the use of monospecific sera, and for interpreting the results should be those approved by the national control authority.

The virus concentration as determined by this test should be the basis for the statements regarding amount of virus of each type referred to in the requirements for labelling in Part A, section 8.

No limits for virus content can be specified for all products. The limits for each virus type for each product should be those specified by the national control authority.

5.4 *Innocuity tests*

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

Care should be taken in the case of preparations that may be innocuous when taken orally but that could cause damage when injected parenterally because of a high salt content or other component.

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)¹ shall apply.

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)² shall apply.

*8. *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)³ shall apply with the addition of the following:

---

The label on the container shall include the following information:
the type or types of poliovirus included in the vaccine; and
the fact that the vaccine is for oral administration only.

The leaflet accompanying the package shall include the following information:
the designation(s) of the strain(s) of poliovirus contained in the vaccine;
the fact that the vaccine was prepared using monkey kidney tissue;
the nature and amount of any preservative or stabilizer present in the vaccine;
the nature and amount of any antibiotics used in the preparation of the vaccine;
and the amount of virus of each type contained in one recommended human dose.

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

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) ² shall apply.

10.1 Storage conditions
Before being distributed by the manufacturing establishment, or before being issued from a depot for the maintenance of reserves of vaccines, all vaccines in bulk form or in final containers shall be kept continuously at a temperature below —20°C. After distribution or issue the vaccine in the final containers shall be stored at a temperature below 0°C.

The temperature at which the final containers are stored should be one that will maintain ice continuously in a solid state and avoid repeated freezing and thawing.

Liquid vaccine, once thawed, may be kept at a temperature between 2°C and 10°C for 30 days; such vaccine may be kept for longer periods of time if a suitable stabilizer has been added during manufacture.

10.2 *Expiry date*

The date after which the vaccine may not be used shall be not more than two years after the last satisfactory virus titration as described in Part A, section 5.3, provided that the vaccine has been stored continuously at a temperature below $-20^\circ$C. The expiry date, however, shall not be more than one year from the date of issue by the manufacturer, provided that the vaccine has been stored below $0^\circ$C.

### Part B. National Control Requirements

**1. General**

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

The national control authority shall give directions to manufacturers concerning the poliovirus strains and seed lots to be used in vaccine production and concerning the recommended human dose. The seed lot for each type shall be prepared by a suitable method and be at the appropriate passage level from the original seed virus (see Part A, section 3.1.3).

The detailed test procedures for production and control shall be those approved by the national control authority. The national control authority shall provide or approve the reference preparations of the 3 poliovirus types for virus titration, neurovirulence tests, and marker tests (see Part A, section 1.3). Such reference material may be part of seed lots approved for vaccine production or live attenuated virus preparations that have been tested in comparison with vaccine shown to be safe and immunogenic in man.

**2. Release and certification**

A vaccine lot shall be released only if it fulfils Part A of the present requirements. Before release of any vaccine lot from a manufacturing establishment the requirements for consistency of production given in Part A, section 9.1, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)\(^1\) shall apply.

A statement signed by the appropriate official of the national control laboratory shall be provided at the request of the manufacturing establishment and shall certify whether or not the lot of vaccine in question meets all national requirements as well as Part A of the present requirements.

---

The certificate shall further state the date of the last satisfactory determination of virus concentration, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document shall be attached.

The purpose of the certificate is to facilitate the exchange of poliomyelitis vaccine (oral) between countries.

Part C. Requirements for Poliomyelitis Vaccine (Oral) Prepared Using Human Diploid Cells

The following additional or alternative requirements are for poliomyelitis vaccine (oral) prepared with human diploid cells and concern the testing of the cell substrate used for production of the vaccine; they should therefore be added to or substituted for the appropriate sections in Parts A and B as indicated. All the other requirements given in Parts A and B of the document are applicable to this vaccine as well.

Modifications affecting Part A. Manufacturing Requirements

1. Definitions

1.4 Terminology

The following shall apply instead of the definition given in Part A, section 1.4:

Single harvest: A virus suspension of one virus type harvested from cell cultures prepared from one ampoule of cell seed.

The following shall be added:

Cell seed: A quantity of cells derived from a single human tissue and of uniform composition, stored frozen at \(-70^\circ\text{C}\) or below in aliquots, one of which would be used for the production of each single harvest.

2. General manufacturing requirements

The following shall be added:

No cell cultures other than those approved by the national control authority for the production of this vaccine shall be introduced or handled in the production area.

3. Production control

3.1 Control of source materials

The following shall apply instead of the requirements given in Part A, section 3.1.2:
3.1.2 Cell seed for the production of cell cultures

The production of the human diploid cell cultures used for vaccine manufacture shall be based on the cell seed system.

The cell seed used for the production of oral poliomyelitis vaccine shall be that approved by and registered with the national control authority. The cells shall have been characterized with respect to their genealogy, growth characteristics, storage conditions, and karyology, and they shall have been shown, by tests in animals and eggs, to be free from adventitious agents.\(^1\) The supernatant fluids shall also have been shown by tests in cell cultures to be free from adventitious agents. The cells shall be shown to be diploid and stable with respect to karyology and morphology and to meet the requirements given in the subsections of Part C, section 3.2.3 throughout their finite life span. The cells shall at no time have shown the properties of a continuous cell line.

The tests in animals and eggs for adventitious agents shall include one of inoculating cells by the intramuscular route into each of the following groups of animals using at least 10\(^6\) cells for each group:

- 2 liters of suckling mice, totalling at least 10 animals, less than 24 hours old;
- 10 adult mice;
- 5 guinea-pigs; and
- 5 rabbits

as well as into the allantoic cavity of 10 embryonated eggs 9–11 days old.

The animals shall be observed for at least 4 weeks and the embryonated eggs shall be examined after not less than 3 days. Any animals that are sick or show any abnormality shall be investigated to establish the cause of illness. The allantoic fluids shall be tested with guinea-pig and chick or other avian red cells for the presence of haemagglutinins.

The cells shall also be shown to be free from potential heterotransplantability by appropriate tests in animals.

Suitable tests using immunosuppressed animals may be made as follows: A quantity of 10\(^6\) cells obtained from cultures at the same passage level as those used for vaccine production are inoculated into each cheek pouch of 6 hamsters, 3 of which have been treated with cortisone, and the animals observed for not less than 4 weeks. Some hamsters of the strain used should have been inoculated with HeLa or KB cells and it should have been shown that tumour formation can be caused by the inoculation of the neoplastic tissue, thus demonstrating the ability

---

\(^1\) Other types of test have been suggested, e.g., examination of ultrathin sections under the electron microscope. Information is insufficient, however, to include such tests in these requirements.
of the strain of animals to give rise to tumours. Any other test using animals treated with immunosuppressive agents and with equal sensitivity to neoplastic cells may be used.

The cells are suitable for vaccine production if at least 80% of the animals inoculated with the cells remain healthy and survive the observation period, none of the animals or eggs show evidence of the presence in the cell cultures of any adventitious agent, and none of the animals show evidence of tumour formation from the cells.

The cell seed shall also have been shown to yield cell cultures capable of producing vaccine that has been found to be safe and antigenic.

3.1.3 Virus seed lot system

The requirements given in this section shall be amended to allow the preparation of seed lots in either human diploid cell cultures or monkey kidney-cell cultures. If human diploid cells are used the requirements given in Part C, sections 3.1.4 and 3.2 shall apply.

3.1.4 Tests on virus seed lots

The requirements given in this section shall apply with the exception that the requirements referred to in Part A, section 3.3 shall be applicable as amended in Part C, section 3.3.

3.2 Production precautions

The following shall apply instead of the requirements given in Part A, section 3.2, with the exception of section 3.2.1 which is not applicable.

3.2.2 Cell cultures used for vaccine production

Only human diploid cell cultures derived from a cell seed approved by the national control authority shall be used for vaccine production. The production of each single harvest shall be initiated from a new ampoule of the cell seed. All processing of the cell seed and subsequent cell cultures shall be done in an area in which no other cells are handled. The cell cultures shall be used only if no changes have occurred in their growth characteristics (including freedom from potential heterotransplantability as shown by the test in Part C, section 3.1.2) and storage conditions and if no changes from the normal karyology have been shown to occur within the total number of population doublings that correspond to the average finite life of the cells as determined under the particular conditions of the production establishment.

It is important that the karyological pattern should have been determined not to differ from that established for the cell

---

1 Population doubling. This may be calculated from an actual cell count of an aliquot or by estimation of the area of expansion of the cell sheet.
seed for the total number of population doublings of the cell cultures and that no changes from the normal karyology should have been shown to occur within a proportion of the total number of population doublings that would be used for vaccine production.

The cells shall, however, not be used beyond two-thirds of the total number of population doublings corresponding to the average finite life of the cells.

3.2.3 Tests of cell cultures used for vaccine production

Each batch of diploid cells used as a substrate for the production of a single harvest of virus shall be tested for freedom from extraneous agents and for retention of normal morphology and karyology. Only cells that have the normal morphological appearance and karyology and that pass the test described below shall be used for vaccine production. The cells shall have been shown to have biological properties unchanged from those of the cell seed as shown by the tests outlined in Part C, section 3.1.2.

In some countries immunobiological tests are made to verify identity of the cells with the cell seed and the purity of the cultures.

Cells sufficient for chromosome monitoring (Part C, section 3.2.3.1) and for preparing control cultures (Part C, section 3.2.4) shall be taken from the pooled material removed from each culture vessel not earlier than two population doublings preceding the doubling level at which cells are to be inoculated with vaccine virus. These cells, or cells subcultured from these cells, shall be used for making preparations for chromosome monitoring. The remaining cells shall be set aside as control material. The supernatant fluids from the production bottles at the cell doubling level at which they are to be inoculated with vaccine virus shall be used for the tests for extraneous agents and for bacteria, fungi, and mycoplasma (Part C, sections 3.2.3.2 and 3.2.3.3).

On the day of inoculation with seed lot virus, each cell culture shall be examined for degeneration caused by an infective agent. If this examination or any of the tests required in this section shows evidence of the presence in a cell culture of any adventitious agent, the poliovirus grown in the whole group of cultures concerned shall not be used for vaccine production.

3.2.3.1 Chromosome monitoring pool — preparation and testing

Preparations shall be made from at least 1% of the sample of pooled cell substrate removed from the culture vessels. Chromosome monitoring shall be done at the stage equivalent to the doubling level at which cells are to be inoculated with vaccine virus, or within three population doublings beyond
this stage, but if the cells are subcultivated they shall be repooled. For determination of the general character of the cell material, a minimum of 300 cells shall be examined for frequency of polyplody and a minimum of 100 metaphase plates for exact counts, and analyses of karyotype shall be performed on at least one selected cell. The metaphase plates shall be examined for characteristics that shall include frequency of chromosome breaks, structural chromosome abnormalities, other abnormalities such as despiralization or marked attenuations of the primary or secondary constrictions, and the presence of minute chromosomes.

For vaccine production examination of the cells is usually made between the 27th and 33rd population doubling and the frequency of cells in metaphase with chromosome breaks should not exceed 9%, with structural abnormalities not more than 4%, and with polyploidy not more than 5% 1. All cells showing abnormalities should be subjected to detailed examination and records should be maintained of the detailed criteria applied to particular abnormalities evaluated in the karyotype analysis.

Permanent stained slide preparations of the chromosome monitoring pool, or photographs of these, shall be maintained as part of the record of the batch of vaccine and for monitoring successive batches.

It is desirable that a portion of the sample of pooled cell substrate removed from the culture vessels be stored frozen so as to retain viability. This would be available for future reference for karyology or for any other purpose relating to the batch of vaccine.

3.2.3.2 Tests of supernatant fluids for extraneous viruses

The pooled supernatant fluids from the production cell cultures taken immediately prior to inoculation of virus for vaccine production shall be tested by the inoculation of 10-ml quantities into cultures from each of the following:

- human embryonic kidney cells,
- Cercocebus or Erythrocebus monkey kidney cells, and
- primary rabbit kidney cells.

---

1 These upper limits correspond to the 99 percentile of the values established from a large number of observations on the cell seed system derived from the human diploid cell strain WI-38. However, greater experience of, and more observations in a number of laboratories on, a particular cell seed may result in revised figures being established for such a percentile. National control authorities should consider all available information in specifying the criteria to be fulfilled (see the additional Requirements to Part B, National Control Requirements given on page 55). Detailed information is available in the Minutes of Meetings of the Cell Culture Committee of the Permanent Section on Microbiological Standardization (I.A.M.S.) and the Workshop on Karyology of Human Diploid Cells, Chatham, Mass., USA, 1971. These values will not necessarily be applicable if another human diploid cell strain is used.
In some countries the national control authority requires that additional tests be made in the same cell strain used for vaccine production of another human diploid cell strain and in rhesus monkey kidney cells, as well as the use of larger volumes of pooled supernatant fluid.

Serum used in the nutrient medium of the cultures other than the monkey kidney-cell cultures shall have been shown to be free from inhibitors to virus growth. Each sample shall be inoculated into bottles of the cell cultures, in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm² per ml of pooled fluid. At least one bottle of the cell cultures shall remain uninoculated and serve as a control.

In the case of the Cercopithecus or Erythrocebus monkey kidney-cell cultures, added serum may be used in the propagation of the cells, provided it does not contain inhibitors to virus growth, but the maintenance medium after inoculation of test material should contain no added serum.

The cultures shall be examined for normal morphology during a period of incubation at 37°C for 14 to 21 days. At the end of the observation period a subculture shall be made from the cercopithecus or erythrocebus cultures in the same cell system and these cultures shall be examined for a further 14 days. Some of the cultures from each monkey kidney shall be tested also for haemadsorbing viruses using appropriate red cells that have not been stored for more than 7 days.

These tests are usually made using guinea-pig red cells. In some countries the national control authority requires that the tests for haemadsorbing viruses be made in addition using red cells from humans (blood group IV O), monkeys and chickens. The cultures should be examined at 3–5 days and again at 12 days. All tests should be read after incubation for not less than 30 minutes at 0–4°C and again after a further incubation for at least 30 minutes at 20°–25°C. In addition the test with monkey cells should be read after a still further incubation for 30 minutes at 34°–37°C.

If the samples of supernatant fluid are not tested immediately they shall be kept at a temperature below −60°C.

3.2.3.3 Tests for bacteria, fungi, and mycoplasma

A volume of 20 ml of the pooled supernatant fluids from the production cell cultures shall be tested for bacteria and mycotic sterility and for mycoplasma. The tests for bacterial and mycotic sterility shall be made as described in Part A, section 5, of the Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Sub-
stances),\(^1\) and the tests for mycoplasma shall be done using both solid and liquid media that have been shown to be capable of supporting the growth of sterol-requiring and non-sterol-requiring mycoplasmas, using 10 ml for each group of tests.

In some countries the volume of pooled supernatant fluids is ultracentrifuged and both the pellet and its supernatant fluid tested for sterility.

### 3.2.4 Tests of control cell cultures

The cells set aside as control material (see Part A, section 3.2.3) shall be treated in a similar manner to the production cell cultures but remain uninoculated as control cultures for the detection of extraneous viruses.

The sample of pooled material taken should be such that the area of cells at the stage equivalent to the doubling level at which cells are to be inoculated with vaccine virus, or beyond, represents not less than 25% of that of the total of cell suspension derived from the ampoule of cell seed at that stage.

These control cell cultures shall be incubated under the same conditions as the inoculated cultures for at least 2 weeks, and shall be examined during this period for evidence of cytopathic changes. For the test to be valid, not more than 20% of the control cell cultures may be discarded for non-specific, accidental reasons.

At the end of the observation period, the control cell cultures shall be examined for degeneration caused by an infective agent. If this examination or any of the tests required in this section shows evidence of the presence in a control culture of any adventitious agent, the poliovirus grown in the corresponding inoculated cultures derived from the same ampoule of cell seed shall not be used for vaccine production.

### 3.2.4.1 Tests for haemadsorbing viruses

At the time of inoculating the production cultures with vaccine virus or at the time of virus harvest, cells comprising 4% of the control cells shall be tested for the presence of haemadsorbing viruses using guinea-pig red cells that have not been stored for more than 7 days.

These tests are usually made using guinea-pig red cells. In some countries the national control authority requires that the tests for haemadsorbing viruses be made in addition using other types of red cells including human (blood group IV O), monkey and chicken or other avian. The cultures should be examined at 3–5 days and again at 12 days. All tests should be read after incubation for 30 minutes at 0°–4°C and again after a further incubation for 30 minutes at 20°–25°C. In

---

3.2.4.2 Tests for other extraneous agents

At the time of harvest, or not more than 7 days after the day of inoculation of the production cultures with seed lot virus, samples of 10 ml of the pooled supernatant fluids from the control cultures shall be taken and tested for extraneous viruses in each of the cell cultures, as described in Part C, section 3.2.3.2. At the end of the observation period for the original control cell cultures similar samples of the pooled fluid shall be taken and the tests referred to in this section shall be repeated.

3.2.5 Temperature of incubation

After inoculation of the production bottles with virus both inoculated and control cell cultures shall at no time be at a temperature outside the range of 33°-35°C for the relevant periods of incubation.

The temperature should not vary by more than ± 0.5°C.

3.3 Control of single harvests

The following modifications shall apply to the sections indicated below:

3.3.1 Single harvest

The following shall be added:

The inoculated cell cultures should be processed in such a manner that each virus suspension harvested remains identifiable as a single harvest and is kept separate from other harvests until all the results of tests prescribed for supernatant fluids in Part C, section 3.2.3.2, have been obtained.

3.3.4 Tests of neutralized single harvests on monkey kidney and human cell cultures

For the requirements prescribed in this section of Part A the volume of each single harvest taken for neutralization and testing shall be at least 10 ml and shall be such that at least a total of 50 ml or the equivalent of 500 doses of final vaccine, whichever is the greater, has been withheld from the corresponding bulk suspension.

The antisera used for neutralization shall be of non-human origin and shall have been prepared in animals other than monkeys, using antigens cultured in non-simian cells.

The neutralized suspension shall be divided into two portions and tested in Cercopithecus or Erythrocebus monkey kidney-cell cultures and human cell cultures sensitive to measles, by culturing and subculturing as described in this section of Part A.
Serum used in the nutrient medium of these cultures shall have been shown to be free from inhibitors of virus growth.

3.3.5 Tests in rabbit kidney-cell cultures for herpes virus and other viruses

For the requirements prescribed in this section of Part A the volume of each single harvest taken for testing shall be at least 5 ml.\textsuperscript{1}

Serum used in the nutrient medium of these cultures shall have been shown to be free from inhibitors of virus growth, including herpes virus.\textsuperscript{2}

8. Labelling

The relevant requirements regarding the leaflet accompanying the package shall be amended to include statements that the vaccine has been prepared using human diploid cells and that the manufacture of the vaccine has been undertaken with the approval of the national control authority (see Part B below).

Modifications affecting Part B. National Control Requirements

1. General

The following requirements shall be added:

The manufacture of oral poliomyelitis vaccine using human diploid cell cultures shall be undertaken only with the approval of the national control authority, and then only under a system of chromosome monitoring that will ensure that the cell cultures used for vaccine production have not undergone changes that may adversely affect the safety and efficacy of the product. The national control authority shall therefore give directions to the manufacturing establishment concerning the suitability of the cell seed to be used for vaccine production and the acceptable cell population-doubling level that must not be exceeded for the cell cultures derived therefrom; such directions shall also include criteria for acceptable karyology. Since the information necessary to determine the acceptability of a particular cell strain can be gained only from detailed study in a number of laboratories over several years, the national control authority shall take into consideration all available information in making a decision.

It is desirable that a sample of the cell seed used for vaccine production consisting of not less than $10^8$ cells should be lodged with the national control authority. The sample should be frozen so as to retain viability and stored at $-70^\circ\text{C}$ or below.

\textsuperscript{1} The tests should be made as for tests for B virus in rabbit kidney-cell cultures, since B virus may also be a possible, though improbable, contaminant.

\textsuperscript{2} See footnote on page 32.
Appendix 1

STUDY GROUP ON REQUIREMENTS FOR BIOLOGICAL SUBstances

Geneva, 7–12 November 1960

The members of this Study Group, which prepared the original draft of Requirements for Poliomyelitis Vaccine (Oral) (Requirements for Biological Substances No. 7), were: Dr M. P. Cumakov, Institute for Poliomyelitis Research, Moscow, USSR; Dr D. G. Evans, National Institute for Medical Research, London, England (Rapporteur); Dr S. Gard, Karolinska Institutet, Stockholm, Sweden; Dr J. H. S. Gear, Poliomyelitis Research Foundation, Johannesburg, South Africa (Chairman); Dr R. Murray, Division of Biologies Standards, National Institutes of Health, Bethesda, Md., USA; Dr F. P. Nagler, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada; Dr F. Plesmyczyk, State Institute of Hygiene, Warsaw, Poland (Vice-Chairman); Dr D. Slonim, Institute of Sera and Vaccines, Prague, Czechoslovakia; Dr M. K. Voroshilova, Institute for Poliomyelitis Research, Moscow, USSR. Dr N. K. Jerne, Chief Medical Officer, Biological Standardization, WHO, acted as Secretary.

Appendix 2

WHO EXPERT GROUP ON REQUIREMENTS FOR BIOLOGICAL SUBSTANCES

Geneva, 16–22 March 1965

The members of this Expert Group, which undertook the 1965 revision of Requirements for Poliomyelitis Vaccine (Oral) (Requirements for Biological Substances No. 7), were: Dr M. P. Cumakov, Director, Institute of Poliomyelitis and Virus Encephalitis, USSR Academy of Medical Sciences, Moscow, USSR (Vice-Chairman); Dr R. Gispen, Director, National Institute of Public Health, Utrecht, Netherlands; Dr J. Kaneko, Chairman, Technical Board of Japan Association of Biological Manufacturers, Hikari Plant, Takeda Chemical Industries Ltd., Hikari, Yamaguchi-ken, Japan; Dr R. L. Kirschstein, Division of Biologies Standards, National Institutes of Health, Bethesda, Md., USA; Dr S. S. Marennikova, Deputy Director, Research Institute of Virus Preparations, Moscow, USSR; Dr F. P. Nagler, Chief, Virus Laboratories, Department of National Health and Welfare, Ottawa, Ontario, Canada (Chairman); Dr F. T. Perkins, Head, Division of Immunological Products Control, Medical Research Council Laboratories, London, England (Rapporteur); Dr R. Soemianto, Director, Pasteur Institute, Bandung, Indonesia; Dr R. Sotier, Professor, Faculty of Medicine, and Director, Virology Section, National Public Health Laboratory, Lyons, France; Dr P. Techinda, Chief, Division of Medical Research, Department of Medical Sciences, Bangkok, Thailand. Secretariat: Dr W. C. Cockburn, Chief, Virus Diseases, WHO, Geneva; Dr A. S. Outechoorn, Chief, Biological Standardization, WHO, Geneva (Secretary).
Appendix 3

PREPARATION OF POLIOMYELITIS VACCINE (ORAL)
USING MONKEY KIDNEY CELL CULTURES

Example of a Flowsheet of Tests in Cell Cultures

Day (0) ———————————————————— (14) ———————————————————— (28)
10 ml MK
10 ml VK
50 ml PK
10 ml HA

Day (0) ———————————————————— (14) ———————————————————— (19) ———————————————————— (28)

Pooled fluid (medium change)

10 ml MK
10 ml VK
50 ml PK
10 ml HA

Day (0) ———————————————————— (14) ———————————————————— (19) ———————————————————— (28)

Control cell cultures (25%)

4% HAEM

Remainder HAEM

Pool fluid (from group at medium change)

10 ml VK
50 ml PK
50 ml HA

Day (0) ———————————————————— (14) ———————————————————— (19) ———————————————————— (28)

Virus inoculation ———————————————————— (14 days after harvest) ———————————————————— (28 days after harvest)

Neutralized single harvest

Production cell culture (70%)

5 ml MK
5 ml VK
10 ml HA

Single harvest

5 ml MK
5 ml VK
10 ml HA

HAEM = test for haemadsorbing viruses; MK = Monkey kidney species used for production; VK = vervet monkey kidney or one sensitive to SV40 virus; RK = rabbit kidney; HA = human amnion or human cells sensitive to measles.

Note: This example includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those authorized by the national control authority, this flow sheet should not be considered as an integral part of the revised requirements and it has been included solely for guidance. Manufacturing establishments should prepare their own flowsheet for clarification of the procedures used.
Appendix 4
PREPARATION OF POLIOMYELITIS VACCINE (ORAL)
USING HUMAN DIPLOID CELLS

Example of a Flowsheet of Tests in Cell Cultures

<table>
<thead>
<tr>
<th>Day</th>
<th>(5)</th>
<th>(7)</th>
<th>(14)</th>
<th>(21)</th>
<th>(28)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAEM</td>
<td>cells</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Control 10%

4% cells

HAEM

Chromosome

monitoring

10 ml RK

10 ml HEK

10 ml PC

10 ml MK

10 ml VK

Pooled

fluid

Virus

inoculation

(5-5)

(12)

(14)

(28)

HAEM

HAE

10 ml RK

10 ml HEK

10 ml PC

10 ml MK

10 ml VK

Pooled

fluids

Production 90%

Suckling mice

Adult mice

Guinea pigs

rabbits

Embryonated eggs

3-4 days

Allantocic fluids

Haemagglutinins

(5)

(12)

(14)

(28)

Harvest

(14 days after harvest)

(30 days after harvest)

5 ml HA

(+ serum)

(5 ml MK

(+ serum)

(5 ml RK

Cell

Neutralized single harvest

HA

MK

HAEM = test for haemadsorbing viruses; RK = rabbit kidney; HEK = human embryonic kidney; PC = production cell strain; MK = rhesus monkey kidney; VK = vervet or patas monkey (Cercopithecus or Erythrocebus) kidney; HA = human amnion or human cells sensitive to measles.

Note. This example includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those authorized by the national control authority, this flowsheet should not be considered as an integral part of the requirements and it has been included solely for guidance. Manufacturing establishments should prepare their own flowsheet for clarification of the procedures used.
Annex 2

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 formulated by international groups of experts and published in the *Technical Report Series* of the World Health Organization are listed hereunder:

<table>
<thead>
<tr>
<th>No.</th>
<th>Year</th>
<th>Requirements for Biological Substances:</th>
</tr>
</thead>
<tbody>
<tr>
<td>178</td>
<td>1959</td>
<td>1. General Requirements for Manufacturing Establishments and Control Laboratories</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Requirements for Poliomyelitis Vaccine (Inactivated)</td>
</tr>
<tr>
<td>179</td>
<td>1959</td>
<td>3. Requirements for Yellow Fever Vaccine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Requirements for Cholera Vaccine</td>
</tr>
<tr>
<td>180</td>
<td>1959</td>
<td>5. Requirements for Smallpox Vaccine</td>
</tr>
<tr>
<td>200</td>
<td>1960</td>
<td>6. General Requirements for the Sterility of Biological Substances</td>
</tr>
<tr>
<td>237</td>
<td>1962</td>
<td>7. Requirements for Poliomyelitis Vaccine (Oral)</td>
</tr>
<tr>
<td>274</td>
<td>1964</td>
<td>8. Requirements for Pertussis Vaccine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9. Requirements for Procaine Benzylpenicillin in Oil with Aluminium Monostearate</td>
</tr>
<tr>
<td>293</td>
<td>1964</td>
<td>10. Requirements for Diphtheria Toxoid and Tetanus Toxoid</td>
</tr>
</tbody>
</table>

— 59 —
323 1966 WHO Expert Group:
Requirements for Biological Substances (Revised 1965)
1. General Requirements for Manufacturing Establishments and Control Laboratories
2. Requirements for Poliomyelitis Vaccine (Inactivated)
7. Requirements for Poliomyelitis Vaccine (Oral)
5. Requirements for Smallpox Vaccine

329 1966 WHO Expert Committee on Biological Standardization:
11. Requirements for Dried BCG Vaccine
12. Requirements for Measles Vaccine (Live) and Measles Vaccine (Inactivated)

361 1967 WHO Expert Committee on Biological Standardization:
13. Requirements for Anthrax Spore Vaccine (Live — for Veterinary Use)
14. Requirements for Human Immunoglobulin
15. Requirements for Typhoid Vaccine
9. Requirements for Procaine Benzylpenicillin in Oil with Aluminium Monostearate (Revisions adopted 1966)

384 1968 WHO Expert Committee on Biological Standardization:
16. Requirements for Tuberculins
17. Requirements for Inactivated Influenza Vaccine

413 1969 WHO Expert Committee on Biological Standardization:
4. Requirements for Cholera Vaccine (Revised 1968)
18. Requirements for Immune Sera of Animal Origin

444 1970 WHO Expert Committee on Biological Standardization:
19. Requirements for Rinderpest Cell Culture Vaccine (Live) and Rinderpest Vaccine (Live)
20. Requirements for Brucella abortus Strain 19 Vaccine (Live — for Veterinary Use)

444 1970 WHO Expert Committee on Biological Standardization:
Development of a National Control Laboratory for Biological Substances (A guide to the provision of technical facilities)

463 1971 WHO Expert Committee on Biological Standardization:
21. Requirements for Snake Antivenins

486 1972 WHO Expert Committee on Biological Standardization:
7. Requirements for Poliomyelitis Vaccine (Oral) (Revised 1971)
NOTE

The lists of international biological standards, international biological reference preparations, and international biological reference reagents previously included as annexes to the reports of the WHO Expert Committee on Biological Standardization will in future be issued as a separate publication. Copies may be obtained direct, or through booksellers, from the agents listed on the back cover of this report, or they may be ordered from: World Health Organization, Distribution and Sales Service, 1211 Geneva 27, Switzerland.

---

1 World Health Organization (1972) *Biological substances — International standards, reference preparations, and reference reagents, 1972, Geneva*. Revised editions, incorporating the latest additions and amendments, will be published every few years. The changes made between editions will be listed in the reports of the Expert Committee.
## INDEX

| Anti-*Clostridium chauvoei* serum | 17 | Long-acting thyroid stimulator | 15 |
| Anti-*ha* (anti-C) incomplete blood-typing serum | 16 | Lynecycline | 9 |
| Anti-*Rh* (anti-D) complete blood-typing serum | 15 | Mei B (melarsoprol) | 12 |
| Anti-*Rh* (anti-D) immunoglobulin | 16 | Minocycline | 10 |
| Anti-*Salmonella pullorum* sera | 18 | MSb | 12 |
| Anti-syphilitic human serum | 18 | Neoarsphenamine | 12 |
| Cholera vaccines | 13 | Oxophenarsine | 12 |
| Cholera vaccines, requirements for | 19 | Pertussis vaccine | 14 |
| Clindamycin | 10 | Poliomyelitis vaccine (oral), requirements for | 18, 20 |
| *Clostridium welchii* (perfringens) types |  | Rabies vaccine | 15 |
| B and D vaccines | 13 | Requirements for biological substances | 59 |
| Dimecamprol | 12 | Spectinomycin | 11 |
| Diphtheria antitoxin for flocculation test | 17 | Sterility, requirements for | 19 |
| Diphtheria toxoid (plain) | 14 | Sulfarsphenamine | 12 |
| Doxycycline | 9 | Tetracycline | 10 |
| Gas-gangrene antitoxin (hystolyticus) | 17 | Typhoid vaccine | 15 |
| Glucagon | 11 | Vitamin B<sub>12</sub> | 12 |
| Gramicidin S | 9 | Vitamin D | 11 |
| Influenza virus haemagglutinin (type A) | 14 |  |  |
## World Health Organization
### Technical Report Series

**Recent reports:**

<table>
<thead>
<tr>
<th>No.</th>
<th>Title</th>
<th>Pages</th>
<th>Price...</th>
</tr>
</thead>
<tbody>
<tr>
<td>463</td>
<td>(1971) WHO Expert Committee on Biological Standardization</td>
<td></td>
<td>$1.75</td>
</tr>
<tr>
<td></td>
<td>Twenty-third Report (91 pages)</td>
<td></td>
<td>5.-</td>
</tr>
<tr>
<td>464</td>
<td>(1971) Joint FAO/WHO Expert Committee on Brucellosis</td>
<td></td>
<td>$1.25</td>
</tr>
<tr>
<td></td>
<td>Fifth Report (76 pages)</td>
<td></td>
<td>4.-</td>
</tr>
<tr>
<td>465</td>
<td>(1971) Application and Dispersal of Pesticides</td>
<td></td>
<td>$1.25</td>
</tr>
<tr>
<td></td>
<td>Eighteenth Report of the WHO Expert Committee on Insecticides (66 pages)</td>
<td></td>
<td>4.-</td>
</tr>
<tr>
<td>466</td>
<td>(1971) Methodology for Family Studies of Genetic Factors</td>
<td></td>
<td>$1.00</td>
</tr>
<tr>
<td></td>
<td>Report of a WHO Scientific Group</td>
<td></td>
<td>3.-</td>
</tr>
<tr>
<td>467</td>
<td>(1971) WHO Expert Committee on Malaria</td>
<td></td>
<td>$1.25</td>
</tr>
<tr>
<td></td>
<td>Fifteenth Report (59 pages)</td>
<td></td>
<td>4.-</td>
</tr>
<tr>
<td>468</td>
<td>(1971) Prevention of Rh Sensitization</td>
<td></td>
<td>$1.00</td>
</tr>
<tr>
<td></td>
<td>Report of a WHO Scientific Group (36 pages)</td>
<td></td>
<td>3.-</td>
</tr>
<tr>
<td>469</td>
<td>(1971) Cerebrovascular Diseases: Prevention, Treatment, and Rehabilitation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Report of a WHO Meeting (57 pages)</td>
<td></td>
<td>4.-</td>
</tr>
<tr>
<td>470</td>
<td>(1971) Health Aspects of the Supply and Use of Non-Human Primates for Biomedical Purposes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Report of a WHO Scientific Group (30 pages)</td>
<td></td>
<td>3.-</td>
</tr>
<tr>
<td>471</td>
<td>(1971) Endocrine Regulation of Human Gestation</td>
<td></td>
<td>$1.00</td>
</tr>
<tr>
<td></td>
<td>Report of a WHO Scientific Group (32 pages)</td>
<td></td>
<td>3.-</td>
</tr>
<tr>
<td>472</td>
<td>(1971) Statistical Indicators for the Planning and Evaluation of Public Health Programmes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fourteenth Report of the WHO Expert Committee on Health Statistics (40 pages)</td>
<td></td>
<td>3.-</td>
</tr>
<tr>
<td>473</td>
<td>(1971) Methods of Fertility Regulation: Advances in Research and Clinical Experience</td>
<td></td>
<td>$1.00</td>
</tr>
<tr>
<td></td>
<td>Report of a WHO Scientific Group (48 pages)</td>
<td></td>
<td>3.-</td>
</tr>
<tr>
<td>474</td>
<td>(1971) Pesticide Residues in Food</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Report of the 1970 Joint FAO/WHO Meeting (44 pages)</td>
<td></td>
<td>3.-</td>
</tr>
<tr>
<td>475</td>
<td>(1971) WHO Expert Committee on Insecticides</td>
<td></td>
<td>$0.60</td>
</tr>
<tr>
<td></td>
<td>Nineteenth Report (20 pages)</td>
<td></td>
<td>2.-</td>
</tr>
<tr>
<td>476</td>
<td>(1971) Family Planning in Health Services</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Report of a WHO Expert Committee (65 pages)</td>
<td></td>
<td>4.-</td>
</tr>
<tr>
<td>477</td>
<td>(1971) Joint FAO/WHO Expert Committee on Nutrition</td>
<td></td>
<td>$1.25</td>
</tr>
<tr>
<td></td>
<td>Eighth Report (80 pages)</td>
<td></td>
<td>4.-</td>
</tr>
<tr>
<td>478</td>
<td>(1971) The Use of Cannabis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Report of a WHO Scientific Group (47 pages)</td>
<td></td>
<td>3.-</td>
</tr>
<tr>
<td>479</td>
<td>(1971) WHO Expert Committee on Yellow Fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third Report (56 pages)</td>
<td></td>
<td>3.-</td>
</tr>
<tr>
<td>480</td>
<td>(1971) Personal Health Care and Social Security</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Report of a Joint ILO/WHO Committee (74 pages)</td>
<td></td>
<td>4.-</td>
</tr>
<tr>
<td>481</td>
<td>(1971) Development of Studies in Health Manpower</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Report of a WHO Scientific Group (56 pages)</td>
<td></td>
<td>3.-</td>
</tr>
<tr>
<td>482</td>
<td>(1971) Principles for the Testing and Evaluation of Drugs for Mutagenicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Report of a WHO Scientific Group (18 pages)</td>
<td></td>
<td>2.-</td>
</tr>
</tbody>
</table>