WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Twenty-third Report

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# CONTENTS

<table>
<thead>
<tr>
<th>GENERAL</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
</tr>
</tbody>
</table>

## PHARMACOLOGICAL SUBSTANCES

### Antibiotics
1. Methacycline ............................................... 10
2. Neomycin B .................................................. 10
3. Neomycin ................................................... 11
4. Tetracycline ................................................ 11
5. Lymecycline ................................................ 11
6. Saramycetin ................................................. 11
7. Candididin .................................................. 12
8. Trichomycin ................................................ 12
9. Penicillin K ................................................ 12
10. Clindamycin ............................................... 12
11. Minocycline ............................................... 12
12. Virginiamycin ............................................. 13
13. Anti-tumour antibiotics — bleomycin ..................... 13

### Hormones and enzymes
14. Glucagon ................................................... 13
15. Human growth hormone .................................... 13
16. Erythropoietin .............................................. 14
17. Human menopausal gonadotrophins ......................... 14
18. Blood coagulation factor VIII (antihaemophilic factor) 14

## IMMUNOLOGICAL SUBSTANCES

### Antigens
19. Pertussis vaccine .......................................... 15
20. Measles vaccine (inactivated) ........................... 15
21. Lecithin (egg) ............................................. 16
22. Rubella haemagglutinin ................................... 16
23. *Clostridium oedematios* (type A) toxoid (adsorbed) 16
24. Plague vaccine ............................................. 17
25. *Clostridium welchii* (perfringens) types B and D vaccines 17
26. Epidemic typhus vaccine .................................. 17
<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>27. Anti-rubella serum</td>
<td>18</td>
</tr>
<tr>
<td>28. Diphtheria antitoxin for flocculation test</td>
<td>18</td>
</tr>
<tr>
<td>29. Rheumatoid arthritis serum</td>
<td>18</td>
</tr>
<tr>
<td>30. <em>Naja</em> and other antivenins</td>
<td>19</td>
</tr>
<tr>
<td>31. Gas-gangrene antitoxin (histolyticus)</td>
<td>19</td>
</tr>
<tr>
<td>32. Human immunoglobulins IgG, IgA and IgM</td>
<td>20</td>
</tr>
<tr>
<td>33. Human immunoglobulin IgD</td>
<td>21</td>
</tr>
<tr>
<td>34. Human immunoglobulin IgE</td>
<td>21</td>
</tr>
<tr>
<td>35. Anti-nuclear-factor serum (homogeneous)</td>
<td>21</td>
</tr>
<tr>
<td>36. Anti-Brucella abortus serum</td>
<td>22</td>
</tr>
</tbody>
</table>

**BIOLOGICAL REFERENCE REAGENTS**

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>37. Anti-cholera sera</td>
<td>22</td>
</tr>
<tr>
<td>38. Anti-leptospira sera</td>
<td>22</td>
</tr>
<tr>
<td>39. Anti-echinococcus serum</td>
<td>23</td>
</tr>
</tbody>
</table>

**INTERNATIONAL REQUIREMENTS FOR BIOLOGICAL SUBSTANCES**

<table>
<thead>
<tr>
<th>Requirements</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>40. Requirements for snake antivenins</td>
<td>23</td>
</tr>
<tr>
<td>41. Requirements for rubella vaccine</td>
<td>23</td>
</tr>
<tr>
<td>42. Requirements for procaine benzylpenicillin in oil with aluminium monostearate</td>
<td>24</td>
</tr>
<tr>
<td>43. Requirements for <em>Brucella melitensis</em> (rev. 1) vaccine</td>
<td>24</td>
</tr>
<tr>
<td>44. Requirements for poliomyelitis vaccine (oral)</td>
<td>24</td>
</tr>
</tbody>
</table>

**ANNEXES**

| Annex 1. Requirements for snake antivenins                                  | 27   |
| Annex 2. Requirements for biological substances and other sets of recommenda-tions | 45   |
| Annex 3. International biological standards and international biological reference preparations 1971 | 47 |
| Annex 4. International biological reference reagents                       | 80   |
| Annex 5. Proposed international biological standards, international biological reference preparations and international biological reference reagents. | 88   |
| Annex 6. Discontinued international biological standards                    | 91   |

**INDEX**

| Index                                                                      | 93   |
WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Geneva, 17 to 25 November 1970

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WHO EXPERT COMMITTEE ON
BIOLOGICAL STANDARDIZATION

Twenty-third Report

The WHO Expert Committee on Biological Standardization met in Geneva from 17 to 25 November 1970. Dr L. Bernard, Assistant Director-General, welcomed the members of the Committee on behalf of the Director-General. He recalled the traditional tasks that the Committee undertook and pointed out that, although the items on the agenda related mainly to the control of biological substances, the subjects before the Committee have increased in recent years, both in number and complexity. Certain new elements therefore have appeared, such as blood coagulation substances, and immunological reagents. It was evident that the Committee had to consider an increasing number of items that were of interest to other programmes of WHO. Over the years, the reports of the WHO Expert Committee on Biological Standardization had provided useful guidance to national authorities concerned with the control of biological products.

GENERAL

Since the days of the WHO Interim Commission, it has been envisaged\(^1\) that each Member State of WHO would develop its own national laboratory for biological substances, which would provide the technical facilities for the control of biological products either manufactured in or imported into that country. Many countries, however, have not yet formally designated national centres for biological standards or national laboratories for the control of biological products, although some functions of control may in fact be performed by individual workers.

It is desirable that national control authorities be advised of the value of setting up laboratory facilities for the control of biological substances, even though they are initially on a modest scale and perhaps only for some selected products. These laboratories, besides fulfilling the needs for control of biological products in the country, could also perform valuable

\(^1\) Off. Rec. Wld Hlth Org., 1948, 11, 10 (Second report of the Expert Committee on Biological Standardization).
functions in diagnosis and research. The Committee in its twenty-second report\(^1\) adopted certain recommendations that could be used in developing such national control laboratories for biological substances.

The Committee was informed that where such laboratories or facilities exist, demands for international biological standards and reference preparations are sometimes made on the erroneous assumption that these materials are available for routine day-to-day use. Further, the international laboratories for biological standards in Copenhagen, London and Weybridge, often receive requests for international preparations from laboratories that are not directly concerned with the national control of biological products. Such laboratories include research institutes, university and hospital laboratories, and manufacturing and testing institutions. As many as possible of such demands are met, provided the requests appear to be made for some reasonable purpose. In this way the international laboratories for biological standards continue the principles enunciated at the Intergovernmental Conference on Biological Standardization, held in Geneva in October 1935. This Conference recommended that international standards should be freely distributed,\(^2\) which has always been interpreted as meaning free of charge and as widely as possible.

The Committee emphasized, however, that international standards and reference preparations should be conserved for the primary purpose for which they are intended, namely for the calibration of national standards and reference preparations or working standards. It is these national preparations that may be used for the routine day-to-day biological assay of preparations to be tested. Serious problems would arise if international standards and reference preparations were distributed indiscriminately in response to all requests, since this would result in stocks becoming exhausted in a short time. Materials for establishment as international preparations have always been obtained as donations—first, by the League of Nations and later by the Interim Commission and WHO. Such materials, however, are sometimes rare and often expensive to produce, and their characterization may demand much work. In addition, before the preparations can be established, or replaced, as international standards or reference preparations, international collaborative assays are made that are often extensive and may take several months or years to complete.

Although national control laboratories can obtain WHO international standards and reference preparations wherever needed for the calibration of national standards and reference preparations, it is the responsibility of the national control laboratories to prepare or obtain their own national


\(^2\) **Bull. Hith Org. L. of N.** 1935, 4, 631. These recommendations were later adopted by the Council of the League of Nations in 1936 and brought to the notice of Member Governments.
reference materials. In some special instances, however, materials suitable for use as national standards may be difficult to produce and characterize. An example of a national institution providing such material, as a service, occurred when the third International Standard for Corticotrophin, Porcine, for Bioassay, was established in 1962.\(^1\) The National Institute for Medical Research, London, when setting up the international standard, prepared several additional lots of material, which were calibrated and made available free of charge to laboratories for use in routine assays. In other instances, where the materials for national use may even be easily produced, the facilities available to a national laboratory may be inadequate to permit suitable preparations to be made. An example of working material of this kind being provided as an international service to such laboratories is the cholera O group 1 serum, which was prepared and made available by the Statens Serum Institut, Copenhagen.\(^5\)

It has not been the practice for WHO to provide national standards for biological substances or working standards for routine use to national control laboratories. Since, however, some laboratories experience difficulties in obtaining working materials in certain circumstances, WHO should consider the possibility of arranging for the supply of suitable preparations to those laboratories that need them. Laboratories that are willing to assist may be invited to contribute materials and to provide facilities for training personnel. In order, however, to avoid excessive demands on a particular laboratory, the means of providing assistance should preferably be co-ordinated on a group or area basis.

The Committee studied the report\(^3\) of a Panel on Radiation Sensitivity of Toxins and Animal Poisons, which was organized by the International Atomic Energy Agency and met in Bangkok from 19 to 22 May 1969. The Panel had made recommendations for research on certain microbial toxins, animal poisons and venoms, antitoxins and antivenins. The Committee noted those recommendations that were related to the standardization of biological substances, comprising a number of toxoids, antitoxins and antivenins that have already been included in the biological standardization programme for several years. Newer methods of preparing toxoids, e.g., by radiation of toxins, could be of interest, in relation to the establishment of standards and the formulation of requirements. The Committee restated its interest\(^4\) in the preparation of venom fractions and in the identification and characterization of these fractions, since these may be of value in the estimation of potency of antivenins.

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The Committee endorsed the suggestion made by the Panel\(^1\) for developing the collaboration of laboratories to obtain the materials necessary for these research and reference purposes and for making collaborative studies, and emphasized the importance of facilities for training personnel who would participate in these studies.

**PHARMACOLOGICAL SUBSTANCES**

**ANTIBIOTICS**

1. Methacycline

The Committee noted\(^2\) the report, now available, of the collaborative assay, referred to in its twenty-second report,\(^3\) of the preparation of methacycline established as the International Reference Preparation of Methacycline.

2. Neomycin B

The Committee noted\(^4\) the results of the collaborative study, requested in its twenty-first report,\(^5\) of the proposed international reference preparation of neomycin B. The study included chemical and physical tests of purity as well as biological comparisons of the preparation with material of similar purity. The results showed that the preparation was essentially pure neomycin B sulfate and confirmed that it was suitable to serve as an international reference preparation. The Committee established this preparation as the International Reference Preparation of Neomycin B and agreed that the international unit should be equivalent to one microgram of neomycin B base. The Committee therefore defined the International Unit for Neomycin B as the activity contained in 0.001492 mg of the International Reference Preparation of Neomycin B.

The Committee emphasized that this preparation is not suitable for the assay of preparations of neomycins other than neomycin B.

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\(^2\) Unpublished working document WHO/BS/70.994.


\(^4\) Unpublished working documents WHO/BS/70.993, WHO/BS/70.1000 and WHO/BS/70.1000 Corr. 1.

3. Neomycin

The Committee noted\(^1\) the results of recent studies by the National Institute for Medical Research, London, which showed that the International Reference Preparation of Neomycin was no longer representative of the preparations of neomycin in use throughout the world. The Committee requested the National Institute for Medical Research to investigate the possibility of replacing the international reference preparation by a more suitable preparation.

4. Tetracycline

The Committee noted\(^2\) that the collaborative assay of the proposed second international standard for tetracycline had been completed. The Committee was informed that in accordance with the authorization given in its twenty-second report\(^3\) the National Institute for Medical Research, London, had established the second International Standard for Tetracycline in replacement of the first international standard and, with the agreement of the participants, had defined the International Unit for Tetracycline as the activity contained in 0.00101833 mg of the International Standard for Tetracycline.

5. Lymeycline

The Committee noted\(^4\) that, as requested in its twenty-first report,\(^5\) the National Institute for Medical Research, London, had obtained a quantity of lymeycline suitable to serve as the replacement of the first International Reference Preparation of Lymeycline.

The Committee was informed that a collaborative assay was in progress and authorized the National Institute for Medical Research to establish this material as the second International Reference Preparation of Lymeycline on the basis of the results of the collaborative assay and to define the international unit with the agreement of the participants.

6. Saramycetin

The Committee was informed that, as requested in its twenty-second report,\(^6\) the WHO Secretariat had investigated the need for an international

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\(^1\) Unpublished working document WHO/BS/70.1001.
\(^2\) Unpublished working document WHO/BS/70.1014.
\(^4\) Unpublished working document WHO/BS/70.1010.
reference preparation of saramycetin. Since this antibiotic is only in limited use for clinical investigations, the Committee agreed that at the present time there was no need for an international reference preparation.

7. Candidin

The Committee noted¹ that, as requested in its twenty-first report,² the National Institute for Medical Research, London, had obtained a preparation of candidin and that studies of this material were continuing.

8. Trichomycin

The Committee noted³ that, as requested in its twenty-first report,⁴ the National Institute for Medical Research, London, had obtained a preparation of trichomycin. The Committee was informed that studies were in progress to determine the suitability of this preparation for use as an international reference preparation.

9. Penicillin K

The Committee was informed that stocks of the International Reference Preparation of Penicillin K were exhausted. Since it is possible to characterize preparations of penicillin K adequately by chemical and physical means the Committee decided not to replace this preparation and discontinued the International Reference Preparation of Penicillin K.

10. Clindamycin

The Committee was informed that there was a need for an international reference preparation of clindamycin. The Committee therefore requested the National Institute for Medical Research, London, to obtain suitable material and to arrange a collaborative assay.

11. Minocycline

The Committee was informed that there was a need for an international reference preparation of minocycline. The Committee therefore requested the National Institute for Medical Research, London, to obtain suitable material and to arrange a collaborative assay.

¹ Unpublished working document WHO/BS/70.1011.
³ Unpublished working document WHO/BS/70.1013.
12. Virginiamycin

The Committee reconsidered the possible need for an international reference preparation of virginiamycin, referred to in its eighteenth report, and agreed that there may now be a need for such a preparation. Since this antibiotic is largely used as a growth-promoting factor in animal feeds, the Committee requested the Central Veterinary Laboratory, Weybridge, in collaboration with the WHO Secretariat to collect further information on the present use and control of this antibiotic.

13. Anti-Tumour Antibiotics — Bleomycin

The Committee was informed that there may be a need for an international reference preparation of bleomycin. Since this is an anti-tumour antibiotic, the Committee requested the WHO Secretariat to consider bleomycin when collecting the information on anti-tumour antibiotics requested in its twenty-second report.

HORMONES AND ENZYMES

14. Glucagon

The Committee noted that, as requested in its nineteenth report, the National Institute for Medical Research, London, had obtained further information on glucagon preparations. The preparations of glucagon available for therapeutic use are solely of animal origin and a quantity of highly purified porcine glucagon is being studied in a national control laboratory to ascertain the suitability of the material in the assay of such preparations.

The Committee also noted that there was a need for international reference material and requested the National Institute for Medical Research to investigate the availability and suitability of the material under study and to arrange a collaborative assay.

15. Human Growth Hormone

The Committee noted the results of the further analysis that had been made, as requested in its twenty-second report, of the data obtained in the

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3 Unpublished working document WHO/BS/70.1002.
5 Unpublished working document WHO/BS/70.1017.
collaborative assay for the establishment of the International Reference Preparation of Growth Hormone, Human, for Immunoassay.

16. Erythropoietin

The Committee noted ¹ that the collaborative assay, referred to in its twenty-first report, ² of the proposed second International Reference Preparation of Erythropoietin, Human, Urinary, for Bioassay, had been completed and that the results showed the preparation to be suitable. The Committee therefore established this material as the second International Reference Preparation of Erythropoietin, Human, Urinary, for Bioassay, in replacement of the first international reference preparation.

The Committee also noted ¹ a proposal, which had been agreed to by the participants in the collaborative assay, for the definition of the international unit equivalent to the existing unit. The Committee therefore defined, on this basis, the International Unit for Erythropoietin, Human, Urinary, for Biossay, as the activity contained in 0.50 mg of the second International Reference Preparation of Erythropoietin, Human, Urinary, for Bioassay.

17. Human Menopausal Gonadotrophins

The Committee noted ³ that stocks of the second International Reference Preparation of Human Menopausal Gonadotrophins (Follicle Stimulating Hormone and Interstitial Cell Stimulating Hormone) Urinary, for Bioassay, were becoming depleted. The Committee was informed that the National Institute for Medical Research, London, had obtained suitable material for a replacement and that a collaborative assay was being arranged.

18. Blood Coagulation Factor VIII

(Antihaemophilic Factor)

The Committee noted ⁴ the results of the collaborative study of the two preparations of blood coagulation factor VIII referred to in its twenty-first report ⁵—a preparation of freeze-dried pooled plasma and a preparation of freeze-dried concentrate. The results showed that the concentrate was suitable for use as a standard in the assay of factor VIII activity by a number of methods and had satisfactory stability. The Committee also considered some further information collected by the WHO Secretariat and agreed

¹ Unpublished working document WHO/BS/70.1015.
³ Unpublished working document WHO/BS/70.1018.
⁴ Unpublished working document WHO/BS/70.999.
that there was a need for an international standard for factor VIII activity to be used in the control of therapeutic factor VIII preparations. The Committee established the concentrate as the International Standard for Blood Coagulation Factor VIII.

The Committee was informed of a proposal for the definition of an international unit, which had been agreed to by the participants in the collaborative study. The Committee, on this basis, defined the International Unit for Blood Coagulation Factor VIII as the activity contained in 14.365 mg of the International Standard for Blood Coagulation Factor VIII.

The Committee emphasized that this international standard should be conserved for the calibration of national standards for the control of Factor VIII preparations used in therapy. Since stocks of the international standard are limited, it cannot also be made available for the calibration of numerous working reference materials for the routine estimation of factor VIII activity in plasma for monitoring therapy, unless such calibration can be effected on a centralized national basis.

IMMUNOLOGICAL SUBSTANCES

ANTIGENS

19. Pertussis Vaccine

The Committee was informed that although the preparation of pertussis vaccine, referred to in its seventeenth report\(^1\) and intended as the replacement of the International Standard for Pertussis Vaccine, had been obtained, the collaborative assay had been deferred in view of the research studies arranged by the WHO Secretariat on certain aspects of the assay of potency of this vaccine. The Committee was also informed that the Statens Serum-institut, Copenhagen, would consider the results of these studies,\(^2\) now available, when designing the collaborative assay of the proposed second international standard.

20. Measles Vaccine (Inactivated)

The Committee reconsidered the question, which had been under study for some years, of the possible need for an international reference preparation of measles vaccine (inactivated). The WHO Secretariat had ascertained that there is now only limited use of this vaccine and the Committee therefore

\(^2\) *Bull. Wild Hlth Org.*, 1971, in press.
decided that there was no need, at present, for an international reference preparation.

21. Lecithin (Egg)

The Committee noted that the collaborative assay, referred to in its twentieth report, of the proposed fourth International Reference Preparation of Lecithin (Egg), which was a pool of three lots of material, had been completed and that the preparation was suitable.

The Committee therefore established this material as the fourth International Reference Preparation of Lecithin (Egg) in replacement of the third international reference preparation.

22. Rubella Haemagglutinin

The Committee noted the results of collaborative studies in which the haemagglutinating activity of a number of rubella virus preparations had been compared. Estimations using a common reference preparation of haemagglutinin showed wide discrepancies. Further, when a common reference preparation of haemagglutinin was used in the haemagglutination inhibition test, there was no advantage over the use of haemagglutinin preparations of local origin. The Committee therefore agreed that the value of an international reference rubella haemagglutinin, envisaged in its twentieth report, was not confirmed.

23. Clostridium oedematiens (Type A) Toxoid (Adsorbed)

The Committee noted that, as requested in its twenty-second report, the WHO Secretariat, with the collaboration of the State Institute for the Control of Medical Biological Preparations, Moscow, had collected information on the use of Clostridium oedematiens (Type A) toxoid (adsorbed). There is only limited use of this toxoid in man, confined to the immunization of those exposed to particular risk of injury and gas-gangrene infection. Since adequate laboratory control of this toxoid is feasible in a single country by the use of a national reference preparation, the Committee agreed that there was no need for an international standard.

1 Unpublished working document WHO/BS/70.1004.
5 Unpublished working document WHO/BS/70.1022.
24. Plague Vaccine

The Committee noted the information on plague vaccine that had been collected in accordance with the request in its twenty-first report. In addition, the WHO Secretariat had asked all Member States for information on the use and control of plague vaccines and a WHO Expert Committee on Plague had recently considered these questions. While different kinds of plague vaccine were made in several countries, there was no general agreement on the effectiveness of the vaccine (live or killed), little preference for a particular kind of vaccine and little information on the effective control of plague vaccine by the use of reference material.

The Committee agreed that at present it was not feasible to establish international reference material for use in laboratory tests or to consider the formulation of requirements for plague vaccine.

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

The Committee had noted in its sixteenth report that there was a need for international standards for Clostridium welchii (perfringens) types B and D vaccines and had requested the Central Veterinary Laboratory, Weybridge, to obtain suitable materials and to arrange collaborative assays.

The Committee noted that materials that may serve for international reference purposes had been obtained and that preliminary studies of their suitability were being made.

26. Epidemic Typhus Vaccine

The Committee was informed that there may be a need for reference material of vaccine against epidemic typhus. The Committee requested the WHO Secretariat to obtain information on the present use and control of such vaccine.

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1 Unpublished working document WHO/BS.70.997.
5 Unpublished working document WHO/BS.70.1007.
ANTIBODIES

27. Anti-Rubella Serum

The Committee noted ¹ the results of the collaborative study in which the preparation of human normal immunoglobulin, obtained as a possible replacement of the International Reference Preparation of Anti-Rubella Serum (discontinued in 1967), was compared with a sample of human convalescent serum. The results of virus neutralization tests showed that the relative potency estimates varied less between laboratories than did the estimates obtained by direct titration. The results of haemagglutination inhibition tests, however, did not show a corresponding reduction in variation. The Committee nevertheless agreed that this preparation—in addition to its use in the virus neutralization test—might serve a useful purpose in studies of the haemagglutination inhibition test. The Committee therefore established this preparation as the second International Reference Preparation of Anti-Rubella Serum.

The Committee also noted ¹ a proposal for the definition of the international unit, which had been agreed to by the participants in the collaborative study and, on this basis, defined the International Unit for Anti-Rubella Serum as the activity contained in 0.14595 mg of the second International Reference Preparation of Anti-Rubella Serum.

28. Diphtheria Antitoxin for Flocculation Test

The Committee was informed that, as requested in its twenty-second report,² the Statens Seruminstitut, Copenhagen, had obtained material for the replacement of the International Reference Preparation of Diphtheria Antitoxin for Flocculation Test. Studies in which a number of different diphtheria toxoids were used has shown the suitability of the preparation to serve as such a replacement.

29. Rheumatoid Arthritis Serum

The Committee noted ³ the report, now available, of the collaborative assay, referred to in its twenty-first report,⁴ of the preparation of rheumatoid

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¹ Unpublished working documents WHO/BS/70.996 and WHO/BS/70.996 Corr. 1.
arthritis serum that had been established as the International Reference Preparation of Rheumatoid Arthritis Serum.

30. *Naja* and other Avivenins

The Committee noted 1 the further report of studies of the International Standard for *Naja* Antivenin. Collaborative studies of this international standard have been conducted by the Statens Serum Institut, Copenhagen, over a number of years and are intended to lead to the development of methods for assaying the potency of *Naja* and related antivenin preparations. The results of assays of these antivenin preparations, using (a) common venoms distributed to the laboratories and (b) venoms of other zoogeographic origin that differed between these laboratories, showed that the relative potencies obtained depended on the particular venoms used. These findings supported the recommendation made in the Requirements for Snake Antivenins (Annex 1 to this report) to base the control of antivenin preparations on the use of appropriate reference venoms. The Committee was informed that there was some evidence that similar discrepancies in the results of potency estimates from the use of different venoms may also occur in the case of assays of *Echis* and *Bitis* antivenins.

The Committee agreed that in view of these findings it would not be advisable at present to consider the establishment of international standards for other snake antivenins to be used for the laboratory control of potency of these products. The Committee also agreed that it would be preferable for studies to be made on the composition of and immunological differences between snake venoms from the same and different zoogeographic areas with a view to developing the most suitable laboratory methods for the control of antivenins.

31. Gas-Gangrene Antitoxin (Histolyticus)

The Committee noted 2 that stocks of the second International Standard for Gas-Gangrene Antitoxin (Histolyticus), established in 1951, are nearly exhausted and that the Statens Serum Institut, Copenhagen, had ascertained that there is still a need for this standard. The Committee was informed that part of the batch of material from which this international standard was prepared had been in use for several years as a national standard and that an offer had been made of a quantity of this material to serve as the replacement of the international standard. The Committee accepted the offer and requested the Statens Serum Institut, Copenhagen, to obtain this material and to arrange a limited collaborative assay.

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1 Unpublished working document WHO/BS/70.1016.
2 Unpublished working document WHO/BS/70.995.
32. Human Immunoglobulins IgG, IgA and IgM

The Committee noted the results of the studies on the pooled human serum, now freeze-dried in batches, referred to in its twenty-first report. A collaborative assay had been arranged by the WHO International Reference Centre for Immunoglobulins, Lausanne, in conjunction with the National Institute for Medical Research, London, in which, using immunodiffusion procedures, a number of comparative estimates were made of the immunoglobulins of the classes IgG, IgA and IgM in human sera. The results showed that the batch designated 67/86, part of which had been offered for international use was suitable to serve as a reference preparation for the estimation of these immunoglobulins in human serum.

The Committee agreed that in view of the heterogeneity of reference materials that have been used in such assays and the difficulties in obtaining adequately defined, purified material, there was a need for an international reference preparation.

The Committee therefore established the material offered as the International Reference Preparation of Human Immunoglobulins IgG, IgA and IgM. The Committee emphasized that this international reference preparation should be reserved for the calibration of working preparations, such as the related batches derived from the same pooled human serum, for use in clinical medicine and research.

In order that results of assays could be conveniently expressed in terms of the international reference preparation, the Committee agreed that international units be defined. The Committee noted a proposal, which was acceptable to the participants in the collaborative study, for the definition of international units that were equivalent to the only known existing national units. The Committee therefore defined the International Unit for Human Immunoglobulin IgG, as the activity contained in 0.8147 mg of the International Reference Preparation of Human Immunoglobulins IgG, IgA and IgM, the International Unit for Human Immunoglobulin IgA as the activity contained in 0.8147 mg of the International Reference Preparation of Human Immunoglobulins IgG, IgA and IgM, and the International Unit for Human Immunoglobulin IgM as the activity contained in 0.8147 mg of the International Reference Preparation of Human Immunoglobulins IgG, IgA and IgM.

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2 Unpublished working document WHO/BS/70.1019.
33. Human Immunoglobulin IgD

The Committee noted ¹ that a pool of human serum containing immunoglobulins of the class IgD had been obtained and freeze-dried by the National Institute for Medical Research, London, and that preliminary tests in collaboration with the WHO International Centre for Immunoglobulins, Lausanne, had shown that this preparation might be suitable to serve as a reference preparation of immunoglobulins of this class. Further collaborative studies of this preparation are in progress.

34. Human Immunoglobulin IgE

The Committee noted ¹ that a pool of human serum containing immunoglobulins of the class IgE had been obtained and freeze-dried by the WHO International Centre for Immunoglobulins, Lausanne, in collaboration with the National Institute for Medical Research, London. Preliminary tests had shown that this preparation might be suitable to serve as a reference preparation of immunoglobulins of this class. Further collaborative studies of this preparation are in progress.

35. Anti-Nuclear-Factor Serum (Homogeneous)

The Committee noted ² the results of the collaborative study, arranged by the National Institute for Medical Research, London, in collaboration with the WHO Secretariat, of the preparation of anti-nuclear-factor serum (homogeneous) referred to in its twenty-first report.³

The results showed that the use of this preparation in comparative assays reduced the variability between laboratories of estimates of anti-nuclear-factor activity in human serum and that the preparation was suitable to serve as an international reference preparation. In view of the importance of estimates of this factor in the diagnosis and prognosis of certain autoimmune diseases, the Committee agreed that such an international preparation would be useful and therefore established the preparation studied as the International Reference Preparation of Anti-Nuclear-Factor Serum (Homogeneous).

In order that the results of assays could be conveniently expressed in terms of the international reference preparation, the Committee agreed that an international unit be defined. The Committee noted ² a suggestion for such a unit, which was acceptable to the participants in the collaborative study, and, on this basis, defined the International Unit for Anti-Nuclear-

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¹ Unpublished working document WHO/BS/70.1019.
² Unpublished working document WHO/BS/70.1005.
Factor Serum (Homogeneous) as the activity contained in 0.186 mg of the International Reference Preparation of Anti-Nuclear-Factor Serum (Homogeneous).

36. Anti-Brucella abortus Serum

The Committee noted the results of studies of the second International Standard for Anti-Brucella abortus Serum. These studies were intended to ascertain the suitability of this standard for use in the complement-fixation test. This assay procedure, which has been increasingly employed in recent years, had not been included in the collaborative assay for the establishment of the international standard and the present studies enabled a comparison to be made of the results of assays using the complement-fixation test with those obtained using the agglutination test. The results confirmed that the international standard is also suitable for use in the complement-fixation test.

BIOLOGICAL REFERENCE REAGENTS

37. Anti-Cholera Sera

The Committee noted that in response to the request in its twenty-first report the WHO Secretariat had considered the question of the provision of agglutinating sera, such as cholera O Group 1, monospecific Inaba and monospecific Ogawa for use in the identification of strains of *Vibrio cholerae* O group 1. The Committee agreed that there was no need for such sera to be established as international reference reagents, since suitable sera could be prepared in individual laboratories. The Committee therefore discontinued the existing International Reference Reagent of Cholera Agglutinating Serum (Ogawa) but requested the Statens Serum-institut, Copenhagen, to continue to distribute the preparation until stocks were exhausted.

38. Anti-Leptospira Sera

The Committee noted that a collaborative study of the three antileptospira sera referred to in its twenty-first report had shown that these preparations were of satisfactory specificity and stability to serve as international

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2 Unpublished working documents WHO/BS/70.1008 and WHO/BS/70.1008 Add. 1.
3 Unpublished working document WHO/BS/70.998.
reference reagents. The Committee therefore established the following preparations of Anti-Leptospira Sera as International Reference Reagents:

Anti-Leptospira interrogans serotype butembo serum
Anti-Leptospira interrogans serotype jules serum
Anti-Leptospira interrogans serotype sumneri serum

39. Anti-Echinococcus Serum

The Committee was informed that the two preparations of human anti-echinococcus serum referred to in its fourteenth and sixteenth reports had been obtained by the WHO Secretariat and that studies were being planned to determine the suitability of the preparations for use in various tests for detecting echinococcus antibodies.

INTERNATIONAL REQUIREMENTS FOR BIOLOGICAL SUBSTANCES

40. Requirements for Snake Antivenins

The Committee studied the Requirements for Snake Antivenins that had been prepared by the WHO Secretariat in collaboration with a number of experts. After making certain modifications, the Committee agreed that the text of these requirements was satisfactory, and that they would be useful for the control of such antivenins produced in different countries.

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

41. Requirements for Rubella Vaccine

The Committee noted that a suggestion had been made for the formulation of requirements for rubella vaccine (live), which is now produced in some countries and controlled nationally. Since there are several problems to be resolved in respect of rubella vaccine, including evaluation of its efficacy, the Committee agreed that the question of formulating such requirements should be deferred.

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4 Unpublished working document WHO/BS/70.1023.
42. Requirements for Procaine Benzylpenicillin in Oil with Aluminium Monostearate

The Committee noted\(^1\) that certain objections had been raised, on mathematical and statistical grounds, in regard to the recommended method of evaluating the results of the blood-level duration test, specified in Part A, section 5.3.2 of the Requirements for Procaine Benzylpenicillin in Oil with Aluminium Monostearate (Requirements for Biological Substances No. 9).\(^2\) The Committee agreed that it was preferable, in the evaluation of the results of this test, to allow the use of any suitable statistical analysis that would permit the calculation of the lower 99 % confidence limit of the ratio obtained by comparing the mean blood-level of penicillin in the group of rabbits receiving the test preparation with the mean blood-level in the group receiving the international reference preparation.

The Committee therefore requested the WHO Secretariat to arrange for the preparation of a suitable modification to the Requirements for Procaine Benzylpenicillin in Oil with Aluminium Monostearate, so as to permit a choice of methods of statistical analysis.

43. Requirements for Brucella melitensis (Rev. 1) Vaccine

The Committee noted\(^3\) a suggestion that there was a need for the formulation of requirements for the control of *Brucella melitensis* (Rev. 1) vaccine, which is used in veterinary medicine. Since the widespread immunization of sheep and goats in some countries is an important means of preventing the transmission of *Brucella melitensis* infection to humans, the Committee agreed that there was a need for such requirements and requested the Central Veterinary Laboratory, Weybridge, in collaboration with the WHO Secretariat, to investigate the possibility of arranging for the formulation of these requirements.

44. Requirements for Poliomyelitis Vaccine (Oral)

The Committee noted\(^4\) a suggestion that, in view of the knowledge and experience gained in the control of poliomyelitis vaccine (oral) since the publication of the revised Requirements for Poliomyelitis Vaccine (Oral) (Requirements for Biological Substances No. 7),\(^5\) there was a need for a further revision of certain sections of the requirements.

\(^1\) Unpublished working document WHO/BS/70.1021.
\(^3\) Unpublished working document WHO/BS/70.1006.
\(^4\) Unpublished working document WHO/BS/70.1003.
The Committee agreed with this suggestion and asked the WHO Secretariat to consider the preparation of such a revision.

ACKNOWLEDGEMENTS

The Committee wishes to record its thanks to the following members of the WHO Secretariat for their special contributions to its deliberations: Dr M. Abdussalam, Chief, Veterinary Public Health; Dr W. C. Cockburn, Chief, Virus Diseases; Dr B. Cvjetanović, Chief, Bacterial Diseases; Dr G. Edsall, Bacterial Diseases (Consultant); Dr D. Rowe, Director of the WHO International Reference Centre for Immunoglobulins; Dr A. Szenberg, Medical Officer, Immunology; Dr S. S. Vrancheva, Medical Officer, Biological Standardization.
Annex 1

REQUIREMENTS FOR SNAKE ANTIVENINS
(Requirements for Biological Substances No. 21)

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction .......................... 27</td>
</tr>
<tr>
<td>General considerations .............. 31</td>
</tr>
<tr>
<td>Part A: Manufacturing requirements .................. 33</td>
</tr>
<tr>
<td>1. Definition .......................... 33</td>
</tr>
<tr>
<td>2. General manufacturing requirements ......... 35</td>
</tr>
<tr>
<td>3. Production control .................. 35</td>
</tr>
<tr>
<td>4. Filling and containers .................. 40</td>
</tr>
<tr>
<td>5. Control tests on filling lot .......... 40</td>
</tr>
<tr>
<td>6. Records ........................... 41</td>
</tr>
<tr>
<td>7. Samples ............................ 41</td>
</tr>
<tr>
<td>8. Labelling .......................... 41</td>
</tr>
<tr>
<td>9. Distribution and shipping .............. 42</td>
</tr>
<tr>
<td>10. Storage and expiry date ............ 42</td>
</tr>
<tr>
<td>Part B: National control requirements .......... 43</td>
</tr>
<tr>
<td>1. General ............................ 43</td>
</tr>
<tr>
<td>2. Release and certification ............... 43</td>
</tr>
<tr>
<td>3. Efficacy and safety of antivenins in the field 44</td>
</tr>
</tbody>
</table>

Introduction

Administration of antivenin has been for many years the most widely accepted form of treatment of snakebite. The nineteenth WHO Expert Committee on Biological Standardization was of the opinion that there was a need for the formulation of requirements

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1 Prepared by the following experts and members of the WHO Secretariat:
   Professor D. G. Evans, London School of Hygiene and Tropical Medicine, London, England (Consultant); Dr P. Krag, Director, Department of Biological Standardization, Statens Seruminstitut, Copenhagen, Denmark (Consultant); Dr A. S. Outsoor, Chief Medical Officer, Biological Standardization, WHO, Geneva, Switzerland; Dr E. B. Seligmann Jr, Chief, Laboratory of Control Activities, Division of Biologic Standards, National Institutes of Health, Bethesda, Md, USA (Consultant); Dr S. S. Vrancheva, Medical Officer, Biological Standardization, WHO, Geneva, Switzerland; Professor A. de Vries, Beilinson Hospital, Petah Tikva, Israel (Consultant).

2 The management of snakebite is not dealt with in this document. No strict rules can be laid down for treatment, but national health authorities may wish to consider making available for use in their countries, particularly where the risk of snakebite is large, recommendations for both the first-aid treatment and the management of snakebite cases. Administration of antivenin should preferably be under medical supervision and after hospitalization, if possible; the preferred route of administration, i.e., intravenous, can then be used.

for snake antivenins. A useful preliminary to this would be the existence of requirements for immune sera of animal origin and these requirements have now been prepared.¹

As a first step a meeting on Requirements for Biological Substances was held in Geneva from 6–11 March 1967 to consider the problem of formulating requirements for snake antivenins. The present document has been largely based on the recommendations drafted by this meeting.

The participants in this meeting were:

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Dr A. H. Mohamed, Professor of Physiology, Faculty of Medicine, Ein-Shamus University, Cairo, UAR
Dr A. S. Outshoorn, Chief, Biological Standardization, WHO, Geneva, Switzerland (Secretary)
Dr C. Puranamanda, Director, Queen Saovabha Memorial Institute, Bangkok, Thailand (Vice-Chairman)
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Dr H. A. Reid, School of Tropical Medicine, Liverpool, England (Rapporteur)
Dr F. F. Rezepov, Deputy Director, Tarasevič State Control Institute for Medical and Biological Preparations, Moscow, USSR
Dr Findlay E. Russell, Director of Laboratory, Laboratory of Neurological Research, Los Angeles, Calif., USA (Consultant)
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Dr J. Uri, Medical Officer, Biological Standardization, WHO, Geneva, Switzerland
Dr A. de Vries, Director, Rogoff Medical Research Institute of the Labour Sick Fund, Beilinson Hospital, Petah Tikva, Israel (Chairman)
Dr O. Zwisler, Behringwerke A.G., Marburg/Lahn, Federal Republic of Germany

The international requirements for snake antivenins that are set out below follow the pattern of the Requirements for Biological Substances Nos. 1–20 already published by WHO,² and in drafting them account has been taken of the recommendations of the meeting on International Requirements for Biological Substances referred to above, the opinions of consultants and other experts, the regulations and requirements for the manufacture and control of antivenins that have been formulated in a number of

countries, as well as information from both published and unpublished reports.

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

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Useful data were also obtained from the panel discussion on Production and Standardization of Antivenins at the Second International Symposium on Animal and Plant Toxins, held from 22 to 28 February 1970 in Tel Aviv, Israel.
General Considerations

The formulation of international requirements for snake antivenins is complicated by the fact that methods of producing and testing antivenins differ greatly throughout the world. Ways of estimating and expressing the neutralizing effect of antivenins against relevant venoms vary and may not always reflect efficacy in man. These differences could jeopardize the effective use of antivenins. Because of venom variations in different species of snakes and even within the same species in the same zoogeographic areas, it is desirable for national control authorities to establish reference venoms to be used in the assay of antivenin potency. Such reference venoms are already available in a few countries and the relevant requirements for potency have been formulated by the national control authorities. It has not been possible, however, for international use, to formulate precise requirements for potency for each and every type of antivenin preparation.

The present requirements have been formulated with a view to ensuring, in general, that the specific immune substances present in antivenin preparations have satisfactory neutralizing properties for the lethal components of venom. Since many of the recommendations in these requirements would also be applicable to antisera against the venoms of scorpions and spiders, these requirements could be modified suitably to serve as requirements for scorpion and spider antivenins.

The WHO Expert Committee on Biological Standardization has for many years been interested in problems of establishing international standards for antivenins and of undertaking studies that might be of value in the production of antivenins and in their control by the use of such standards. Although the International Standard for Naja Antivenin has been established and the International Unit has been defined, studies have not confirmed the feasibility of international use of this standard in the control of potency. Because of the complex nature of venoms, there is a need for studies on the separation and characterization of venom components as well as for monospecific sera against components for use in such studies.

Several animal species are used for antivenin production, but horses are most commonly used. Species such as monkeys known to harbour viruses infective to man should not be used for antivenin production. Antivenins of human origin are not available and it may not be feasible to produce them for general use. Appropriate antivenins prepared in

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1 The World Health Organization is attempting to compile detailed data on the characteristics of national reference venoms used in various countries. National control authorities are requested to send any available information on such reference venoms to the World Health Organization, 1211 Geneva 27, Switzerland.

animals other than horses may be valuable for snakebite victims to whom the administration of horse serum is contraindicated. It is recommended, however, that persons who know they are hypersensitive to horse protein or who are prone to serious allergenic manifestations should take every possible precaution to avoid being bitten by venomous snakes.

Although many laboratories now make purified antivenin preparations, some unpurified antivenins are still available. Since intravenous administration is the route of choice, studies for the development of purified antivenins should be encouraged and workers in laboratories producing antivenins should do their best to familiarize themselves with available methods of purification and should work towards the development of better methods. It is highly desirable that ultimately only purified antivenins of high activity and good stability should be used.

Polyvalent antivenins are frequently produced for use in the treatment of snakebite when the species of snake involved has not been identified. Although such a procedure may sometimes be unavoidable, it is by no means ideal and should not be regarded as the procedure of choice; it is much better to give antivenin of a specificity appropriate to the venom(s) of the snake(s) in the zoogeographic area. In order to enable effective monovalent or group-specific antivenins to be prepared, national health authorities should make surveys of the various zoogeographic areas within their countries so that for each area relevant information is available on the particular snake(s), as well as on their venom characteristics. In some countries such information has already been gathered and has proved of value. National control authorities should ensure that antivenins produced are appropriate to the particular types of venoms to be encountered. Appropriate antivenins should also be available for particular circumstances, such as for use in zoos or laboratories where snakes not peculiar to the country or region are kept.

Studies are being made in some countries on the feasibility of active immunization against snake venom poisoning. The position at the present time is that with care it may be possible to immunize persons against a particular venom but persons at risk may encounter many species of snakes. Further, an adequate quantity of antibody must be available at the time of the bite, if protection is to be achieved, and this would necessitate frequent immunization of the subject with the attendant dangers of vital tissue damage and sensitization. Moreover nothing is known of how humans would react to such prolonged immunization. The conclusion is that active immunization against snake venom would be of limited value only, even if relatively safe and effective immunizing agents could be produced.

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 used as 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 snake antivenins 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 that the safety and potency of the antivenins are 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 antivenin is manufactured.

Part A. Manufacturing Requirements

1. Definition

1.1 International name and proper name

The international name shall be Antivenenum, followed by the zoological name(s) of the species of snake(s) from which the venom was derived for immunizing animals for the production of antivenin, followed by the species of animal in which the antivenin was made.

Examples of monovalent and polyvalent antivenins are given below to illustrate the procedure to be adopted:

Antivenenum Naja naja (equinum)
Antivenenum Bitis lachesis, Bitis gabonica (equinum)

In general the preparations are made in horses (equinum) but other animal species may also be used, e.g., cattle (bovinum) or goat (caprinum). Polyvalent antivenin may be a mixture of antivenins, each specific against a single snake species, produced in different animals of the same species (e.g., horses), or an antivenin prepared in animals of a particular species by immunization against venoms of more than one species of snake.

The proper name shall be the equivalent of the international name in the language of the country where the antivenin will be used, or if the appropriate health authorities permit, in the language of the country of origin.

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

1.2 Descriptive definition

Antivenenum is a preparation of natural serum, or of purified immunoglobulins, or of treated immunoglobulins obtained from serum containing
specific antibodies against snake venom. The preparation shall satisfy all the requirements formulated below.

In some countries the manufacture of unprocessed serum preparations is allowed, but because antivenins are often given intravenously and the dangers of untoward reactions are great their use should be discouraged.

Some preparations are available in the dried form.

1.3 International standards and units, and national reference preparations

The establishment of international reference venom preparations is not envisaged at the present time. National control authorities should provide each of the appropriate reference venoms, or approve of and give instructions regarding the use of such reference venoms to manufacturers, for potency testing of antivenins (see Part A, section 3.5.5.1).

The International Standard for *Naja* Antivenin was established in 1964 and the International Unit (IU) was defined.\(^1\) The preparation is dispensed in ampoules containing purified, dried, polyvalent horse serum (containing antibodies against venom from *Naja naja*, *Naja naja* and *Hemachatus hemachatus*). Studies\(^2\) have shown that it is not feasible to use this standard on an international basis for the assay of potency of naja antivenins.

The above standard is 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. The international standard is intended for studies leading to the development of methods for the use of such a preparation in evaluating the potency of *Naja* and related antivenin preparations and to improve procedures in the manufacture and laboratory control of such antivenins.

1.4 Terminology

*Source material* is a quantity of unprocessed serum or plasma obtained from a single immunized animal, or a pool of such quantities.

*Bulk material* is unprocessed or processed serum or plasma derived from one or more source materials.

*Final bulk* is the sterile preparation of serum or of immunoglobulin material made from one or more bulks and present in the single container from which the final containers are filled directly or through one or more intermediate containers.

*Antivenin lot* is the finished material in final containers which has at some stage been processed together and has, therefore, a uniform composition. An antivenin lot consists of one or more filling lots.

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\(^2\) Unpublished working document WHO/BS/70.1016.
Filling lot (final lot) is a collection of sealed final containers which are homogeneous with respect to the risk of contamination during filling and, if applicable, during drying. A filling lot must, therefore, have been filled in one working session and, if applicable, dried together.

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 antivenins, with the addition of the following:

A special room that can be washed down with a disinfectant shall be used for the collection of blood from the animals.

The processing of the sera shall be done in an area separate from that housing the animals and from that used for the collection of blood.

3. Production control

3.1 Control of venoms and animals

3.1.1 Control of venoms used for immunization

The snakes from which venom is taken for the production of antivenin shall be collected over the zoogeographic range of the relevant species of snake.

If the use of the antivenin is to be restricted to a country or limited area, the collection of snakes can be limited to that area, provided that this is indicated in the labelling (see Part A, section 8).

The zoological identity of the snake and the area of collection shall be recorded to ensure that only venoms are pooled which are species-specific and antigenically representative of the relevant zoogeographic area. Snakes kept for the collection of venom shall be maintained in a manner that will ensure their continued suitability for venom collection and only healthy snakes shall be used for this purpose.

In the absence of requirements specified by the national control authority for the keeping (and feeding) of snakes to provide venom, appropriate professional advice should be sought.

Venoms should be collected before the snakes are fed. Care must be taken not to injure the mouth or venom glands during the collection procedure and to avoid any bleeding. Dilution of the venom by other salivary secretions should be avoided.

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Venoms shall be processed under conditions that minimize the risk of microbial and other contamination.

Only venom that has the normal physical characteristics of venom from the particular species of snake shall be used.

Each venom collection shall be identifiable by name and batch number.

Venom should be dried as soon as possible after collection. Drying from the frozen state is preferable; if this is not possible the venom should be dried in a vacuum desiccator. Dried venoms are best stored in sealed containers in the dark at \(5^\circ \pm 3^\circ\text{C}\).

For each batch of venom the lethal toxicity for animals (LD<sub>50</sub>) should be determined and the batch should also be characterized on the basis of its physical, chemical and biological properties (see Part A, section 3.5.5.1).

If venom is obtained from an outside source the manufacturer should assure himself that the material conforms to the recommendations described above.

Only venom solutions that have been sterilized or largely freed from contaminating micro-organisms by a method approved by the national control authority shall be used for immunization of animals.

Some venom solutions may be sterilized by membrane filtration but the choice of method will depend on the kind of venom used and the experience of the laboratory.

It is desirable that the immunization procedure should start with detoxified venom or small doses of unmodified venom. Some manufacturers use venom to which suitable adjuvants have been added.

3.1.2 Control of venom fractions used for immunization

If venom fractions are used for immunization they shall be prepared from venoms that meet all the requirements specified in section 3.1.1 above. For each batch of venom fraction the specific activity shall be determined (see Part A, section 3.5.5.1).

3.1.3 Animals used for the production of sera

The requirements in Part A, section 3.1.2 of the Requirements for Biological Substances No. 18 (Requirements for Immune Sera of Animal Origin) \(^1\) shall apply to animals used for the production of sera intended for the preparation of antivenins.

3.2 Production precautions

The general production precautions as formulated in Part A, section 3 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Labora-

tories) shall apply to the manufacture of snake antivenins. In addition, care shall be taken in handling dry venoms to prevent the inhalation of aerosols.

3.3 Control of source material

3.3.1 Collection and storage of source material

The requirements in Part A, section 3.3.1 of Requirements for Biological Substances No. 18 (Requirements for Immune Sera of Animal Origin) shall apply.

The yield of plasma per animal can be significantly increased by the use of plasmaphoresis.

3.3.2 Potency test

It is advisable to verify that the source material contains an adequate amount of specific antibody (see Part A, section 3.5.5.2).

3.4 Control of bulk material

3.4.1 Purification process

If a purified bulk is prepared, then the requirements in Part A, section 3.4.1 of Requirements for Biological Substances No. 18 (Requirements for Immune Sera of Animal Origin) shall apply.

No particular purification process is recommended in these requirements. The national control authority should assess the suitability of a particular process for obtaining a potent and safe preparation.

3.4.2 Storage of bulk material

Bulk material shall be stored under conditions that prevent bacterial multiplication and minimize deterioration.

3.5 Preparation and control of final bulk

3.5.1 Preparation

The final bulk shall be prepared from either purified or unpurified bulk material by a method approved by the national control authority. It shall consist of material from one animal species only.

3.5.2 Preservatives, stabilizing agents and other added substances

The requirements in Part A, section 3.5.2 of Requirements for Biological Substances No. 18 (Requirements for Immune Sera of Animal Origin) shall apply.

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3.5.3 *Hydrogen ion concentration*

The requirements in Part A, section 3.5.3 of Requirements for Biological Substances No. 18 (Requirements for Immune Sera of Animal Origin)¹ shall apply.

3.5.4 *Sterility test*

The requirements in Part A, section 5 of Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances)² shall apply.

3.5.5 *Potency test*

3.5.5.1 *Reference venom*

The potency testing of antivenins shall be based on the use of reference venoms established or approved by the national control authority. Each reference venom shall be a homogeneous pool from snakes of a single species or single variety from the appropriate zoogeographic area.

After drying, venoms should be finely ground and blended to provide a homogeneous mixture.

Only venoms that meet all the requirements of Part A, section 3.1.1, shall be used for reference purposes.

Each batch of reference venom shall be identifiable by name and batch number.

Each batch of reference venom shall have its lethal toxicity for animals determined by a suitable method. The method used shall be one approved by the national control authority.

In determining lethal toxicity, due regard should be paid to factors such as the species of animals, their sex and weight, the route of injection, the rate of injection when the intravenous route is used, specific toxic signs, the interval to death, and the period of observation. The test should be made using sufficient venom dose levels and animals at each level to permit evaluation of a major part of the mortality curve (at least 20–80% mortality) and an estimation of the statistical variation.

It is desirable that each batch of reference material should also be characterized on the basis of its physical, chemical, and biological properties, including immunological activity (e.g., against a characterized antivenin preparation, see 3.5.5.3 below) and pharmacological characteristics. Such information would be of value when replacing a reference batch.

In some cases reference venom fractions may also be used for the assessment of the neutralizing effect of antivenins against

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specific activities of such fractions (fibrinolytic, haemolytic, haemorrhagic or neurotoxic). Such reference fractions should be prepared from a homogeneous pool of venom from snakes of a single species or variety and should meet the requirements of Part A, section 3.1.2. For each batch of reference venom fraction, the relevant activities should be determined.

3.5.5.2 Potency assay

Each final bulk shall be tested for its capacity to neutralize the lethal effect of each corresponding reference venom using an assay method approved by the national control authority.

In assays of the neutralization of lethal effects due regard should be paid to factors such as the species of animals, the incubation time of the venom/antivenin mixtures, the route of injection, the rate of injection when the intravenous route is used, the interval to death, and the period of observation. The test should be made using a sufficient number of antivenin dilutions and animals at each dilution to permit evaluation of a major part of the mortality curve (at least 20–80% mortality) and an estimation of the statistical variation.

In some cases, in determining the capacity to neutralize the lethal effect of venom it may be necessary to perform the titration of antivenin at two venom LD$_{50}$ levels.$^1$

It is desirable to record the signs of specific toxicity shown by the animals used in the test as a check on variations in quality batches of antivenins.

The potency of the antivenin under test shall be expressed in terms of the number of LD$_{50}$ and the equivalent weight in mg of reference venom neutralized by a specified quantity of the antivenin based on the protection of a stated proportion of animals (e.g., 50%). Such potency shall be expressed for each reference venom used.

If two venom levels are used then the potency should be expressed for each of these venom levels.

For those final bulks that in addition are tested for neutralizing effects against venom fractions, specific assays should be used designed to measure the desired activity (see section 3.5.5.1 above). The potency may be expressed for each reference venom fraction used in a similar way to that used in expressing the protective potency against lethal effect.

It is not possible to specify precise requirements for acceptable neutralizing capacity for every type of antivenin preparation. The potency of an antivenin preparation should be sufficiently high to make possible the administration to man of a minimal amount of the preparation to neutralize the maximal amount of venom from one snakebite.

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$^1$ In the case of some Naja venoms, 3 LD$_{50}$ and 21 LD$_{50}$ might be chosen. In this case, the 50% survival (ED$_{50}$) will indicate amounts of antivenin that would neutralize 2 LD$_{50}$ and 20 LD$_{50}$ respectively.
3.5.5.3 Tests by comparison with an antivenin preparation

A characterized antivenin preparation in a stable form may be used for the comparison of batches of antivenin preparations made by a particular manufacturing process or in relation to a particular zoogeographic area of distribution of certain snakes. It should preferably be freeze-dried and stored in the dark at 5°±3°C. Such an antivenin may be monovalent or polyvalent with known antibody content of sufficient activity to neutralize the reference venom, where necessary at the two LD₅₀ levels used in the potency test (see section 3.5.5.2 above).

The comparison made should be of the neutralizing effect of the antivenin under test with that of the characterized antivenin against the same venom or venoms, and preferably against relevant reference venoms used for the potency determination of antivenin preparations.

3.5.6 Additional tests on final bulk

If the final bulk is to be sent to another institute for final processing and filling, it shall be tested for abnormal toxicity, pyrogenicity, and total solids and protein content, as given in Part A, sections 5.3, 5.5 and 5.6, respectively of Requirements for Biological Substances No. 18 (Requirements for Immune Sera of Animal Origin),¹ and the results shall accompany the final bulk to the other institute.

4. Filling and containers

The requirements 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.

5. Control tests on filling lot

The requirements given in the sub-sections of Part A, section 5, of the Requirements for Biological Substances No. 18 (Requirements for Immune Sera of Animal Origin)³ shall apply, with the exception that the potency test shall be made as described in Part A, section 3.5.5.2, of these requirements on at least one filling lot derived from an antivenin lot.

In the case of a dried product it is advisable that a potency test should be performed on each filling lot.

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)² shall apply, with the addition of the following:

The label on the container shall show:

- the animal source of the preparation if the international name of the preparation is not used;
- the volume of fluid content or, for dried preparations, the nature and volume of the solvent to be used for reconstitution;

The label on the package may show and the leaflet accompanying the package shall show:

- the potency of the antivenin (see Part A, section 3.5.5.2);
- the nature of the preparation, i.e., natural serum or purified immunoglobulin or treated immunoglobulin and, if purified or treated, the nature of the process;
- the method of reconstitution, if dried, and the solvent to be used if none is included in the package;
- if the use of the antivenin is restricted to a particular country or area (see Part A, section 3.1.1), the name of such country or area.

The leaflet accompanying the package shall include:

- any additional details concerning the potency of the antivenin that may be relevant to its use;

the identity of each reference venom against which the potency of the antivenin preparation has been expressed;
further information to guide the clinician, i.e., indications for giving antivenins;
recommended human dose according to certain factors, such as clinical manifestations, the time elapsed from the bite, route of administration, adverse reactions;
a list of species of snakes for which cross-protection may be expected;
the main precautions to be employed in administering antivenin.¹

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)² 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

Antivenins shall be stored in the dark at a temperature of 5⁰ ± 3⁰C.

10.2 Expiry date

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

It is usual for expiry dates of 3–5 years, or even more, to be assigned to many antivenin products, subject to a satisfactory demonstration to the national control authority of stability of the product.

¹ These should include precautions against untoward allergic reactions, particularly if there is any history of allergies from previous serum injections.
Part B. National Control Requirements

1. General

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

In addition, the national control authority shall specify the requirements to be fulfilled for the method of preparation of the bulk material and final bulk (Part A, sections 3.4.1 and 3.5.1) and for potency testing (Part A, sections 3.5.5.2 and 5) and shall provide each national reference venom, or shall give directions to manufacturers concerning each appropriate reference venom, for the potency testing of antivenins (Part A, sections 1.3 and 3.5.5.1).

The national control authority shall take into account all available information on the distribution of the various species of snake and the antigenic differences of venoms within species in the relevant zoogeographic areas, so as to ensure that the antivenins used for treatment of snakebite are appropriate to the particular venoms encountered.

When antivenins are prepared in one country for use in another, the national control authority of the country in which the product is to be used shall ensure that potency testing has been done with reference venoms appropriate to the zoogeographic areas in which the antivenins are to be used.

2. Release and Certification

Preparations of antivenins shall be released only if they fulfil Part A of the present requirements.

A statement signed by the appropriate official in charge of the national control laboratory shall be provided at the request of the manufacturing establishment and shall certify whether or not the lot of antivenin in question meets all national requirements as well as Part A of the present requirements. The certificate shall also state the name and identify of each reference venom against which a satisfactory potency test has been performed, the expiry date of the product, 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 antivenins between countries.

3. Efficacy and safety of antivenins in the field

Since there is little information on the efficacy and safety of antivenins in the field it would be valuable if the appropriate health authorities could establish a record-keeping system that would provide for a central compilation of pertinent records on snakebite cases in the country. For each case treated, records should be made of, for example, the identity and description of the snake; length of time prior to receiving antivenin; treatment prior to receiving antivenin; condition of the patient at the time of receiving antivenin, including site of bite; details of the administration of antivenin; description of product used, including manufacturer and lot number; dosage given and route of administration; reactions to the antivenin; details of other treatment; details of patient's progress during and following treatment; and final outcome.
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:

No.  Year  Requirements for Biological Substances:
178  1959  1. General Requirements for Manufacturing Establishments and Control Laboratories
        2. Requirements for Poliomyelitis Vaccine (Inactivated)
179  1959  3. Requirements for Yellow Fever Vaccine
        4. Requirements for Cholera Vaccine
180  1959  5. Requirements for Smallpox Vaccine
200  1960  6. General Requirements for the Sterility of Biological Substances
237  1962  7. Requirements for Poliomyelitis Vaccine (Oral)
274  1964  8. Requirements for Pertussis Vaccine
        9. Requirements for Procaine Benzylpenicillin in Oil with Aluminium Monostearate
293  1964  10. Requirements for Diphtheria Toxoid and Tetanus Toxoid
323  1966  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

        — 45 —
WHO Expert Committee on Biological Standardization:
11. Requirements for Dried BCG Vaccine
12. Requirements for Measles Vaccine (Live) and Measles Vaccine (Inactivated)

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)

WHO Expert Committee on Biological Standardization:
16. Requirements for Tuberculin
17. Requirements for Inactivated Influenza Vaccine

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

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)

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

WHO Expert Committee on Biological Standardization:
21. Requirements for Snake Antivenins
Annex 3

INTERNATIONAL BIOLOGICAL STANDARDS
AND
INTERNATIONAL BIOLOGICAL REFERENCE PREPARATIONS
1971

The primary purpose underlying the establishment of International Biological Standards and International Biological Reference Preparations is to provide a means of ensuring uniformity throughout the world in the designation of potency of preparations that are used in the prophylaxis, therapy, or diagnosis of human and animal disease, and that cannot be characterized adequately by chemical and physical means. A secondary purpose is the facilitation of work out of which clinical application may arise. International Units have been defined, wherever possible, to enable the potency of biological preparations to be expressed in these units or their equivalents. The World Health Assembly has recommended that Member States of the Organization give official recognition to existing international standards and units.

The International Laboratories for Biological Standards at the Statens Serum Institut, Copenhagen, Denmark, at the National Institute for Medical Research, London, England, and at the Central Veterinary Laboratory, Weybridge, Surrey, England, are custodians of International Biological Standards and International Biological Reference Preparations; they distribute samples of these preparations, free of charge, to national control laboratories for biological products. Such samples are intended for use in laboratory assays only and must not be administered to humans unless by special authorization.

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### International Laboratory for Biological Standards, Statens

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<th>Substance</th>
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<th>Form in which dispensed</th>
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<tr>
<td>Old tuberculin (OT)</td>
<td>0.011111 µl</td>
<td>Ampoules containing 2 ml of old tuberculin (90 000 International Units (IU) per ml)</td>
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<td>Purified protein derivative of mammalian tuberculin</td>
<td>0.000028</td>
<td>Ampoules containing 10 mg of PPD plus 4 mg of salts (500 000 IU per ampoule)</td>
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<td>Purified protein derivative of avian tuberculin</td>
<td>0.0000726</td>
<td>Ampoules containing 10 mg of PPD plus 26.3 mg of salts (500 000 IU per ampoule)</td>
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<td>Tetanus toxoid</td>
<td>0.03</td>
<td>Ampoules containing 25 mg of alcohol-purified tetanus toxoid plain plus glycine (833 IU per ampoule)</td>
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<td>Tetanus toxoid, adsorbed</td>
<td>0.6667</td>
<td>Ampoules containing 80 mg of tetanus toxoid adsorbed to aluminium hydroxide, plus an equal part of guinea-pig serum dried (120 IU per ampoule)</td>
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<td>Diphtheria toxoid, plain</td>
<td>0.50</td>
<td>Ampoules containing 50 mg of alcohol-purified diphtheria toxoid plus glycine (100 IU per ampoule)</td>
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<td>Diphtheria toxoid, adsorbed</td>
<td>0.75</td>
<td>Ampoules containing 80 mg of diphtheria toxoid adsorbed to aluminium hydroxide, plus an equal part of guinea-pig serum dried (107 IU per ampoule)</td>
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<td>Schick test toxin (diphtheria)</td>
<td>0.0042</td>
<td>Ampoules containing 0.005 mg of purified diphtheria toxin plus 1 mg of bovine albumin and 2.74 mg of phosphate buffer salts (900 IU per ampoule)</td>
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<td>Pertussis vaccine</td>
<td>1.5</td>
<td>Ampoules containing 52 mg of dried vaccine (34.7 IU per ampoule)</td>
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<td>Ampoules containing 20 mg of dried vaccine (1.6 x 10⁹ organisms per ampoule)</td>
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<td>Cholera vaccine (Ogawa)</td>
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<td>Ampoules containing 20 mg of dried vaccine (1.6 x 10⁹ organisms per ampoule)</td>
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<td>Cardiolipin</td>
<td>—</td>
<td>Ampoules containing 4 ml, 8 ml or 16 ml of a solution of purified cardiolipin in ethanol (6.0 mg of cardiolipin per ml, as calculated from the phosphorus content)</td>
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<td>Lecithin (beef heart)</td>
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<td>Bottles containing 30 ml of a solution of purified beef-heart lecithin in ethanol (30.3 mg of lecithin per ml)</td>
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<td>Lecithin (egg)</td>
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<td>Ampoules containing 4 ml or 16 ml of a solution of purified egg lecithin in ethanol (29.60 mg of lecithin per ml as calculated from the dry weight estimate)</td>
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<td>Rabies vaccine</td>
<td>—</td>
<td>Ampoules containing 121 mg of a freeze-dried suspension of rabbit brain infected with fixed rabies virus and inactivated by ultraviolet irradiation</td>
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<td>Smallpox vaccine</td>
<td>—</td>
<td>Ampoules containing 14 mg of freeze-dried smallpox vaccine</td>
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<td>Typhoid vaccine (acetone-</td>
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<td>Ampoules containing 11 mg of dried vaccine</td>
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<td>Typhoid vaccine (heat-phenol-</td>
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<td>Ampoules containing 34 mg of freeze-dried vaccine</td>
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<td>inactivated)</td>
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<td>Poliomyelitis vaccine (inactivated)</td>
<td>—</td>
<td>Ampoules containing 10 ml of trivalent inactivated poliomyelitis vaccine, frozen</td>
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<tr>
<td>BCG vaccine</td>
<td>—</td>
<td>Ampoules containing dried BCG vaccine derived from 2.5 mg (semi-dry weight) of bacillary mass of BCG and 5 mg of sodium glutamate (total weight of dried material 5.72 mg per ampoule).</td>
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<tr>
<td>Influenza virus haemagglutinin</td>
<td>0.093661</td>
<td>Ampoules containing 18.7322 mg of freeze-dried, purified influenza virus (Type A strain Singapore 1/57 30338), propagated in embryonated hens' eggs, killed by formalin and suspended in phosphate-buffered saline with 1% bovine serum albumin (200 IU per ampoule).</td>
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### ANTIGENS II

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<td>Swine erysipelas vaccine</td>
<td>0.50</td>
<td>Ampoules containing 499 mg of dried vaccine, derived from formaldehyde-treated <em>Erysipelothrix rhusiopathiae</em> type B, adsorbed to aluminium hydroxide (1000 IU per ampoule)</td>
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<td>Newcastle disease vaccine (inactive)</td>
<td>1.0</td>
<td>Ampoules containing 525 mg of freeze-dried vaccine derived from formaldehyde-treated allantoic fluid of eggs infected with strains of Newcastle disease virus adsorbed to aluminium hydroxide (525 IU per ampoule)</td>
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<td>Newcastle disease vaccine (live)</td>
<td>—</td>
<td>Ampoules containing 109.5 mg of freeze-dried allantoic fluid derived from eggs infected with the virus (Hitchner B1 strain).</td>
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<td><em>Clostridium oedematiens</em> (alpha) toxoid</td>
<td>—</td>
<td>Ampoules containing 53.4 mg of freeze-dried toxoid</td>
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### ANTIBODIES I

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<td>Tetanus antitoxin</td>
<td>0.03384</td>
<td>Ampoules containing 47 mg of freeze-dried hyperimmune horse serum (1400 IU per ampoule)</td>
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<td>Diphtheria antitoxin</td>
<td>0.0628</td>
<td>Bottles containing 10 ml of a solution of dried hyperimmune horse serum in saline containing 60% v/v of glycerol (10 IU per ml)</td>
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<td>Anti-dysentery serum (Shiga)</td>
<td>0.05</td>
<td>Bottles containing 10 ml of a solution of dried hyperimmune horse serum in saline containing 60% v/v of glycerol (200 IU per ml)</td>
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<tr>
<td>Gas-gangrene antitoxin (perfringens) (<em>Clostridium welchii</em> type A antitoxin)</td>
<td>0.3346</td>
<td>Bottles containing 90.35 mg of dried hyperimmune horse serum (270 IU per ampoule)</td>
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## ANTIGENS II

**Central Veterinary Laboratory, Weybridge, Surrey, England**

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## ANTIBODIES I

**Seruminstitut, Amager Boulevard, 80, Copenhagen, Denmark**

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<td>2nd Standard 1935 (0.2660 mg)</td>
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</tr>
<tr>
<td>3rd Standard 1943 (0.3477 mg)</td>
<td></td>
</tr>
<tr>
<td>4th Standard 1953 (0.1132 mg)</td>
<td></td>
</tr>
<tr>
<td>5th Standard 1963</td>
<td></td>
</tr>
<tr>
<td>Substance</td>
<td>International Unit of present standard (μg)</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Gas-gangrene antitoxin (<em>vibrio septique</em>)</td>
<td>0.118</td>
</tr>
<tr>
<td>Gas-gangrene antitoxin (oedematiens)</td>
<td>0.0828</td>
</tr>
<tr>
<td>Gas-gangrene antitoxin (histolyticus)</td>
<td>0.2</td>
</tr>
<tr>
<td>Gas-gangrene antitoxin (Sordelli)</td>
<td>0.1334</td>
</tr>
<tr>
<td>Staphylococcus α antitoxin</td>
<td>0.2376</td>
</tr>
<tr>
<td>Scarlet fever streptococcus antitoxin</td>
<td>0.049</td>
</tr>
<tr>
<td>Anti-streptolysin O</td>
<td>0.0213</td>
</tr>
<tr>
<td>Anti-pneumococcus serum (type 1)</td>
<td>0.0886</td>
</tr>
<tr>
<td>Anti-pneumococcus serum (type 2)</td>
<td>0.0894</td>
</tr>
<tr>
<td>Anti-Q-fever serum</td>
<td>0.1017</td>
</tr>
<tr>
<td>Years of establishment (in brackets, unitage of previous standards)</td>
<td>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1st Standard 1934 (0.2681 mg)</td>
<td><em>Bull. Hlth Org. L. o. N.</em>, 1938, 7, 698, 807; 1939, 8, 856; 1945/46, 12, 21</td>
</tr>
<tr>
<td>2nd Standard 1952 (0.1135 mg)</td>
<td><em>Bull. Hlth Org. L. o. N.</em>, 1935, 4, 6, 68, 514; 1938, 7, 702, 845; 1945/46, 12, 32</td>
</tr>
<tr>
<td>2nd Standard 1951 (0.5000 mg)</td>
<td><em>Bull. Hlth Org. L. o. N.</em>, 1935, 4, 4, 48, 512</td>
</tr>
<tr>
<td>Substance</td>
<td>International Unit of present standard (IU)</td>
</tr>
<tr>
<td>----------------------------------------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Anti-rabies serum</td>
<td>1.0</td>
</tr>
<tr>
<td>Anti-A blood-typing serum</td>
<td>0.3465</td>
</tr>
<tr>
<td>Anti-B blood-typing serum</td>
<td>0.3520</td>
</tr>
<tr>
<td>Anti-Rh(b) (anti-D) incomplete blood-typing serum</td>
<td>0.95</td>
</tr>
<tr>
<td>Syphilitic human serum</td>
<td>3.617</td>
</tr>
<tr>
<td>Anti-poliovirus serum (type 1)</td>
<td>10.78</td>
</tr>
<tr>
<td>Anti-poliovirus serum (type 2)</td>
<td>10.46</td>
</tr>
<tr>
<td>Anti-poliovirus serum (type 3)</td>
<td>10.48</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em> Type A antitoxin</td>
<td>0.1360</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em> Type B antitoxin</td>
<td>0.1740</td>
</tr>
</tbody>
</table>
## TWENTY-THIRD REPORT

### ANTIBODIES I (contd)

**Seruminstitut, Amager Boulevard, 80, Copenhagen, Denmark**

<table>
<thead>
<tr>
<th>Years of establishment (in brackets, uniasco of previous standards)</th>
<th>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</th>
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<tbody>
<tr>
<td>Substance</td>
<td>International Unit of present standard (μg)</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em> Type C antitoxin</td>
<td>0.0800</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em> Type D antitoxin</td>
<td>0.0121</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em> Type E antitoxin</td>
<td>0.0691</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em> Type F antitoxin</td>
<td>7.44</td>
</tr>
<tr>
<td><em>Naja</em> antivenin</td>
<td>2.69</td>
</tr>
<tr>
<td>Anti-smallpox serum</td>
<td>0.08416</td>
</tr>
<tr>
<td>Anti-toxoplasma serum</td>
<td>0.09067</td>
</tr>
<tr>
<td>Diphtheria antitoxin for flocculation test</td>
<td>—</td>
</tr>
<tr>
<td>Anti-typhoid serum</td>
<td>—</td>
</tr>
<tr>
<td>Anti-yellow-fever serum</td>
<td>0.5</td>
</tr>
<tr>
<td>Years of establishment (in brackets, unitage of previous standards)</td>
<td>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</td>
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### ANTIBODIES I (contd)  
**Held and**  
**International Laboratory for Biological Standards, Statens**

<table>
<thead>
<tr>
<th>Substance</th>
<th>International Unit of present standard (μg)</th>
<th>Form in which dispensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-measles serum</td>
<td>9.378</td>
<td>Ampoules containing 93.8 mg of dried human serum (10 IU per ampoule)</td>
</tr>
<tr>
<td>Anti-staphylococcal P-V leucocidin serum</td>
<td>0.3565</td>
<td>Ampoules containing 53.5 mg of freeze-dried horse serum (150 IU per ampoule)</td>
</tr>
<tr>
<td>Rheumatoid arthritis serum</td>
<td>0.171</td>
<td>Ampoules containing 17.1 mg of freeze-dried pooled human serum (100 IU per ampoule)</td>
</tr>
<tr>
<td>Anti-rubella serum</td>
<td>0.14595</td>
<td>Ampoules containing 145.95 mg of freeze-dried human normal immunoglobulin (1000 IU per ampoule)</td>
</tr>
<tr>
<td>Human serum immunoglobulins</td>
<td></td>
<td>Ampoules containing 81.47 mg of the freeze-dried residue from diluted pooled human serum (100 IgG IU, 100 IgA IU and 100 IgM IU per ampoule)</td>
</tr>
<tr>
<td>IgG, IgA and IgM&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.8147</td>
<td><strong>Anti-nuclear-factor serum</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>(homogeneous)</td>
<td>0.186</td>
<td>Ampoules containing 18.6 mg of the freeze-dried residue of 0.2 ml of pooled human serum (100 IU per ampoule)</td>
</tr>
</tbody>
</table>

### ANTIBODIES II  
**Held and**  
**International Laboratory for Biological Standards,**

<table>
<thead>
<tr>
<th>Substance</th>
<th>International Unit of present standard (μg)</th>
<th>Form in which dispensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Brucella abortus serum</td>
<td>0.09552</td>
<td>Ampoules containing 95.52 mg of freeze-dried bovine serum (1000 IU per ampoule)</td>
</tr>
<tr>
<td><em>Clostridium welchii</em> (perfringens) type B antitoxin</td>
<td>0.0137</td>
<td>Ampoules containing 68.5 mg of dried hyper-immune horse serum (5000 IU per ampoule)</td>
</tr>
</tbody>
</table>

<sup>1</sup> A preparation from the same batch of material as this international reference preparation is available from The Director, Division of Biological Standards, National Institute of Medical Research, Mill Hill, London N.W.7, England. See also information on availability of material in *Bull. Wld Hlth Org.*, 1970, 42, 535, Annex 1.
### Antibodies I (contd)

**Serum Institute, Amager Boulevard, 80, Copenhagen, Denmark**

<table>
<thead>
<tr>
<th>Years of Establishment (in brackets, unitage of previous standards)</th>
<th>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</th>
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### Antibodies II

**Central Veterinary Laboratory, Weybridge, Surrey, England**

<table>
<thead>
<tr>
<th>Years of Establishment</th>
<th>References</th>
</tr>
</thead>
</table>

\(^2\) Serum from the same batch of material as this international reference preparation is available from the Director, Division of Biological Standards, National Institute of Medical Research, Mill Hill, London N.W.7, England.
### ANTIBODIES II (contd)

**International Laboratory for Biological Standards,**

<table>
<thead>
<tr>
<th>Substance</th>
<th>International Unit of present standard (mg)</th>
<th>Form in which dispensed</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium welchii</em> (perfringens) type D antitoxin</td>
<td>0.0657</td>
<td>Ampoules containing 65.7 mg of dried hyper-immune horse serum (1000 IU per ampoule)</td>
</tr>
<tr>
<td>Swine erysipelas serum (anti-N)</td>
<td>0.14</td>
<td>Ampoules containing 87.9 mg of dried hyper-immune horse serum (628 IU per ampoule)</td>
</tr>
<tr>
<td>Anti-swine-fever serum</td>
<td>0.89</td>
<td>Ampoules containing 889.5 mg of freeze-dried pig serum (1000 IU per ampoule)</td>
</tr>
<tr>
<td>Anti-canine-distemper serum</td>
<td>0.0897</td>
<td>Ampoules containing 89.7 mg of freeze-dried hyperimmune horse serum (1000 IU per ampoule)</td>
</tr>
<tr>
<td>Anti-canine-hepatitis serum</td>
<td>0.0796</td>
<td>Ampoules containing 79.6 mg of freeze-dried hyperimmune horse serum (1000 IU per ampoule)</td>
</tr>
<tr>
<td>Anti-Newcastle-disease serum</td>
<td>0.1734</td>
<td>Ampoules containing 55.5 mg of freeze-dried chicken serum (320 IU per ampoule)</td>
</tr>
<tr>
<td>Anti-<em>Mycoplasma gallisepticum</em> serum</td>
<td>0.0556</td>
<td>Ampoules containing 55.6 mg of freeze-dried chicken serum (1000 IU per ampoule)</td>
</tr>
</tbody>
</table>

### ANTIBIOTICS I

**International Laboratory for Biological Standards, National**

<table>
<thead>
<tr>
<th>Substance</th>
<th>International Unit of present standard (mg)</th>
<th>Form in which dispensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin</td>
<td>0.001282</td>
<td>Ampoules containing 175 mg of streptomycin sulfate (780 IU per mg)</td>
</tr>
<tr>
<td>Dihydrostreptomycin</td>
<td>0.001219</td>
<td>Ampoules containing 200 mg of dihydro-streptomycin sulfate (820 IU per mg)</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>0.01351</td>
<td>Ampoules containing 100 mg of zinc bacitracin (74 IU per mg)</td>
</tr>
<tr>
<td>Years of establishment (in brackets, unitage of previous standards)</td>
<td>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------------</td>
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distributed by

ANTIBIOTICS I

Institute for Medical Research, Mill Hill, London, N.W.7, England

<table>
<thead>
<tr>
<th>Years of establishment (in brackets)</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>2nd Standard 1958</strong></td>
<td></td>
</tr>
<tr>
<td><strong>2nd Standard 1966</strong></td>
<td></td>
</tr>
<tr>
<td><strong>2nd Standard 1964</strong></td>
<td></td>
</tr>
<tr>
<td>Substance</td>
<td>International Unit of present standard (mg)</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.00101833</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>0.001</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>0.0011364</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.001053</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>0.000119</td>
</tr>
<tr>
<td>Nystatin</td>
<td>0.000333</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.001064</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.000993</td>
</tr>
<tr>
<td>Oleandomycin</td>
<td>0.001176</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>0.001031</td>
</tr>
<tr>
<td>Colistin</td>
<td>0.00004878</td>
</tr>
<tr>
<td>Rolitetracycline</td>
<td>0.001004</td>
</tr>
<tr>
<td>Years of establishment (in brackets, unitage of previous standards)</td>
<td>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Substance</td>
<td>International Unit of present standard (mg)</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>0.001232</td>
</tr>
<tr>
<td>Kanamycin B</td>
<td></td>
</tr>
<tr>
<td>Viomycin</td>
<td>0.0012285</td>
</tr>
<tr>
<td>Neomycin</td>
<td>0.00147</td>
</tr>
<tr>
<td>Neomycin B</td>
<td>0.001492</td>
</tr>
<tr>
<td>Ristocetin</td>
<td></td>
</tr>
<tr>
<td>Ristocetin B</td>
<td></td>
</tr>
<tr>
<td>Gramicidin S</td>
<td>0.001002</td>
</tr>
<tr>
<td>Gramicidin</td>
<td>0.001</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>0.0003125</td>
</tr>
<tr>
<td>Demethylchlortetracycline</td>
<td>0.001</td>
</tr>
<tr>
<td>Triacetyloleandomycin</td>
<td>0.0012</td>
</tr>
<tr>
<td>Procaine benzylpenicillin in oil with aluminium monostearate</td>
<td></td>
</tr>
</tbody>
</table>

1 The International Nonproprietary Name of this substance is bekamycin (see *WHO Chronicle*, 1970, 24, 415).
distributed by Institute for Medical Research, Mill Hill, London, N.W.7, England

<table>
<thead>
<tr>
<th>Years of establishment (in brackets, unitage of previous standards)</th>
<th>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</th>
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<tbody>
<tr>
<td>2nd Reference Preparation 1969</td>
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* The International Nonproprietary Name of this substance has been changed to troleandomycin (see Amendment to page 94 of Cumulative List No. 2, 1967; *WHO Chronicle*, 1970, 24, 435).
<table>
<thead>
<tr>
<th>Substance</th>
<th>International Unit of present standard (µg)</th>
<th>Form in which dispensed</th>
</tr>
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<tbody>
<tr>
<td>Paromomycin</td>
<td>0.001333</td>
<td>Ampoules containing 75 mg of paromomycin sulfate (750 IU per mg)</td>
</tr>
<tr>
<td>Colistin methane sulfonate</td>
<td>0.00007874</td>
<td>Ampoules containing 75 mg of colistin methane sulfonate (12 700 IU per mg)</td>
</tr>
<tr>
<td>Cefalotin</td>
<td>0.0010661</td>
<td>Ampoules containing 50 mg of sodium cefalotin (938 IU per mg)</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>0.0011351</td>
<td>Ampoules containing 50 mg of lincomycin hydrochloride (881 IU per mg)</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>0.001087</td>
<td>Ampoules containing 80 mg of capreomycin sulfate (920 IU per mg)</td>
</tr>
<tr>
<td>Rifamycin SV</td>
<td>0.001127</td>
<td>Ampoules containing 100 mg of sodium rifamycin SV (887 IU per mg)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>0.00156</td>
<td>Ampoules containing 50 mg of gentamycin sulfate (641 IU per mg)</td>
</tr>
<tr>
<td>Lymecycline</td>
<td>0.001107</td>
<td>Ampoules containing 50 mg of lymecycline base (903 IU per mg)</td>
</tr>
<tr>
<td>Methacycline</td>
<td>0.001082</td>
<td>Ampoules containing 50 mg of methacycline hydrochloride (924 IU per mg)</td>
</tr>
</tbody>
</table>

**ANTIBIOTICS II**

<table>
<thead>
<tr>
<th>Substance</th>
<th>International Unit of present standard (µg)</th>
<th>Form in which dispensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tylosin</td>
<td>0.001</td>
<td>Ampoules containing 40 mg of tylosin base (1000 IU per mg)</td>
</tr>
<tr>
<td>Hygromycin B</td>
<td>0.0008928</td>
<td>Ampoules containing 40 mg of hygromycin B (1120 IU per mg)</td>
</tr>
<tr>
<td>Nisin</td>
<td>0.001</td>
<td>Ampoules containing 85 mg of nisin (1000 IU per mg)</td>
</tr>
</tbody>
</table>

1 In some countries this antibiotic is known as colistin sulphonate or colistimethate.

2 The International Nonproprietary Name of this substance is rifamycin (see Cumulative List No. 2, 1967, p. 54; *WHO Chronicle*, 1963, 17, 397).
### ANTIBIOTICS I (contd)

**Institute for Medical Research, Mill Hill, London, N.W.7, England**

<table>
<thead>
<tr>
<th>Years of establishment (in brackets, unitage of previous standards)</th>
<th>References (WHO/BS refers to unpublished working documents of the WHO Expert Committee on Biological Standardization)</th>
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</thead>
</table>

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### ANTIBIOTICS II

**Central Veterinary Laboratory, Weybridge, Surrey, England**


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*The International Nonproprietary Name of this substance has been changed to gentamicin (see Corrigendum to page 45 of Cumulative List No. 2, 1967, *WHO Chronicle*, 1969, 23, 446).*

*The International Nonproprietary Name of this substance is metacycline (see Cumulative List No. 2, 1967, p. 58; *WHO Chronicle*, 1962, 16, 390).*
<table>
<thead>
<tr>
<th>Substance</th>
<th>International Unit of present standard (mg)</th>
<th>Form in which dispensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytocin and vasopressin (antidiuretic hormone), bovine, for bioassay</td>
<td>0.5</td>
<td>Ampoules containing 30 mg of acetone-dried powder of whole posterior pituitary gland of the ox (2 oxytocic IU, 2 vasopressor IU, and 2 antidiuretic IU per mg)</td>
</tr>
<tr>
<td>Prolactin, ovine, for bioassay</td>
<td>0.04545</td>
<td>Ampoules containing 10 mg of freeze-dried purified prolactin from anterior pituitary gland of the sheep (22 IU per mg)</td>
</tr>
<tr>
<td>Corticotrophin, porcine, for bioassay</td>
<td>1.0</td>
<td>Ampoules containing 5 mg of purified corticotrophin from anterior pituitary gland of the pig with lactose, freeze-dried (1 IU per mg)</td>
</tr>
<tr>
<td>Thyrotrophin, bovine, for bioassay</td>
<td>13.5</td>
<td>Ampoules containing ten 20-mg tablets of a blend of 1 part of purified thyrotrophin from anterior pituitary gland of the ox and 19 parts of lactose (approximately 1.48 IU per tablet)</td>
</tr>
<tr>
<td>Growth hormone, bovine, for bioassay</td>
<td>1.0</td>
<td>Ampoules containing 30 mg of dried growth hormone from anterior pituitary gland of the ox (1 IU per mg)</td>
</tr>
<tr>
<td>Growth hormone, human, for immunoassay</td>
<td>24.29</td>
<td>Ampoules containing 8.5 mg of purified growth hormone from human anterior pituitary gland with sucrose and buffer salts, freeze-dried (0.350 IU per ampoule)</td>
</tr>
<tr>
<td>Human menopausal gonadotrophins (follicle stimulating hormone and interstitial cell stimulating hormone), urinary, for bioassay</td>
<td>0.2295</td>
<td>Ampoules containing 9 mg of active principle from urine of post-menopausal women with lactose, freeze-dried (40 follicle stimulating hormone IU and 40 interstitial cell stimulating hormone IU per ampoule)</td>
</tr>
<tr>
<td>Serum gonadotrophin, equine, for bioassay</td>
<td>0.003569</td>
<td>Ampoules containing 5.71 mg of active material from the serum of pregnant mares with lactose, freeze-dried (1600 IU per ampoule)</td>
</tr>
<tr>
<td>Years of establishment (in brackets, unitage of previous standards)</td>
<td>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
</tbody>
</table>
| 1st Standard 1925 (0.5 mg)  
2nd Standard 1942 (0.5 mg)  
| 1st Standard 1939 (0.1 mg)  
| 1st Standard 1950 (1.00 mg)  
2nd Standard 1955 (0.88 mg)  
| 1st Reference Preparation 1959  
| 1st Standard 1939 (0.25 mg)  
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<tr>
<th>Substance</th>
<th>International Unit of present standard (IU)</th>
<th>Form in which dispensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chorionic gonadotrophin, human, for bioassay</td>
<td>0.001279</td>
<td>Ampoules containing 7 mg of active principle from human urine of pregnancy, with lactose, freeze-dried (5300 IU per ampoule)</td>
</tr>
<tr>
<td>Insulin, bovine and porcine, for bioassay</td>
<td>0.04167</td>
<td>Ampoules containing 110-125 mg of recrystallized insulin, 52% from bovine and 48% from porcine pancreas (24 IU per mg)</td>
</tr>
<tr>
<td>Erythropoietin, human, urinary, for bioassay</td>
<td>0.50</td>
<td>Ampoules containing 2 mg of the freeze-dried residue from an extract of human urine with 3 mg of sodium chloride (10 IU ampoule)</td>
</tr>
<tr>
<td>Heparin</td>
<td>0.0077</td>
<td>Ampoules containing 20 mg of sodium salt of purified active principle from bovine lung tissue (130 IU per mg)</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>0.000025</td>
<td>Bottles containing 6 g of a solution of vitamin D₃ in vegetable oil (1000 IU per g)</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>−</td>
<td>Ampoules containing ten 20-mg tablets of vitamin B₁₂ with sodium chloride (approximately 9.3 μg of cyanocobalamin per tablet)</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>0.1</td>
<td>Ampoules containing ten 20-mg tablets of dried bovine testicular hyaluronidase diluted with lactose (approximately 200 IU per tablet)</td>
</tr>
<tr>
<td>Streptokinase-streptodornase</td>
<td></td>
<td>Ampoules containing 1 mg of active material with 5.5 mg of lactose, freeze-dried (3100 streptokinase IU and 2400 streptodornase 1U per ampoule)</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>0.002090</td>
<td>Ampoules containing 37.35 mg of the freeze-dried residue from a concentrate of human blood coagulation factor VIII (2.6 IU per ampoule)</td>
</tr>
<tr>
<td>Streptodornase</td>
<td>0.002700</td>
<td>Ampoules containing 37.35 mg of the freeze-dried residue from a concentrate of human blood coagulation factor VIII (2.6 IU per ampoule)</td>
</tr>
<tr>
<td>Blood coagulation factor VIII</td>
<td>14.365</td>
<td>Ampoules containing 37.35 mg of the freeze-dried residue from a concentrate of human blood coagulation factor VIII (2.6 IU per ampoule)</td>
</tr>
</tbody>
</table>
# Reference

<table>
<thead>
<tr>
<th>Years of establishment (in brackets, unitage of previous standards)</th>
<th>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</th>
</tr>
</thead>
</table>
| **1st Standard 1939 (0.1 mg)**  
| **1st Standard 1925 (0.12500 mg)**  
2nd Standard 1935 (0.04550 mg)  
3rd Standard 1952 (0.04082 mg)  
| **1st Reference Preparation 1965**  
(1.45 mg)  
| **1st Standard 1942 (0.0077 mg)**  
| **1st Standard 1931 (0.1 mg)**  
[Irradiated ergosterol]  
### HORMONES, VITAMINS AND ENZYMES (contd)

**International Laboratory for Biological Standards, National**

<table>
<thead>
<tr>
<th>Substance</th>
<th>International Unit of present standard (mg)</th>
<th>Form in which dispensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urokinase</td>
<td>0.001410</td>
<td>Ampoules containing 6.77 mg of purified urokinase extracted from human urine, with lactose, freeze-dried (4800 IU per ampoule)</td>
</tr>
</tbody>
</table>

### MISCELLANEOUS I

**International Laboratory for Biological Standards, National**

<table>
<thead>
<tr>
<th>Substance</th>
<th>InternatInternational Unit of present standard (mg)</th>
<th>Form in which dispensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digitalis</td>
<td>76.9</td>
<td>Ampoules containing 2500 mg of dry powdered leaves of <em>Digitalis purpurea</em> (0.01316 IU per mg)</td>
</tr>
<tr>
<td>Neoarsphenamine</td>
<td>-</td>
<td>Ampoules containing 300 mg of neoarsphenamine</td>
</tr>
<tr>
<td>Sulfarsphenamine</td>
<td>-</td>
<td>Ampoules containing 300 mg of sulfarsphenamine</td>
</tr>
<tr>
<td>Oxophenarsine</td>
<td>-</td>
<td>Sets of three ampoules containing (a) 120 mg of oxophenarsine hydrochloride, (b) 100 mg of anhydrous sodium carbonate, and (c) 500 mg of anhydrous sucrose</td>
</tr>
<tr>
<td>Mel B</td>
<td>-</td>
<td>Ampoules containing 100 mg of melaminyl-4-phenylarsenodithioglycerol</td>
</tr>
<tr>
<td>MSb</td>
<td>-</td>
<td>Ampoules containing 500 mg of sodium p-melaminylphenylstibonate polymer</td>
</tr>
<tr>
<td>Dimercaprol</td>
<td>-</td>
<td>Ampoules containing 2 ml of 2,3-dimercapto propane</td>
</tr>
<tr>
<td>Protamine</td>
<td>-</td>
<td>Ampoules containing 60 mg of protamine of piscine origin</td>
</tr>
<tr>
<td>Pyrogen</td>
<td>-</td>
<td>Ampoules containing 2 mg of purified 'O' somatic antigen of <em>Shigella dysenteriae</em>, freeze-dried</td>
</tr>
<tr>
<td>Years of establishment (in brackets, unitage of previous standards)</td>
<td>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

**Institute for Medical Research, Mill Hill, London, N.W.7, England**

<table>
<thead>
<tr>
<th>1st Standard 1926 (100.0 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd Standard 1936 (80.0 mg)</td>
</tr>
<tr>
<td>3rd Standard 1949</td>
</tr>
</tbody>
</table>

| 1st Reference Preparation 1925 |
| 2nd Reference Preparation 1935 |
| 3rd Reference Preparation 1940 |

| 1st Reference Preparation 1925 |
| 2nd Reference Preparation 1936 |
| 3rd Reference Preparation 1951 |

| 1st Reference Preparation 1951 |
| 1st Reference Preparation 1954 |


## MISCELLANEOUS II

**International Laboratory for Biological Standards, Statens**

<table>
<thead>
<tr>
<th>Substance</th>
<th>International Unit of present standard (mg)</th>
<th>Form in which dispensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opacity reference preparation</td>
<td>--</td>
<td>Ampoules containing 15 ml of a suspension of Pyrex-glass particles in water (10 IU of opacity per ml)</td>
</tr>
</tbody>
</table>

## MISCELLANEOUS III

**Held and**

**Rijks Instituut voor de Volksgezondheid**

| Haemoglobin cyanide reference preparation | -- | Ampoules containing 10 ml of haemoglobin cyanide solution |

---

1 This Institute is custodian and distributor of haemoglobin cyanide reference preparation.
**MISCELLANEOUS II**

**Seruminstitut, Amager Boulevard, 80, Copenhagen, Denmark**

<table>
<thead>
<tr>
<th>Years of establishment (in brackets, unissue of previous standards)</th>
<th>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd Reference Preparation 1962</td>
<td></td>
</tr>
<tr>
<td>3rd Reference Preparation 1965</td>
<td></td>
</tr>
</tbody>
</table>

**MISCELLANEOUS III**

**held, Sterrenbos 1, Utrecht, Netherlands**

<table>
<thead>
<tr>
<th>Years of establishment (in brackets, unissue of previous standards)</th>
<th>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

on behalf of WHO. Samples can be obtained only by application to this address.
Annex

INTERNATIONAL BIOLOGICAL

This category of international biological substances was established for the purpose of providing biological diagnostic reagents of high specificity for the identification of micro-organisms or their products (specific antisera), as well as other important biological materials used in the diagnosis of disease. Since these

REFERENCE REAGENTS I

International Laboratory for Biological Standards, Statens

<table>
<thead>
<tr>
<th>Substance</th>
<th>Form in which dispensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-tick-borne encephalitis sera:</td>
<td></td>
</tr>
<tr>
<td>Anti-tick-borne encephalitis serum (lopping III (Moredun) virus)</td>
<td>Ampoules containing 1 ml of freeze-dried sheep serum</td>
</tr>
<tr>
<td>Anti-tick-borne encephalitis serum (Russian spring-summer encephalitis (Sophyn and Absettarov) virus)</td>
<td>Ampoules containing 2 ml of freeze-dried sheep serum</td>
</tr>
<tr>
<td>Anti-trichinella human serum</td>
<td>Ampoules containing 1 ml of freeze-dried pooled human serum</td>
</tr>
<tr>
<td>Enterovirus antisera: ¹</td>
<td></td>
</tr>
<tr>
<td>Coxsackie virus antisera ¹ types:</td>
<td></td>
</tr>
<tr>
<td>A1, A2, A3, A4, A5, A6, A7, A8</td>
<td></td>
</tr>
<tr>
<td>A9</td>
<td></td>
</tr>
<tr>
<td>A10, A11, A12, A13, A14, A15, A16, A17, A18, A19, A20, A21, A22, A24</td>
<td>Ampoules containing 0.5 ml of freeze-dried monkey serum</td>
</tr>
<tr>
<td>B1, B2, B3</td>
<td></td>
</tr>
<tr>
<td>B4, B5</td>
<td></td>
</tr>
<tr>
<td>B6</td>
<td></td>
</tr>
</tbody>
</table>

¹ Antisera prepared from the same batch of material as these international reference reagents are available in the WHO virus reference centres and in the Research Reference
4

REFERENCE REAGENTS

Reference reagents are not used for the quantitative assay of the activity of biological products, an international unit is not assigned to them. However, they serve usefully for long-term reference purposes.

distributed by

REFERENCE REAGENTS I

Seruminstitut, Amager Boulevard, 80, Copenhagen, Denmark

<table>
<thead>
<tr>
<th>Years of establishment</th>
<th>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Reference Reagent 1969</td>
<td></td>
</tr>
<tr>
<td>1st Reference Reagent 1965</td>
<td></td>
</tr>
<tr>
<td>1st Reference Reagent 1969</td>
<td></td>
</tr>
<tr>
<td>1st Reference Reagent 1966</td>
<td></td>
</tr>
<tr>
<td>1st Reference Reagent 1969</td>
<td></td>
</tr>
</tbody>
</table>

Reagents Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md., USA.
REFERENCE REAGENTS I (contd)  Held and International Laboratory for Biological Standards, Statens

<table>
<thead>
<tr>
<th>Substance</th>
<th>Form in which dispensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterovirus antisera&lt;sup&gt;1&lt;/sup&gt; (continued):</td>
<td></td>
</tr>
<tr>
<td>Echo virus antisera&lt;sup&gt;2&lt;/sup&gt; types 1, 2, 3, 4, 5, 6, 6&lt;sup&gt;11&lt;/sup&gt;, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 26, 27, 28, 29, 30, 31 and 33 Poliovirus antisera types 1, 2 and 3 Reovirus antisera type 1</td>
<td>Ampoules containing 0.5 ml of freeze-dried monkey serum</td>
</tr>
<tr>
<td>Adenovirus antisera：&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Types 1, 2, 3, 5, 6, 7a, 8, 9, 10, 11, 13, 15 and 17 Types 12 and 18</td>
<td>Ampoules containing 0.5 ml of freeze-dried horse serum Ampoules containing 0.5 ml of freeze-dried horse serum</td>
</tr>
<tr>
<td>Parainfluenza virus antisera：&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Types 1, 2 and 3</td>
<td>Ampoules containing 0.5 ml of freeze-dried horse serum</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em> antiserum&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Antisera prepared from the same batch of material as these international reference reagents are available in the WHO virus reference centres and in the Research Reference Reagents Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md., USA.
## Reference Reagents I (contd)

**Seruminstitut, Amager Boulevard, 80, Copenhagen, Denmark**

<table>
<thead>
<tr>
<th>Years of establishment</th>
<th>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</th>
</tr>
</thead>
</table>

* Antisera prepared from the same batch of material as these international reference reagents are available in the WHO virus reference centres and in the Center for Disease Control, Atlanta, Ga, USA.
<table>
<thead>
<tr>
<th>Substance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-Leptospira sera:</strong></td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>azlicki</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>castellonis</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>sejroe</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>mini</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>australis</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>copenhageni</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>tarassovi</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>autumnalis</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>rachmati</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>pomona</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>bataviae</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>heidelberg</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>andamanica</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>javanica</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>pyrogenes</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>naum</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>mankarso</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>sarmis</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>pol</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>schuefferi</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>muenchen</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>cynopteri</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>bangkhan</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>wolffi</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>hardt</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>kremastos</em> serum</td>
<td></td>
</tr>
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<td><em>Anti-Leptospira</em> interrogans serotype <em>benjamin</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>zanoni</em> serum</td>
<td></td>
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<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>medianensis</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>pauldus</em> serum</td>
<td></td>
</tr>
<tr>
<td><em>Anti-Leptospira</em> interrogans serotype <em>semarang</em> serum</td>
<td></td>
</tr>
</tbody>
</table>

1 The WHO/FAO and WHO Leptospirosis Reference Laboratories are co-custodians of these international reference sera. Samples can be obtained only by application to the following WHO/FAO Leptospirosis Laboratories: Laboratory of the Queensland Department of Health and Home Affairs, Brisbane, Queensland, Australia; Istituto Superiore di Sanità, Viale Regina Elena 299, Rome, Italy; Israeli Institute for Biological Research, Ness-Ziona, Israel; National Institute
<table>
<thead>
<tr>
<th>Form in which dispensed</th>
<th>Years of establishment</th>
<th>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype canicola serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype grippotyphosa serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype icterohaemorrhagiae serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype atlantae serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype georgia serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype bratislava serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype erinacei-aurati serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype coxi serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype fagis serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype worsfoldi serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype malaya serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype butembo serum</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 The WHO/FAO and WHO Leptospirosis Reference Laboratories are co-custodians of these international reference sera. Samples can be obtained only by application to the following WHO/FAO Leptospirosis Laboratories: Laboratory of the Queensland Department of Health and Home Affairs, Brisbane, Queensland, Australia; Istituto Superiore di Sanità, Viale Regina Elena 299, Rome, Italy; Israeli Institute for Biological Research, Ness-Ziona, Israel; National Institute
distributed by

Reference Laboratories ¹

<table>
<thead>
<tr>
<th>Form in which dispensed</th>
<th>Years of establishment</th>
<th>References (WHO/BS refers to unpublished working documents of the WHO Expert Committees on Biological Standardization)</th>
</tr>
</thead>
</table>

of Health, Tokyo, Japan; Institute for Tropical Hygiene and Geographical Pathology (Royal Tropical Institute), Mauritskade, 57A, Amsterdam, Netherlands; The London School of Hygiene and Tropical Medicine, Keppel Street, London W.C.1., England; Division of Veterinary Medicine, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington 12, D.C., USA; WHO Leptospirosis Reference Laboratory, Gamaleja Institute of Epidemiology and Microbiology, Moscow, USSR.
Annex 5

PROPOSED INTERNATIONAL BIOLOGICAL STANDARDS,
INTERNATIONAL BIOLOGICAL REFERENCE PREPARATIONS
AND
INTERNATIONAL BIOLOGICAL REFERENCE REAGENTS

A. IMMUNOLOGICAL SUBSTANCES

<table>
<thead>
<tr>
<th>Substance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-<em>Clostridium chauvoei</em> serum</td>
<td><em>Wild Hlth Org. techn. Rep. Ser.</em>, 1964, 293, 20; WHO/BS 690, 979</td>
</tr>
<tr>
<td>Substance</td>
<td>References</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype djasiman serum</td>
<td>WHO/BS 437, 489, 664</td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype biggis serum</td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype hamtoni serum</td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype birkini serum</td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype borinacana serum</td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype ndambari serum</td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype ballum serum</td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype abram's serum</td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype alexi serum</td>
<td></td>
</tr>
<tr>
<td>Anti-Leptospira interrogans serotype fort-bragg serum</td>
<td></td>
</tr>
</tbody>
</table>
## B. PHARMACOLOGICAL SUBSTANCES

<table>
<thead>
<tr>
<th>Substance</th>
<th>References</th>
</tr>
</thead>
</table>
Annex 6

DISCONTINUED INTERNATIONAL BIOLOGICAL STANDARDS

The International Biological Standards (or Reference Preparations) for the following substances, which can now be characterized completely by chemical or physical tests or for which there has been little demand, have been discontinued. (References: *Wild Hlth Org. techn. Rep. Ser.*, 1952, 56, 14; 1953, 68, 25; 1957, 127, 9, 19; 1969, 413, 14, 21)

Samples of the substances marked with an asterisk are now available at the WHO International Reference Centre for Chemical Reference Substances, Apotekens Centrallaboratorium, Box 333, Solna 3, Sweden.

Although there are no longer international standards for vitamin A or for provitamin A, the international units for these substances are still used extensively. The WHO Expert Committee on Biological Standardization has therefore redefined the International Unit for Vitamin A as the activity of 0.000344 mg of pure all-trans vitamin A acetate, and the International Unit for Provitamin A as the activity of 0.0006 mg of pure all-trans beta carotene.

<table>
<thead>
<tr>
<th>Substance</th>
<th>International Unit (mg)</th>
<th>Adopted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic acid</td>
<td>—</td>
<td>1925</td>
</tr>
<tr>
<td>Ouabain</td>
<td>—</td>
<td>1928</td>
</tr>
<tr>
<td>Provitamin A (β-carotene)</td>
<td>0.0006</td>
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</tr>
</tbody>
</table>

INDEX

Adenovirus antisera .................................. 82
Amphotericin ........................................ 66
Androsterone ......................................... 91
Antiarachne ........................................... 88
Antidiuretic hormones ................................ 72
Antithaemophilic factor ................................ 14, 74
Anti-nuclear-factor serum
(homogeneous) ......................................... 21, 62
Anti-streptolysin O .................................... 56
Anti-tumour antibodies ................................ 13
Antivenins, snake .................................... 23
Arsphenamine .......................................... 91
Bacitracin .............................................. 64
BCG vaccine ........................................... 52
Biological reference reagents ........................ 22, 23, 80
Bicocytin .............................................. 13
Blood coagulation factor VIII ....................... 14, 74
Blood-typing sera .................................... 58, 88
Brucella serum ........................................ 22, 62
Brucella vaccine ....................................... 46
Candidin ............................................... 12, 90
Canine-distemper serum ................................ 64
Canine-hepatitis serum ................................ 64
Capreomycin .......................................... 70
Carbolicin ............................................. 50
Cefalotin ............................................... 70
Chloramphenicol ...................................... 91
Chlorotetracycline .................................... 66
Cholera antigens ....................................... 91
Cholera agglutinating sera ........................... 22
Cholera vaccines ....................................... 50
Clindamycin .......................................... 12, 90
Clostridium antitoxins ................................ 19, 54, 58, 60, 62, 64, 88
Clostridium vaccines and toxoids .................... 16, 17, 54, 88
Coxsackie virus antisera ............................. 80
Colistin ................................................ 66, 70
Corticosteroids ...................................... 72
Demethylchlortetracycline ............................ 68
Dihydrostreptomycin ................................... 64
Digitalis ............................................... 76
Dimercaprol .......................................... 76
Diphtheria antitoxin .................................. 18, 54, 60
Diphtheria toxin ...................................... 48
Diphtheria toxoid ..................................... 48
Discontinued biological standards ................. 91
Doxycycline .......................................... 90
Dysentery antitoxin .................................. 54
Echinococcus serum .................................. 23, 88
Echovirus antisera ................................... 82
Enterovirus antisera .................................. 80, 82
Epidemic typhus vaccine ............................. 17
Erythromycin ........................................ 66
Erythropoietin ....................................... 14, 74
Gas-gangrene antitoxins ................................ 19, 54, 56
Gentamycin ............................................ 70
Glucagon ............................................... 13, 90
Gonadotrophins ...................................... 14, 72, 74, 90
Gramicidin .......................................... 68
Growth hormones ..................................... 13, 72
Haemoglobin reference preparation ................ 78
Heparin ................................................ 74
Hyaluronidase ........................................ 74
Hygromycin .......................................... 70
Immunglobulins ...................................... 20, 62, 89
Influenza virus haemagglutinin ...................... 52
Insulins ............................................... 74, 90
Kanamycins ........................................... 68
Lecithins .............................................. 16, 50
Leptospira sera ....................................... 22, 84, 86, 89
Lincocycin ............................................ 70
Long-acting thyroid stimulator ....................... 90
Lynceyceline ......................................... 11, 70
Lysine vasopressin ................................... 90
Measles serum ........................................ 15, 82
Measles vaccine ...................................... 15, 46
Melanins .............................................. 76
Methacycline .......................................... 10, 70
Minocycline .......................................... 12, 90
Mycoplasma sera ..................................... 64, 82
Nefarpenamine ........................................ 76
Neomycin ............................................. 10, 68
Newcastle-disease serum ............................. 64
Newcastle-disease vaccines .......................... 54
Nisin .................................................... 70
<table>
<thead>
<tr>
<th>Biological Standardization</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
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<td>Oestradiol</td>
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<tr>
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<td>66</td>
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<td>Opacity reference preparation</td>
<td>78</td>
</tr>
<tr>
<td>Ouabain</td>
<td>91</td>
</tr>
<tr>
<td>Oxaphenerazine</td>
<td>76</td>
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<tr>
<td>Oxytetracycline</td>
<td>66</td>
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<tr>
<td>Oxycodin</td>
<td>72</td>
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<tr>
<td>Parainfluenza virus sera</td>
<td>82</td>
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<tr>
<td>Paromomycin</td>
<td>70</td>
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<tr>
<td>Penicillius</td>
<td>12, 91</td>
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<tr>
<td>Pertussis vaccine</td>
<td>15, 50</td>
</tr>
<tr>
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<td>17</td>
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<tr>
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<td>56</td>
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<tr>
<td>Poliomyelitis sera</td>
<td>52, 58, 82</td>
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<tr>
<td>Poliomyelitis vaccines</td>
<td>24</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>66</td>
</tr>
<tr>
<td>Procaine benzylpenicillin in oil with aluminium monostearate</td>
<td>24, 68</td>
</tr>
<tr>
<td>Progesterone</td>
<td>91</td>
</tr>
<tr>
<td>Prolactin</td>
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<td>Protamine</td>
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<td>Reovirus antisera</td>
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<td>Requirements for biological substances</td>
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<tr>
<td>Respiratory virus sera</td>
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<td>70</td>
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<tr>
<td>Rinderpest vaccines</td>
<td>46, 88</td>
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