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**REQUIREMENTS FOR
BIOLOGICAL SUBSTANCES**

- 1. General Requirements for Manufacturing Establishments and Control Laboratories**
- 2. Requirements for Poliomyelitis Vaccine (Inactivated)**

Report of a Study Group

	Page
1. General considerations	3
2. Recommendation of an international study of potency tests	5
3. Revision of text of the requirements for poliomyelitis vaccine	6
Annex 1. General requirements for manufacturing establishments and control laboratories (Requirements for Biological Substances No. 1)	7
Annex 2. Requirements for poliomyelitis vaccine (inactivated) (Requirements for Biological Substances No. 2) . .	17

WORLD HEALTH ORGANIZATION

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**STUDY GROUP ON GENERAL REQUIREMENTS FOR
MANUFACTURING ESTABLISHMENTS AND CONTROL LABORATORIES
AND ON REQUIREMENTS FOR POLIOMYELITIS VACCINE**

Geneva, 2-7 June 1958

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REQUIREMENTS FOR BIOLOGICAL SUBSTANCES

- 1. General Requirements for Manufacturing Establishments and Control Laboratories**
- 2. Requirements for Poliomyelitis Vaccine (Inactivated)**

Report of a Study Group

The Study Group on General Requirements for Manufacturing Establishments and Control Laboratories and on Requirements for Poliomyelitis Vaccine met in Geneva, from 2-7 June 1958.

Dr P. Dorolle, Deputy Director-General, opened the meeting on behalf of the Director-General of the World Health Organization and welcomed the members of the Group.

The Deputy Director-General outlined the task of the Study Group which was to draw up an international recommendation on the requirements which should be fulfilled by a preparation of poliomyelitis vaccine in order to ensure that the product is a safe, reliable and potent prophylactic agent. An international recommendation on requirements would tend to facilitate the exchange of vaccines between different countries, and would provide guidance to responsible workers who wish to start the production of poliomyelitis vaccine and who may have difficulties in deciding upon appropriate methods of assay and control.

The Deputy Director-General hoped that the Study Group would come to agreement on the many tests that are necessary in the control of the manufacture of poliomyelitis vaccines. The successful accomplishment of its task would be an essential step in the WHO project of promoting greater uniformity in the field of assay methods and requirements for important biological preparations.

1. GENERAL CONSIDERATIONS

The Study Group noted the report of the Study Group on Requirements for Biological Substances (1957)¹ and agreed that requirements for poliomyelitis vaccine could be fitted into the framework suggested in that report.

In its discussions of requirements that could be internationally recommended, the Study Group first considered the second draft of General

¹ Unpublished working document WHO/BS/IR/27

Requirements for Manufacturing Establishments and Control Laboratories¹ and made minor amendments to the text. The revised requirements are given in Annex 1 to this report.

The Study Group further considered a proposed first draft of requirements for poliomyelitis vaccine,² the recommendations of the second report of the Expert Committee on Poliomyelitis,³ a document on sterility control,⁴ several documents concerning potency control,⁵ as well as a large amount of unpublished data that had been collected by the members of the Group.

The Study Group surveyed the regulations and requirements for the manufacture and control of poliomyelitis vaccine that had been adopted in some countries. Most of these had been modelled on the requirements originally formulated in the United States of America. However, a survey of the documents submitted to the Study Group⁶ and of the additional information presented by members of the Group showed that there were many differences between the requirements now in use in individual countries. The Group agreed that an important purpose would be served by the formulation of essential requirements for the manufacture and control of poliomyelitis vaccine which would be internationally acceptable.

The Study Group drafted the requirements for poliomyelitis vaccine given in Annex 2 which it considered to be generally applicable to all vaccines in current use and which are therefore based on inactivated, trivalent vaccines only. The Group agreed that situations could arise in which a largely non-immune population might be threatened by an epidemic of one particular poliovirus type, and that in such situations the use of monovalent, or even divalent, vaccines might appear desirable.

¹ WHO Secretariat, unpublished working document WHO/BS/IR/31

² WHO Secretariat, unpublished working documents WHO/BS/IR/29 & WHO/BS/IR/30

³ *Wld Hlth Org. techn. Rep. Ser.*, 1958, 145

⁴ Eissner, G. & Bonin, O., unpublished working document WHO/BS/IR/36

⁵ Gard, S., Johnsson, T., Lycke, E., Melén, B., Olin, G., Salenstadt, R. & Wrangle, G., unpublished working document WHO/BS/IR/32; de Somer, P., unpublished working document WHO/BS/IR/33; Lépine, P., Roger, F. & Sautter, V., unpublished working document WHO/BS/IR/34; Krech, U., unpublished working document WHO/BS/IR/38; Benyesh, M. & Melnick, J. L., unpublished working document WHO/BS/IR/39; United States of America, Division of Biologics Standards, National Institutes of Health, unpublished working document WHO/BS/IR/42; Prigge, R., Günther, O. & Bonin, O., unpublished working document WHO/BS/IR/43

⁶ Lafontaine, A., unpublished working document WHO/BS/IR/40 (Belgian requirements); Canada, Laboratory of Hygiene, unpublished working document WHO/BS/IR/41; von Magnus, H., unpublished working document WHO/BS/IR/35 (Danish requirements); Lépine, P., unpublished working document WHO/Polio/23 (French requirements); Federal Republic of Germany, Paul-Ehrlich-Institut, Frankfurt-am-Main, unpublished third draft of the provisional regulations for the national control of poliomyelitis vaccines; Penso, G., unpublished working document WHO/BS/IR/37 (Italian requirements); England and Wales, *Therapeutic Substances Amendment Regulations, 1956*, Schedule, Part XVII; United States of America, *Code of Federal Regulations, 1956*, Title 42, Chapter I, Part 73

Such monovalent, or divalent, vaccines should meet all the relevant requirements set out in Annex 2.

While discussing the formulation of requirements for the safety of poliomyelitis vaccine, the Study Group considered the significance of the possible occurrence of lymphocytic choriomeningitis virus, B-virus and other simian agents in the tissue cultures used for vaccine production. It considered that the two viruses mentioned, although pathogenic to man, did not present any serious safety problem since they were far less stable in the presence of formaldehyde, or other chemical agents, than poliovirus. Tests for the presence of B-virus, if considered desirable, should be performed as soon as possible after harvesting the poliovirus that is to be processed for inclusion in the vaccine.

2. RECOMMENDATION OF AN INTERNATIONAL STUDY OF POTENCY TESTS

At the present time, international requirements of antigenic potency of poliomyelitis vaccine could not be formulated in satisfactory detail. The Study Group agreed that decisions on definite levels of acceptance for potency would be premature, partly because of the diversity of principles and techniques applied in currently used laboratory tests, and partly because the available information on the correlation between the results of laboratory potency tests on a vaccine and its performance in the field is insufficient.

The Study Group noted that efforts were being made in various countries to improve the basis for potency control, and it expressed the opinion that international co-operation would at this stage be possible and desirable. The Group recommended the organization of a study in which several laboratories of different countries, at present using different methods, would participate. This international study should be designed to compare the data obtained by different laboratories when testing the same vaccine by the same specified method or methods, as well as by the routine method of each laboratory. In order that the information obtained should be of the greatest value, this international study should include a common reference vaccine and, under code numbers, another vaccine and dilutions thereof, to be evaluated by each of the participants. It would be desirable to test the same vaccine in man.

The Group noted that a batch of poliomyelitis vaccine was being prepared on behalf of the National Institutes of Health, Bethesda, Maryland, USA to serve as a preliminary reference preparation in a national study of potency tests, and that some of this material might be made available for use in the proposed international study.

An important factor in the outcome of the international study would be the storage- and transport-stability of the vaccines to be tested, and

especially the stability of the reference vaccine. Studies of stability should therefore be conducted on the vaccines after transport or after exposure to temperature degradation and a comparison made with a stable infective antigen.

The Group also noted that the Expert Committee on Biological Standardization had already initiated efforts towards the establishment of a stable freeze-dried trivalent poliomyelitis vaccine, and it therefore recommended that the attention of the Expert Committee on Biological Standardization be drawn to the present recommendation in order to permit the inclusion of a freeze-dried antigen, if available, in the international study.

The participating laboratories¹ should be asked to test the vaccines by a specified guinea-pig potency test.² Also arrangements should be made so that a specified chick test² and a specified antibody-combining test² would be performed by a number of the participating laboratories. In addition, every laboratory should at the same time test all vaccines submitted for this study by the method it uses routinely.

The Study Group recommended that the World Health Organization make a suitable arrangement for initiating an international study as outlined, and for collecting and evaluating the results. Pending the outcome of this proposed study, international recommendations on potency requirements could not be made more specific than those appearing in Annex 2 to the present report.

3. REVISION OF TEXT OF THE REQUIREMENTS FOR POLIOMYELITIS VACCINE

The Study Group discussed what procedures could be recommended for revisions of the text of the Requirements for Poliomyelitis Vaccine now drawn up (Annex 2 to this report), and it recommended that the Secretariat of the World Health Organization submit all comments that might be forthcoming from members of the Group, or from other experts, to appropriate study groups or expert committees for consideration, in order to arrive at definitive requirements.

At appropriate intervals the requirements should be re-examined and provision should be made for the issue of a revised text whenever this became necessary.

¹ The following laboratories offered to participate in carrying out the tests required for this international study: Institut d'Hygiène et d'Epidémiologie, Brussels, Belgium; Statens Seruminstitut, Copenhagen, Denmark; Institut Pasteur, Paris, France; Paul-Ehrlich-Institut, Frankfurt-am-Main, Federal Republic of Germany; Istituto Superiore di Sanità, Rome, Italy; The Poliomyelitis Research Foundation Laboratories, Johannesburg, Union of South Africa; Statens Bacteriologiska Laboratorium, Stockholm, Sweden; Biological Standards Control Laboratories, London, England; Division of Biologics Standards, National Institutes of Health, Bethesda Md., USA; Moscow Institute for Poliomyelitis Prophylactics, Moscow, USSR.

² Recommended detailed specifications of all test procedures involved are available, and should be furnished to all participating laboratories.

Annex 1

GENERAL REQUIREMENTS FOR MANUFACTURING ESTABLISHMENTS AND CONTROL LABORATORIES (REQUIREMENTS FOR BIOLOGICAL SUBSTANCES No. 1)*

	Page
General considerations	7
Part A. General requirements for manufacturing establishments	
1. Personnel	8
2. Buildings and equipment	9
3. Production control	10
4. Filling and containers	11
5. Tests	12
6. Records	12
7. Samples	12
8. Labelling.	13
9. Distribution and shipping	13
10. Storage and expiry date.	13
Part B. General requirements for control laboratories	
1. Administration and personnel	13
2. Buildings and equipment	14
3. Scope of activities	14

General Considerations

The procedures required for controlling biological substances during manufacture are different from the control procedures applied to final products by control authorities. Control at the manufacturing level is a matter of national concern, whereas control of final products, including imported products, by a control authority may have international as well as national implications.

* The original draft of these requirements was prepared by a Study Group on Requirements for Biological Substances which met in Geneva from 7-12 October 1957. The members of this Study Group were: Dr M. L. Ahuja, Medical Adviser to the High Commissioner for India, London, England; Dr J. Desbordes, Service central de la Pharmacie, Bureau des Sérums et Vaccins, Paris, France; Dr G. Eissner, Paul-Ehrlich-Institut, Frankfurt-am-Main, Federal Republic of Germany; Dr J. H. Gaddum, Director, Pharmacological Laboratory, University New Buildings, Edinburgh, Scotland; Dr L. Greenberg, Chief, Biologics Control Laboratories, Laboratory of Hygiene, Ottawa, Canada; Dr D. Ikić, Director, Institute for the Production of Sera and Vaccines, Zagreb, Yugoslavia; Dr M. Kurokawa, Chief, Department of General Assay, National Institute of Health, Tokyo, Japan; Dr A. Lafontaine, Directeur, Institut d'Hygiène et d'Epidémiologie, Brussels, Belgium (Chairman); Dr O. Maaløe, Director, Department of Biological Standards, Statens Seruminstitut, Copenhagen, Denmark; Dr G. Penso, Chief, Laboratory of Microbiology, Istituto Superiore di Sanità, Rome, Italy; Dr W. L. M. Perry, Director, Department of Biological Standards, National Institute for Medical Research, London, England; Dr J. T. Tripp, Division of Biologics Standards, National Institutes of Health, Bethesda, Md., USA (Vice-Chairman). Dr N. K. Jerne, Chief, Section of Biological Standardization, WHO, acted as Secretary.

The general requirements given in Part A are applicable to all manufacturing situations.

In an ideal situation the same control measures would be exercised by the governments of all countries. In such circumstances there would be no problem in the free exchange of biological substances between countries, and the control authority in any one would be faced only with the problem of controlling substances manufactured within its own jurisdiction. It is, however, essential to realize that it will be many years before such an ideal situation can possibly be brought about ; in the interim it will continue to be necessary for the national control authority to deal not only with the substances manufactured within its own jurisdiction, but also with substances imported from other countries.

The general requirements given in Part B should apply to all control laboratories operating under present conditions. These general requirements should operate, regardless of the number or kind of biological substances being controlled, and whether these substances have been manufactured within the country or imported.

Each of the following sections constitutes a recommendation. The parts of each section which 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 which are printed in small type concern points on which comments seemed desirable.

In case individual countries should wish to adopt these requirements as the basis for their national regulations concerning general requirements for the manufacture and control of biological products, it is recommended that a clause be included which would permit modifications, on the condition that such modifications had been demonstrated, to the satisfaction of the national control authority, to ensure that the degree of safety and the potency of the products are at least equal to those provided by the requirements formulated below. In any such cases, the World Health Organization should 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 biological substance is manufactured.

Part A. General Requirements for Manufacturing Establishments

1. Personnel

Manufacturing shall be supervised by a person who has been trained in the techniques used in manufacturing biological substances and the scientific knowledge upon which the manufacture of these products is based. This person shall have sufficient authority to enforce discipline

among employees, who shall include specialists with training appropriate to the products made in the establishment.

Thus, in dealing with the problems of manufacture, a training is needed in some or all of the following fields: bacteriology, biometry, chemistry, medicine, pharmacy, veterinary medicine and virology.

The staff making control tests should be separate from the manufacturing unit and not responsible to the person in charge of production.

All staff engaged in manufacture, testing, and animal care should be vaccinated with appropriate specific vaccines, and should submit to a yearly tuberculosis control.

2. Buildings and equipment

2.1 Buildings

Laboratories, operating rooms, animal rooms and all other rooms and buildings used for the manufacture of biological products shall be so designed and constructed of such materials that the highest standards of cleanliness and sanitation can be maintained and freedom from dust, insects and vermin ensured. All such buildings shall be equipped with hot and cold running water and drainage. Adequate precautions shall be taken to avoid contamination of the drainage system with dangerous effluents and also to avoid airborne dissemination of pathogenic microbes and viruses. Staff changing rooms, etc., shall be provided as needed. All buildings and rooms shall be clean and sanitary at all times. If rooms intended for the manufacture of biological substances are used for other purposes, they shall be cleaned thoroughly and, if necessary, sterilized prior to resumption of manufacture of biological substances in them.

2.2 Constant temperature rooms

Adequate refrigerator space, as well as incubators or warm rooms, capable of being maintained at a uniform temperature within any required range shall be provided.

Refrigerators and incubators should maintain a uniform temperature in all parts of the interior and preferably be equipped with recording thermometers.

2.3 Sterile rooms

Sterile transfer and processing rooms shall be of minimum size for their function and have low ceilings and smooth surfaces to permit thorough cleaning before each use.

These rooms should be essentially dust free and preferably supplied with filtered air at a pressure higher than adjacent rooms.

Staff working in these rooms shall be provided with a special changing room.

2.4 *Washing and sterilization equipment*

Adequate facilities shall be available for washing apparatus. Steam autoclaves, dry heat sterilizers, and bacterial retaining filters shall be available for sterilizing supplies, media and apparatus.

Autoclaves should preferably be equipped with recording thermometers. Other means of sterilization, including ultraviolet irradiation and chemical sterilization, have special applications and when appropriate are used with proper controls.

2.5 *Animal quarters*

Quarters for animals shall be designed in a manner and constructed of materials that permit maintenance in a clean and sanitary condition free from insects and vermin. Facilities for animal care shall include isolation units for quarantine of incoming animals, and vermin-free food storage.

There should be provision for the disinfection of cages, if possible by steam, and an incinerator for disposing of waste and of dead animals.

3. Production control

3.1 *Production methods*

Written procedures shall be prepared for each product, describing each step in production and testing. Operators shall not deviate from this written procedure without the approval of the responsible authority.

3.2 *Cleanliness*

Apparatus, equipment and materials used in manufacturing shall be clean and, if necessary, sterile and free from pyrogenic contamination.

3.3 *Orderliness*

All containers of biological substances, regardless of the stage of manufacture, shall be identified by securely attached labels.

3.4 *Precautions against contamination*

All procedures with spore-forming micro-organisms or viruses shall be confined to separate areas with complete equipment used exclusively in those areas.

Separate facilities shall be provided for work with each virus and care shall be taken to prevent aerosol formation (especially by centrifugation and blending), which might lead to transfer of virus from one production unit to another.

Pathological specimens sent in for diagnosis shall be permitted only in separate areas not used for manufacturing biological substances.

Employees should stay in their own work areas, and wear protective clothing, including shoes, caps, etc., which should remain in the area. Employees suffering from an infective illness should not be permitted to work until completely recovered.

Visitors should be as few as possible and they should not normally be permitted to enter sterile rooms.

3.5 *Animal care*

Animals used for production purposes, or for test purposes, shall show no signs of communicable disease, and shall be adequately housed at all times. They shall be provided with a well-balanced diet, and be kept clean and sanitary.

Animals intended for use in production or in tests should be observed daily during a quarantine period of not less than one week. In some instances it is desirable to maintain the animal rooms constantly at the optimum temperature for the particular species and test, and it may also be necessary to maintain pure strains of test animals.

Animals or animal carcasses shall not be removed from the establishment if capable of transmitting disease.

Animals that die from infection are destroyed, preferably in an incinerator.

4. **Filling and containers**

4.1 *Filling rooms*

Filling shall be performed in rooms reserved for this purpose. These shall be sterile rooms equipped specifically for transferring measured quantities of finished biological substances from bulk containers to the final containers. Strict dust control measures and aseptic techniques shall be enforced to ensure that the product is not contaminated during the filling process.

These measures include, for instance, laying of dust by steam or spray, proper protective clothing for workers, etc.

4.2 *Filling procedures*

Filling operations shall be conducted in such a way as to avoid any contamination or alteration of the product.

4.3 *Containers*

The final container shall be sealed as soon as possible after filling. Closures shall be of material that does not have a deleterious effect upon the biological substance, and shall be designed to maintain a hermetic seal throughout the dating period.

5. Tests

All tests of a specific biological substance, requiring the use of living micro-organisms, shall be carried out in rooms separate from those used for production.

Preferably, all tests should be carried out in such separate rooms.

The descriptions of the tests necessary to establish the safety, purity and potency of each lot of a biological substance will be given in the requirements to be formulated for the particular biological substance.

6. Records

6.1 *Production protocols and distribution records*

Records shall be permanent and clearly indicate all steps in processing, testing, filling and distribution. They shall be retained throughout the dating period of a lot or batch of a biological product and be available at all times for inspection by the control authorities.

Records must make it possible to trace all steps in the manufacture and testing of a batch, and should include records of sterilization of all apparatus and materials used in its manufacture. Distribution records must be kept in a manner that permits rapid recall of any particular batch, if necessary.

6.2 *Records of cultures*

Records shall be kept of the complete passage history of all cultures kept in the establishment. Cultures shall be labelled and stored in a safe, orderly manner.

7. Samples

Samples from each lot shall be taken in a sufficient amount to satisfy the requirements for samples of the national control laboratory. Additional samples shall be retained throughout the dating period as reference material, in a manner which ensures the identity of the lot.

Manufacturers should retain sufficient additional samples to permit repeating the control tests.

8. Labelling

Pending the formulation of general requirements for labelling applicable to all biological products, the information to be printed on the label affixed to each container and package, and on the leaflet which may accompany the containers will be given in the requirements for individual biological substances.

9. Distribution and shipping

9.1 Release for distribution

A lot of a biological substance shall not be released until all the required tests have been performed, summarized and reviewed and until any other official control requirement is satisfied. These tests shall always include an identity test performed on the contents of a finished package from each filling, to confirm the accuracy of the labelling.

9.2 Shipping

Biological substances shall be shipped with precautions to ensure that the product retains its potency upon arrival at its destination.

Rules cannot be laid down to cover all situations; this requires the continuous exercise of judgement.

10. Storage and expiry date

10.1 Storage conditions

Biological products shall be stored at all times at controlled temperatures within a range which ensures optimal stability.

During distribution, short periods at ambient temperatures may have to be permitted.

10.2 Expiry date

The expiry date of a biological product shall be defined and fixed with the approval of the national control authority.

Part B. General Requirements for Control Laboratories

1. Administration and personnel

The control laboratory shall be operated by or on behalf of the national control authority.

Authority for taking measures designed to ensure that biological substances used in a country are safe, potent and biologically pure, normally rests upon the health department

of the Government of that country. This authority must obviously be delegated to the expert in charge of the control laboratory, who should have full authority and full responsibility.

The head of the control laboratory shall be a person qualified and experienced in the control of biological substances.

The staff of the control laboratory shall include experts in all disciplines required to cover the biological substances which the laboratory must control, both those that are manufactured in the country and those imported for use.

It will therefore usually be necessary for the staff to include persons trained in some or all of the following fields: bacteriology, biometry, chemistry, medicine, pharmacy, pharmacology, veterinary medicine, and virology.

2. Buildings and equipment

The requirements in respect of buildings and equipment described in Part A, section 2 shall apply in a general way to a control laboratory. Instruments and apparatus shall be of high precision.

All instruments and apparatus should be calibrated and checked at regular intervals.

Technical library facilities, including both books and journals, should always be available.

3. Scope of activities

The most effective method of control is undoubtedly that provided by a system which includes licensing of manufacturers, and routine inspection of their establishments as well as control tests of their finished products.

All of these methods of control should be under the direction of the control laboratory. The requirements that should be met by manufacturing establishments have been outlined in detail in Part A and should be enforced by the control laboratory.

3.1 Licensing and inspection

Manufacturers shall be licensed in respect of each individual biological substance which they manufacture and methods shall exist for withdrawing the licence for that substance in the event of failure to meet the appropriate general and special requirements.

Routine inspection of all manufacturing establishments should be carried out by the expert staff of the control laboratory, preferably at intervals of not more than one year.

3.2 Tests by control laboratory

The control laboratory shall be staffed and equipped in such a way as to be able to carry out effectively all the required tests on samples of

the finished products, as well as on samples taken at an intermediate stage of manufacture.

The tests carried out by the control laboratory on the final products will usually be identical with those which are required of the manufacturer, but the control laboratory should have discretionary power to vary the tests applied and to decide whether to apply tests to all or only to selected batches.

Control tests on the final product are sometimes closely similar to those applied during manufacture; but this is not always the case, since the marketed forms of biological substances, such as mixtures with other active ingredients or with adjuvants and preservatives, may greatly complicate the problem of carrying out the necessary tests. It will, in general, be impracticable to give guidance on the ways in which the numerous marketed preparations of any biological substance should be treated in order to make the tests proposed for the parent substance applicable. Control laboratories will therefore be forced to develop their own technique for this purpose—possibly in the first place by enquiring of the original manufacturer.

The control laboratory shall devise effective internal control measures to permit objective interpretation of tests and evaluation of its own reliability in performing all tests.

The inclusion of replicate coded samples in products to be tested; the simultaneous independent testing of the same batch of substance; and routine checks on sensitivity and calibration of instruments are measures that may be applied as self-imposed "controls" for the control laboratory.

Healthy animals of various species and unquestioned strains shall be available in adequate numbers for an effective performance of the tests to be undertaken.

Test animals must conform to stricter requirements than those used in manufacturing control because the number of samples is limited and maximum reliability of the tests is demanded. Animals should be maintained under optimum nutritional and environmental conditions before and during tests. It is essential for test animals to be kept free from infectious diseases and this is best accomplished by strict quarantine measures.

3.3 *Release and certification*

A lot of a biological substance shall be released only if it fulfils the requirements adopted by the national control laboratory.

In certain circumstances, the official in charge of the national control laboratory shall provide a statement, at the request of the manufacturing

laboratory, certifying whether or not a given lot of a biological substance meets all appropriate requirements.

It is in general impracticable and may in the future become unnecessary for a control laboratory to attempt licensing or inspection of manufacturers outside its own jurisdiction. For the control of imported products it is therefore primarily dependent upon tests on the final products themselves, supplemented by protocols of tests carried out by the manufacturer and, in certain circumstances, by a certificate to the effect that the control authority of the country of origin has found the product satisfactory.

3.4 *Research and training*

It is desirable so to organize the control laboratory that opportunity is provided for research in addition to routine testing. Encouragement of research activities will not only lead to the development of better methods of control, but will also help the laboratory to retain an interested, efficient, and highly qualified staff. The number of specialists in the control of biological substances needed by any country is too small to justify specific university courses in this field. It is therefore necessary for the control laboratory itself to adopt a vigorous training programme, covering both the technical and administrative aspects of control procedures. This generally will be best accomplished by direct supervision of junior staff during the actual performance of duties, but may be supplemented where conditions permit by more formal instruction.

Annex 2

REQUIREMENTS FOR POLIOMYELITIS VACCINE (INACTIVATED) (REQUIREMENTS FOR BIOLOGICAL SUBSTANCES No. 2)

	Page
General considerations	17
Part A. Manufacturing requirements	
1. Definition	18
2. General manufacturing requirements	19
3. Production control	19
4. Filling and containers	24
5. Control tests on final product	25
6. Information required by national control laboratory	28
7. Samples required by national control laboratory .	28
8. Labelling.	28
9. Distribution and shipping	29
10. Storage and expiry date	29
Part B. National control requirements	
1. General	30
2. Tests to be applied by national control laboratory in the country in which the vaccine is produced .	30
3. Release and certification.	30

General Considerations

The recommendation of international requirements for inactivated poliomyelitis vaccine is complicated by the fact that a number of different manufacturing and testing procedures are in use in various countries. The procedures differ mainly in the incorporation of different virus strains, the inactivation and filtration methods, and the use of preservatives and adjuvants in the final vaccine. In spite of these differences it is felt that certain essential requirements concerning manufacture and control can be formulated. The present recommendations are based on methods currently in use and future revisions will be necessary.

Each of the following sections constitutes a recommendation. The parts of each section which 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 which are printed in small type concern points on which comments seemed desirable.

In case individual countries should wish to adopt these requirements as the basis for their national regulations concerning poliomyelitis vaccine, it is recommended that a clause be included which would permit modifications of manufacturing details on the condition that such modifications had been demonstrated, to the satisfaction of the national control authority, to ensure that the degree of safety and the potency of the vaccine are at least equal to those provided by the requirements formulated below. In any such cases, the World Health Organization should be informed of the action taken.

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

Part A. Manufacturing Requirements

1. Definition

1.1 *International name and proper name*

The international name shall be "Vaccinum poliomyelitidis inactivatum". The proper name shall be the equivalent of the international name in the language of the country of origin.

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

1.2 *Descriptive definition*

Vaccinum poliomyelitidis inactivatum shall consist of an aqueous suspension of poliovirus hominis¹ types 1, 2 and 3 grown in monkey-kidney tissue cultures and inactivated by a suitable method. The preparation shall satisfy all the requirements formulated below.

1.3 *International standards or reference preparations and international units*

The WHO Expert Committee on Biological Standardization has taken steps to establish an International Standard for Poliomyelitis Vaccine.²

The Expert Committee has established International Reference Preparations of Antipoliomyelitis Sera of Types 1, 2 and 3.³

Samples of these International Reference Preparations may be obtained from the International Laboratory for Biological Standards, Statens Seruminstitut, Copenhagen, Denmark by the national laboratory for biological standards of any country.

¹ International Nomenclature Committee, Sub-Committee on Viruses (1954) *Int. Bull. bact. Nomencl.*, 4, 109

² *Wld Hlth Org. techn. Rep. Ser.*, 1958, 147, 13; 1959, 172, 11

³ *Wld Hlth Org. techn. Rep. Ser.*, 1959, 172, 15

2. General manufacturing requirements

The general manufacturing requirements contained in Requirements for Biological Substances No. 1¹ shall apply to establishments manufacturing poliomyelitis vaccine, with the addition of the following:

Manufacture of poliomyelitis vaccine shall take place in completely separate areas using separate personnel and equipment. The areas where processing of inactivated poliomyelitis vaccine takes place shall be separate from those where work with active virus is performed.

Written procedures for the preparation of poliomyelitis vaccine adopted by the manufacturer shall be submitted for approval to the national control authorities. Proposals for modifications shall be submitted for approval to the national control authorities before their implementation.

It is particularly important that production and control functions be organized as two separate units of the manufacturing establishment with independent responsibilities.

3. Production control

3.1 Control of source materials

3.1.1 Virus strains

Strains of poliovirus used in the production of vaccine shall be identified by historical records, infectivity tests, and by immunological methods. Any strain which will yield a vaccine meeting the requirements set forth in the present document may be used. Production of vaccine shall be based on a seed virus system; the poliovirus used for vaccine production shall not have passed more than ten subcultures, counted from a strain culture on which the original laboratory and field tests were done.

Preference should be given to strains of low pathogenicity to monkeys.

Each new seed lot of a strain of low pathogenicity should be retested for virulence before being used as seed virus. Samples of the strains used should be deposited in the national control laboratory. Strains of poliovirus currently used in production of vaccine may be obtained by application direct or through the World Health Organization to specialized laboratories.*

¹ See Annex 1.

* The following laboratories have expressed their willingness to supply samples of strains for this purpose: Institut d'Hygiène et d'Epidémiologie, Brussels, Belgium; Statens Seruminstitut, Copenhagen, Denmark; Institut Pasteur, Paris, France; Paul-Ehrlich-Institut, Frankfurt-am-Main, Federal Republic of Germany; Laboratory of Microbiology, Istituto Superiore di Sanità, Rome, Italy; The Poliomyelitis Research Foundation Laboratories, Johannesburg, South Africa; Statens Bacteriologiska Laboratorium, Stockholm, Sweden; Biological Standards Control Laboratories, Medical Research Council, London, N.W.3, England; Division of Biologics Standards, National Institutes of Health, Bethesda, Md., USA; Moscow Institute for Poliomyelitis Prophylactics, Moscow, USSR.

3.1.2 *Monkeys*

Suitable species of monkeys, in good health, shall be used as the source of kidney tissue for the production of poliovirus. Each animal shall be examined at necropsy for signs of disease and, if there is any pathological lesion of significance with regard to their use in the preparation of the vaccine, the kidneys shall be discarded. Kidney tissue from monkeys that have been used previously for experimental purposes shall not be used. An exception can be made in the case of monkeys used for the safety or potency tests with negative clinical findings.

It is recommended that monkeys be kept in as small groups as possible in order to reduce dissemination of infections within the colony.

3.1.3 *Tissue culture for virus production*

Virus for the preparation of vaccine shall be grown by aseptic methods in cultures of monkey-kidney cells that have not been propagated in series. The maintenance medium shall contain no protein. If animal serum is used in the propagation of cells, the final vaccine shall not contain more animal serum than one part per million.

Suitable antibiotics in minimum concentrations required for sterility may be used. If penicillin is used its concentrations may not exceed 200 International Units per ml. Non-toxic pH indicators may be added, e.g., phenol red in a concentration of 0.002%.

3.2 *Production precautions*

The general production precautions as formulated in Part A, section 3 of the General Requirements for Manufacturing Establishments and Control Laboratories¹ shall apply to the manufacture of poliomyelitis vaccine.

It is recognized that staff members working with active poliovirus may be exposed to the danger of infection. Accordingly, it is recommended that all personnel be immunized, if necessary, with poliomyelitis vaccine.

A further hazard exists because of the occurrence of B-virus infection in some monkeys. In order to minimize the transmission of such infections it is recommended that the handling of these animals be reduced to a minimum, and that workers use protective clothing and other protective devices where possible. It is further recommended that research be conducted towards developing prophylactic agents and protective sera against this infection.

Past experience has shown that monkeys may also be a source of infection due to *Mycobacterium tuberculosis*, and adequate precautionary measures against such infections should be taken.

¹ See Annex 1.

3.3 Control at the monovalent stages of the product

3.3.1 Treatment before inactivation

Prior to inactivation each monovalent virus pool shall be filtered or clarified.

The importance of filtration or clarification of the crude virus suspensions as a means of improving the regularity of the inactivation process has been clearly established. Generally, filters are used in series or filtration is performed stepwise through filters of decreasing porosity. Seitz S1 pads are most widely used as final filters, but satisfactory results have also been reported with other filter types, as well as with clarification procedures not involving filtration.

3.3.2 Virus titration

After filtration or clarification and before the initiation of inactivation a sample shall be taken of each monovalent pool for titration of infective poliovirus using tissue culture methods. This titration shall be carried out in tenfold dilution steps using ten tubes per dilution, or any other arrangement of tubes and dilutions yielding equal precision.

Each monovalent pool should show a titre of not less than 10^6 TCID₅₀* per ml using a batch of tissue culture of normal sensitivity. This should be confirmed in a parallel titration of a reference poliovirus preparation, the titre of which has been previously determined using monkey-kidney tissue cultures.

The main purpose of determining the titre of virus pools destined for inactivation is to select pools which can be expected to meet potency requirements after inactivation.

At the present time international reference preparations of poliovirus are not available. Pending the establishment of such international reference preparations, it is desirable for national control laboratories to issue reference virus preparations to manufacturers.

Although there may be theoretical reasons for using such reference preparations for all three types of poliovirus, the purpose of checking the sensitivity of tissue cultures may be met by the use of a reference preparation of a single type. A reference preparation of type I poliovirus has proved satisfactory in this respect.

3.3.3 Tests for *M. tuberculosis* and *B-virus*

Prior to inactivation, adequate samples of each monovalent pool shall be withdrawn for tests in guinea-pigs and in suitable culture media for the absence of *M. tuberculosis*. If the presence of *M. tuberculosis* is demonstrated, any product made from the pool shall be discarded.

Tests for B-virus, if performed at this stage, should be made on samples drawn from monovalent pools before initiation of inactivation, preferably as soon as possible after harvesting.

* TCID₅₀ = Tissue Culture Infective Dose 50%

3.3.4 *Time of inactivation*

Inactivation shall be initiated as soon as possible and not later than 72 hours after filtration or clarification.

It is preferable to start inactivation within 24 hours. Since the purpose of the filtration or clarification step is to remove particulate matter and other interfering substances which may diminish the effectiveness of the inactivation process, and since aggregates tend to increase on standing after filtration or clarification, efforts should be made to keep within this time limit.

3.3.5 *Inactivation procedure*

The virus in the monovalent pools shall be inactivated through the use of an agent or a method which has been demonstrated to be consistently effective in the hands of the manufacturer.

The progress of inactivation should be followed by suitably spaced determinations of virus titres.

Formaldehyde is used as inactivating agent in the production of all current vaccines. Most manufacturers have encountered irregularities in the inactivation process, which are not fully explained. On the basis of the observation that a decrease in the concentration of free formaldehyde occurs in the course of the process, it has been recommended that tests for free formaldehyde be performed at intervals and its concentration maintained at the desired level by intermittent readjustments.

Introduction of a second filtration during the inactivation is another, more widely used, procedure which can effectively reduce the frequency of such irregularities and which is a requirement in some countries. In order to avoid a loss of antigen due to filtration, some producers use a combination of initial formaldehyde treatment with some other method of inactivation.

It should be emphasized that the degree of consistency in producing successfully inactivated pools is an important factor in judging the safety of a given product. However, definite requirements in this respect cannot be formulated. In some countries, a series of five consecutive trivalent or fifteen consecutive monovalent lots, consisting of five lots of each type, must have been successfully processed before any vaccine from a given manufacturer may be released.

3.3.6 *Test for effective inactivation*

Two samples of at least 500 ml from each monovalent pool shall be effectively tested after removal or neutralization of the formaldehyde by inoculation into tissue cultures for the absence of infective poliovirus. One sample shall be taken at the end of the inactivation period and the other at an interval prior to the end, equivalent at least to the time required

to reduce the initial virus activity by a factor of 10^6 . Each sample shall be inoculated into bottles of tissue cultures derived from at least two different batches of cells. Not more than 100 ml shall be inoculated into each bottle. The dilution of the vaccine in the nutrient fluid shall not exceed 1 : 4 and the area of the cell sheet shall be at least 3 cm² per ml of vaccine. One or more bottles of each batch of cultures shall be set aside to serve as uninoculated control bottles with the same medium.

The formaldehyde in samples of vaccine for tissue culture tests is usually neutralized at the time of sampling by addition of bisulfite. Usually, the samples are subsequently dialyzed. Dialysis is recommended as, in safety tests, it is desirable to use the highest possible concentrations of vaccine, preferably undiluted.

It is possible to conduct tissue-culture tests on non-dialyzed material ; however, this is often found to be toxic to cells even when diluted 1 : 4. If in such tests non-specific degeneration of cells occurs, or if the sensitivity of the tissue-culture system is reduced, the test should be repeated on dialyzed material.

The primary tissue-culture bottles shall be observed for at least two weeks. Not less than two subcultures shall be made from each original bottle, one at the end of the observation period and the other one week earlier. The subcultures shall be observed for at least one week.

If cytopathogenic effects occur in any of the cultures, the decision regarding the further use of the pool shall be deferred until the matter is resolved.

If active poliovirus is isolated, the monovalent pool shall not be used unless the national control authorities permit reprocessing. If satisfactory inactivation is not obtained after one reprocessing, this pool shall not be used for the preparation of vaccine. If, however, satisfactory inactivation is obtained after one reprocessing, this pool shall be used only with the approval of the national control laboratory.

The isolation of active poliovirus from a monovalent pool must in any case be regarded as a break in the consistency record.

If viruses other than poliovirus are present, the pool shall not be used unless it can be demonstrated that such viruses have originated from a source other than the monovalent pool being tested.

The problem of detecting residual active poliovirus in a vaccine is not the same as that of measuring infective virus in untreated suspensions. Poliovirus that has been exposed to the action of formaldehyde without becoming inactivated has been shown to require a much longer time to produce cytopathogenic changes than does untreated virus. For this reason it is desirable that tissue cultures in tests for the presence of

residual active virus be observed for as long a time as is technically possible. A satisfactory tissue-culture system for this purpose depends, therefore, not only on the sensitivity of the cells used for the preparation of the cultures but also on the nutrient fluid.

Kidney cells from some monkey species, for instance from the genera *Macaca*, *Cercopithecus* and *Papio*, appear to be more sensitive than others. If other tissue culture systems are used, they should have been shown to possess at least the same sensitivity as those specified above.

It has been recommended that the sensitivity of each tissue-culture batch be tested by using it for a titration of a reference poliovirus preparation (cf., section 3.3.2) and that the tissue cultures be challenged at the end of the test with a small dose of the reference virus.

Cultures can be maintained in good condition for long periods of time by the addition to the medium of serum, albumin preparations, amniotic fluid, etc. Any such additional components of the medium should have first been shown to be free from virus inhibitors and antibodies.

Maintenance of the cultures in good condition may require frequent changes of culture medium. However, it should be borne in mind that by early changes of fluid unadsorbed virus might be removed and the validity of the test thus impaired.

3.4 *Control on trivalent bulk product*

3.4.1 *Test in tissue cultures for infective poliovirus*

A sample of at least 1500 ml shall be effectively tested for the absence of infective virus by the procedure given in section 3.3.6. If active poliovirus is isolated this trivalent bulk product shall not be used.

3.4.2 *Monkey safety test*

See section 5.3.1

3.4.3 *Preservatives and other substances added*

Preservatives or other substances that might be added to or combined with the vaccine shall have been shown to have no deleterious effect on the product.

4. **Filling and containers**

The requirements concerning filling and containers given in Part A, section 4 of the General Requirements for Manufacturing Establishments and Control Laboratories¹ shall apply with the addition of the following:

All filling operations shall take place in areas that are completely separate from those where work with active virus is performed. The

¹ See Annex 1.

containers shall be of colourless and fully transparent glass so as to permit the detection of visible changes in the vaccine.

Single- and multiple-dose containers may be used.

5. Control tests on final product

5.1 Identity test

A specific identity test shall be done on samples of vaccine from the final containers.

The potency test as described in section 5.4 may serve as the identity test.

5.2 Sterility tests

The vaccine in the final containers shall be sterile.

Samples from final containers selected at random from each filling of each lot shall be tested for sterility. The random sampling shall be made in such a manner that all stages of the filling from the bulk container will have an equal chance of being represented. Not less than ten samples from each filling shall be tested. If the volume of the final container is 1.0 ml or less, the entire contents of the containers shall be cultured. If the volume of the final container is greater than 1.0 ml the volume cultured shall be not less than 1.0 ml.

The vaccine samples shall be inoculated into fluid thioglycollate medium or any other medium equally effective for the growth of aerobes and anaerobes and into Sabouraud medium or any other medium equally effective for the growth of yeasts and fungi. The final dilution of the vaccine in the sterility media shall be such that the preservative no longer exerts bacteriostatic activity. The media inoculated for the detection of bacteria shall be incubated at 30°-32°C for not less than one week or they shall be divided into two portions one being incubated at 35°-37°C and the other at 15°-22°C for not less than one week. The media inoculated for the detection of yeasts and fungi shall be incubated at 20°-25°C for not less than two weeks. Other methods may be used if they have been demonstrated to be at least equally effective.

General requirements for sterility of biological preparations will be formulated as Requirements for Biological Substances No. 6.

5.3 Safety tests

5.3.1 Monkey safety test

A test shall be made in *Macaca* or equally susceptible monkeys for the absence of infective poliovirus in vaccine from the final containers, or in the trivalent bulk product.

It is highly desirable that the monkey safety test be performed on vaccine from final containers. Circumstances may exist,

however, in which the performance of this test on vaccine from final containers is not desirable, e.g., in the case of vaccine to which adjuvants have been added, as this may reduce the infectivity of any active virus present and thus diminish the sensitivity of the test. In such circumstances, a monkey safety test on the bulk trivalent pool would be acceptable, providing that special care is taken to exclude contamination with poliovirus of the vaccine in the final container during the late stages of manufacture.

A total of not less than 20 monkeys in overt good health shall be used.

It is recommended that monkeys be conditioned for at least four weeks before they are subjected to the safety test procedure.

A pre-injection serum sample from each animal must contain no neutralizing antibody against any of the three poliovirus types in a dilution of 1:4 when tested against not more than 1000 TCID₅₀ of virus.

Vaccine shall be injected, under deep anaesthesia, by combined intracerebral, intraspinal and intramuscular routes into monkeys. The intracerebral injection shall consist of 0.5 ml into the thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 ml into the lumbar spinal cord enlargement and may be divided between more than one site. The intramuscular injection shall consist of 1.0 ml into the right leg. At the time of inoculation of vaccine, an intramuscular injection of 200 mg of cortisone acetate shall be given and an intramuscular injection of 300 000 units of procaine penicillin. The cortisone acetate may be given in divided doses over a period of several days, starting two days before the inoculation of vaccine. The monkeys shall be observed for 17-19 days and symptoms suggestive of poliomyelitis be recorded. Provided that at least 60% of the animals survive the first 48 hours after injection, those animals that do not survive this 48-hour period may be replaced by an equal number of test animals. If less than 60% of the original test animals survive the first 48 hours, or if the number of animals that survive the entire test period without significant weight loss is less than 80% of the initial number, the test must be repeated.

At the end of the observation period samples of nervous tissue shall be taken for virus recovery and identification. Histological sections from both spinal cord enlargements shall be examined.

Doubtful histopathological findings necessitate (a) examination of samples of sections from several regions of the brain and spinal cord, and (b) attempts at virus recovery from the nervous tissues previously removed from the animal.

Evidence of intraspinal trauma due to injection must be observed in at least 80% of the animals.

The monkey safety test shall be considered negative if the histological and other studies leave no doubt that poliomyelitis infection did not occur. Otherwise, this vaccine shall not be used.

Doubts have been expressed as to whether the monkey safety test is necessary in addition to the tissue-culture test. Experience, however, would indicate that the monkey test is an essential part of the safety-testing procedures, since it was in monkeys that virus was isolated from vaccine suspected of causing infection, whereas in tissue culture isolations were made only with difficulty.

Laboratories using poliovirus strains of low pathogenicity for vaccine preparation have found that *Macaca irus* tends to be more susceptible than *Macaca mulatta* to such strains.

5.3.2 *Innocuity test*

Absence of toxicity of the vaccine in the final containers shall be demonstrated by tests in guinea-pigs and mice.

5.4 *Potency tests*

The final product shall pass a potency test approved by the national control authority.

Pending the establishment of a stable international reference vaccine and an acceptable level of potency that can be internationally recommended the immunizing power of the final product shall be tested by a method involving injection of vaccine into groups of animals and the determination of the antibody response. The method shall be so designed that numerical values for the immunizing effect against each of the three types of poliovirus can be calculated.

The values obtained should not be significantly inferior to the average values obtained by the same method using a vaccine that has previously been shown to induce antibody formation after two inoculations in a high proportion of children initially devoid of detectable antibodies.

A large body of data has been accumulated on different antigenicity tests using monkeys, guinea-pigs, chicks and rats. Some of these data show a correlation between the results obtained in laboratory animals and the capacity of a vaccine to induce antibodies in man.

The two methods most extensively used are : (a) a monkey potency test involving three inoculations of undiluted vaccine into 12 or more monkeys and the determination of the antibody titres obtained in comparison with known reference sera ; and (b) a guinea-pig potency test involving two inoculations of each of three vaccine dilutions into groups of ten or more guinea-pigs, and a determination of the fraction of these animals surpassing a chosen antibody level.

Similar tests in guinea-pigs and in chicks, as well as *in vitro* tests for the presence of antigen, are being used and studied in several laboratories.

At present, acceptance criteria based on the outcome of the various tests available cannot be validly compared. This can only be achieved on the basis of comparisons of the relative potencies, obtained using different methods and assay designs which include a common stable reference antigen, with the performance of the vaccines in the field.

5.5 Total nitrogen content

Poliomyelitis vaccine shall not contain more than 0.35 mg of total nitrogen per ml.

6. Information required by national control laboratory

Protocols showing the history of each lot of vaccine and the results of all prescribed tests in the degree of detail required by the national control laboratory shall be submitted for approval.

In addition, information shall be provided to the national control laboratory concerning the results of tests for the absence of virus in any pool that has been processed, irrespective of whether the material was incorporated into a finished vaccine or not.

7. Samples required by national control laboratory

For each lot of vaccine, samples shall be submitted to the national control laboratory in sufficient volumes to permit a repetition of all tests. One sample shall be taken from the bulk vaccine at the latest possible stage of production, but before final preservatives, adjuvants, or other substances are added. The formaldehyde in such samples shall be neutralized at the time of sampling by the addition of bisulfite. Another sample shall be taken of the finished product in the final containers, and shall be representative of each separate run of fillings. All samples shall be stored at a temperature between 0° and 10°C.

In the case of poliomyelitis vaccine, the samples required are relatively large in size, as much as 2500 ml of the trivalent bulk vaccine and 200 ml in final containers from each lot being needed to perform the tests for potency and safety.

Accidents, contaminations, technical difficulties, etc., may necessitate repetition of any of the required tests. Manufacturers should therefore follow the practice of retaining additional samples.

8. Labelling

The following information shall appear on the label affixed to each container :

the name and address of the manufacturer ;
the international name and/or the proper name of the product ;
the number of the lot, and the number under which the lot was released ;
the date after which the preparation may not be used.

Moreover, this label, or the label of the carton enclosing one or more final containers, or the leaflet accompanying the containers shall contain the following additional information :

the fact that the vaccine fulfils the requirements of this document ;
the nature of the preparation and the method used for killing the virus ;
the nature and quantity of the preservative, if any, and the remaining amount of formaldehyde, if left un-neutralized ;
the nature and quantities of antibiotics used in the preparation of the vaccine ;
a statement that the storage temperature should always remain between 0°C and 10°C, but that, during normal distribution, a total period not exceeding seven days at ambient temperatures may be permitted ;
the recommended individual dose, and the mode of administration.

The above requirements for labelling have been drafted pending the formulation of general requirements on labelling applicable to all biological products.

9. Distribution and shipping

Part A, section 9 of the General Requirements for Manufacturing Establishments and Control Laboratories¹ shall apply.

10. Storage and expiry date

10.1 Storage conditions

Poliomyelitis vaccine shall be stored at all times at a temperature between 0° and 10°C.

10.2 Expiry date

The expiry date shall not be more than 12 months after the date of the last satisfactory potency test, the date of a potency test being that on which the test animals were inoculated with the vaccine. The expiry date shall not, however, be more than 6 months from the date at which the vaccine was issued by the manufacturer or, if the national control authority so decides, not more than 12 months after the date of release.

¹ See Annex 1.

Part B. National Control Requirements

1. General

The general requirements for control laboratories contained in Requirements for Biological Substances No. 1, Part B,¹ shall apply.

2. Tests to be applied by national control laboratory in the country in which the vaccine is produced

Section 3.2 of Requirements for Biological Substances No. 1, Part B, shall apply.

3. Release and certification

No lot of poliomyelitis vaccine shall be released which does not fulfil the requirements set forth in Part A.

A statement signed by the appropriate official in charge of the national control laboratory shall be provided at the request of the producing laboratory and shall certify whether or not the lot of vaccine in question meets all national requirements as well as the requirements set forth in this document. The certificate shall also state the date of the last satisfactory potency test, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document shall be attached.

The purpose of the certificate is to facilitate the exchange of biological substances between countries.

¹ See Annex 1.

**REQUIREMENTS FOR
POLIOMYELITIS VACCINE
(INACTIVATED)**

(Requirements for Biological Substances No. 2)



WORLD HEALTH ORGANIZATION

PALAIS DES NATIONS

GENEVA

1960

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For the composition of the Study Group see page 14.

**REQUIREMENTS FOR POLIOMYELITIS VACCINE
(INACTIVATED)
(REQUIREMENTS FOR BIOLOGICAL SUBSTANCES No. 2)**

	Page
General considerations	1
Part A. Manufacturing requirements	
1. Definition	2
2. General manufacturing requirements	3
3. Production control	3
4. Filling and containers	8
5. Control tests on final product	9
6. Information required by national control laboratory	12
7. Samples required by national control laboratory .	12
8. Labelling	12
9. Distribution and shipping	13
10. Storage and expiry date	13
Part B. National control requirements	
1. General	14
2. Tests to be applied by national control laboratory in the country in which the vaccine is produced .	14
3. Release and certification	14

General Considerations

The recommendation of international requirements for inactivated poliomyelitis vaccine is complicated by the fact that a number of different manufacturing and testing procedures are in use in various countries. The procedures differ mainly in the incorporation of different virus strains, the inactivation and filtration methods, and the use of preservatives and adjuvants in the final vaccine. In spite of these differences it is felt that certain essential requirements concerning manufacture and control can be formulated. The present recommendations are based on methods currently in use and future revisions will be necessary.

Each of the following sections constitutes a recommendation. The parts of each section which 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 which are printed in small type concern points on which comments seemed desirable.

In case individual countries should wish to adopt these requirements as the basis for their national regulations concerning poliomyelitis vaccine, it is recommended that a clause be included which would permit modifications of manufacturing details on the condition that such modifications had been demonstrated, to the satisfaction of the national control authority, to ensure that the degree of safety and the potency of the vaccine are at least equal to those provided by the requirements formulated below. In any such cases, the World Health Organization should be informed of the action taken.

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

Part A. Manufacturing Requirements

1. Definition

1.1 *International name and proper name*

The international name shall be "Vaccinum poliomyelitis inactivatum". The proper name shall be the equivalent of the international name in the language of the country of origin.

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

1.2 *Descriptive definition*

Vaccinum poliomyelitis inactivatum shall consist of an aqueous suspension of poliovirus hominis¹ types 1, 2 and 3 grown in monkey-kidney tissue cultures and inactivated by a suitable method. The preparation shall satisfy all the requirements formulated below.

1.3 *International standards or reference preparations and international units*

The WHO Expert Committee on Biological Standardization has taken steps to establish an International Standard for Poliomyelitis Vaccine.²

The Expert Committee has established International Reference Preparations of Antipoliomyelitis Sera of Types 1, 2 and 3.³

Samples of these International Reference Preparations may be obtained from the International Laboratory for Biological Standards, Statens Seruminstitut, Copenhagen, Denmark by the national laboratory for biological standards of any country.

¹ International Nomenclature Committee, Sub-Committee on Viruses (1954) *Int. Bull. bact. Nomencl.*, 4, 109

² *Wld Hlth Org. techn. Rep. Ser.*, 1958, 147, 13; 1959, 172, 11

³ *Wld Hlth Org. techn. Rep. Ser.*, 1959, 172, 15

2. General manufacturing requirements

The general manufacturing requirements contained in Requirements for Biological Substances No. 1¹ shall apply to establishments manufacturing poliomyelitis vaccine, with the addition of the following:

Manufacture of poliomyelitis vaccine shall take place in completely separate areas using separate personnel and equipment. The areas where processing of inactivated poliomyelitis vaccine takes place shall be separate from those where work with active virus is performed.

Written procedures for the preparation of poliomyelitis vaccine adopted by the manufacturer shall be submitted for approval to the national control authorities. Proposals for modifications shall be submitted for approval to the national control authorities before their implementation.

It is particularly important that production and control functions be organized as two separate units of the manufacturing establishment with independent responsibilities.

3. Production control

3.1 Control of source materials

3.1.1 Virus strains

Strains of poliovirus used in the production of vaccine shall be identified by historical records, infectivity tests, and by immunological methods. Any strain which will yield a vaccine meeting the requirements set forth in the present document may be used. Production of vaccine shall be based on a seed virus system; the poliovirus used for vaccine production shall not have passed more than ten subcultures, counted from a strain culture on which the original laboratory and field tests were done.

Preference should be given to strains of low pathogenicity to monkeys.

Each new seed lot of a strain of low pathogenicity should be retested for virulence before being used as seed virus. Samples of the strains used should be deposited in the national control laboratory. Strains of poliovirus currently used in production of vaccine may be obtained by application direct or through the World Health Organization to specialized laboratories.*

¹ See *Wld Hlth Org. techn. Rep. Ser.*, 1959, 178, Annex 1.

* The following laboratories have expressed their willingness to supply samples of strains for this purpose: Institut d'Hygiène et d'Epidémiologie, Brussels, Belgium; Statens Seruminstitut, Copenhagen, Denmark; Institut Pasteur, Paris, France; Paul-Ehrlich-Institut, Frankfurt-am-Main, Federal Republic of Germany; Laboratory of Microbiology, Istituto Superiore di Sanità, Rome, Italy; The Poliomyelitis Research Foundation Laboratories, Johannesburg, South Africa; Statens Bacteriologiska Laboratorium, Stockholm, Sweden; Biological Standards Control Laboratories, Medical Research Council, London, N. W. 3, England; Division of Biologics Standards, National Institutes of Health, Bethesda, Md., USA; Moscow Institute for Poliomyelitis Prophylactics, Moscow, USSR.

3.1.2 *Monkeys*

Suitable species of monkeys, in good health, shall be used as the source of kidney tissue for the production of poliovirus. Each animal shall be examined at necropsy for signs of disease and, if there is any pathological lesion of significance with regard to their use in the preparation of the vaccine, the kidneys shall be discarded. Kidney tissue from monkeys that have been used previously for experimental purposes shall not be used. An exception can be made in the case of monkeys used for the safety or potency tests with negative clinical findings.

It is recommended that monkeys be kept in as small groups as possible in order to reduce dissemination of infections within the colony.

3.1.3 *Tissue culture for virus production*

Virus for the preparation of vaccine shall be grown by aseptic methods in cultures of monkey-kidney cells that have not been propagated in series. The maintenance medium shall contain no protein. If animal serum is used in the propagation of cells, the final vaccine shall not contain more animal serum than one part per million.

Suitable antibiotics in minimum concentrations required for sterility may be used. If penicillin is used its concentrations may not exceed 200 International Units per ml. Non-toxic pH indicators may be added, e.g., phenol red in a concentration of 0.002%.

3.2 *Production precautions*

The general production precautions as formulated in Part A, section 3 of the General Requirements for Manufacturing Establishments and Control Laboratories¹ shall apply to the manufacture of poliomyelitis vaccine.

It is recognized that staff members working with active poliovirus may be exposed to the danger of infection. Accordingly, it is recommended that all personnel be immunized, if necessary, with poliomyelitis vaccine.

A further hazard exists because of the occurrence of B-virus infection in some monkeys. In order to minimize the transmission of such infections it is recommended that the handling of these animals be reduced to a minimum, and that workers use protective clothing and other protective devices where possible. It is further recommended that research be conducted towards developing prophylactic agents and protective sera against this infection.

Past experience has shown that monkeys may also be a source of infection due to *Mycobacterium tuberculosis*, and adequate precautionary measures against such infections should be taken.

¹ See *Wld Hlth Org. techn. Rep. Ser.*, 1959, 178, Annex 1.

3.3 Control at the monovalent stages of the product

3.3.1 Treatment before inactivation

Prior to inactivation each monovalent virus pool shall be filtered or clarified.

The importance of filtration or clarification of the crude virus suspensions as a means of improving the regularity of the inactivation process has been clearly established. Generally, filters are used in series or filtration is performed stepwise through filters of decreasing porosity. Seitz S1 pads are most widely used as final filters, but satisfactory results have also been reported with other filter types, as well as with clarification procedures not involving filtration.

3.3.2 Virus titration

After filtration or clarification and before the initiation of inactivation a sample shall be taken of each monovalent pool for titration of infective poliovirus using tissue culture methods. This titration shall be carried out in tenfold dilution steps using ten tubes per dilution, or any other arrangement of tubes and dilutions yielding equal precision.

Each monovalent pool should show a titre of not less than 10^6 TCID₅₀* per ml using a batch of tissue culture of normal sensitivity. This should be confirmed in a parallel titration of a reference poliovirus preparation, the titre of which has been previously determined using monkey-kidney tissue cultures.

The main purpose of determining the titre of virus pools destined for inactivation is to select pools which can be expected to meet potency requirements after inactivation.

At the present time international reference preparations of poliovirus are not available. Pending the establishment of such international reference preparations, it is desirable for national control laboratories to issue reference virus preparations to manufacturers.

Although there may be theoretical reasons for using such reference preparations for all three types of poliovirus, the purpose of checking the sensitivity of tissue cultures may be met by the use of a reference preparation of a single type. A reference preparation of type I poliovirus has proved satisfactory in this respect.

3.3.3 Tests for *M. tuberculosis* and B-virus

Prior to inactivation, adequate samples of each monovalent pool shall be withdrawn for tests in guinea-pigs and in suitable culture media for the absence of *M. tuberculosis*. If the presence of *M. tuberculosis* is demonstrated, any product made from the pool shall be discarded.

Tests for B-virus, if performed at this stage, should be made on samples drawn from monovalent pools before initiation of inactivation, preferably as soon as possible after harvesting.

* TCID₅₀ = Tissue Culture Infective Dose 50%

3.3.4 *Time of inactivation*

Inactivation shall be initiated as soon as possible and not later than 72 hours after filtration or clarification.

It is preferable to start inactivation within 24 hours. Since the purpose of the filtration or clarification step is to remove particulate matter and other interfering substances which may diminish the effectiveness of the inactivation process, and since aggregates tend to increase on standing after filtration or clarification, efforts should be made to keep within this time limit.

3.3.5 *Inactivation procedure*

The virus in the monovalent pools shall be inactivated through the use of an agent or a method which has been demonstrated to be consistently effective in the hands of the manufacturer.

The progress of inactivation should be followed by suitably spaced determinations of virus titres.

Formaldehyde is used as inactivating agent in the production of all current vaccines. Most manufacturers have encountered irregularities in the inactivation process, which are not fully explained. On the basis of the observation that a decrease in the concentration of free formaldehyde occurs in the course of the process, it has been recommended that tests for free formaldehyde be performed at intervals and its concentration maintained at the desired level by intermittent readjustments.

Introduction of a second filtration during the inactivation is another, more widely used, procedure which can effectively reduce the frequency of such irregularities and which is a requirement in some countries. In order to avoid a loss of antigen due to filtration, some producers use a combination of initial formaldehyde treatment with some other method of inactivation.

It should be emphasized that the degree of consistency in producing successfully inactivated pools is an important factor in judging the safety of a given product. However, definite requirements in this respect cannot be formulated. In some countries, a series of five consecutive trivalent or fifteen consecutive monovalent lots, consisting of five lots of each type, must have been successfully processed before any vaccine from a given manufacturer may be released.

3.3.6 *Test for effective inactivation*

Two samples of at least 500 ml from each monovalent pool shall be effectively tested after removal or neutralization of the formaldehyde by inoculation into tissue cultures for the absence of infective poliovirus. One sample shall be taken at the end of the inactivation period and the other at an interval prior to the end, equivalent at least to the time required

to reduce the initial virus activity by a factor of 10^6 . Each sample shall be inoculated into bottles of tissue cultures derived from at least two different batches of cells. Not more than 100 ml shall be inoculated into each bottle. The dilution of the vaccine in the nutrient fluid shall not exceed 1 : 4 and the area of the cell sheet shall be at least 3 cm² per ml of vaccine. One or more bottles of each batch of cultures shall be set aside to serve as uninoculated control bottles with the same medium.

The formaldehyde in samples of vaccine for tissue culture tests is usually neutralized at the time of sampling by addition of bisulfite. Usually, the samples are subsequently dialyzed. Dialysis is recommended as, in safety tests, it is desirable to use the highest possible concentrations of vaccine, preferably undiluted.

It is possible to conduct tissue-culture tests on non-dialyzed material; however, this is often found to be toxic to cells even when diluted 1 : 4. If in such tests non-specific degeneration of cells occurs, or if the sensitivity of the tissue-culture system is reduced, the test should be repeated on dialyzed material.

The primary tissue-culture bottles shall be observed for at least two weeks. Not less than two subcultures shall be made from each original bottle, one at the end of the observation period and the other one week earlier. The subcultures shall be observed for at least one week.

If cytopathogenic effects occur in any of the cultures, the decision regarding the further use of the pool shall be deferred until the matter is resolved.

If active poliovirus is isolated, the monovalent pool shall not be used unless the national control authorities permit reprocessing. If satisfactory inactivation is not obtained after one reprocessing, this pool shall not be used for the preparation of vaccine. If, however, satisfactory inactivation is obtained after one reprocessing, this pool shall be used only with the approval of the national control laboratory.

The isolation of active poliovirus from a monovalent pool must in any case be regarded as a break in the consistency record.

If viruses other than poliovirus are present, the pool shall not be used unless it can be demonstrated that such viruses have originated from a source other than the monovalent pool being tested.

The problem of detecting residual active poliovirus in a vaccine is not the same as that of measuring infective virus in untreated suspensions. Poliovirus that has been exposed to the action of formaldehyde without becoming inactivated has been shown to require a much longer time to produce cytopathogenic changes than does untreated virus. For this reason it is desirable that tissue cultures in tests for the presence of

residual active virus be observed for as long a time as is technically possible. A satisfactory tissue-culture system for this purpose depends, therefore, not only on the sensitivity of the cells used for the preparation of the cultures but also on the nutrient fluid.

Kidney cells from some monkey species, for instance from the genera *Macaca*, *Cercopithecus* and *Papio*, appear to be more sensitive than others. If other tissue culture systems are used, they should have been shown to possess at least the same sensitivity as those specified above.

It has been recommended that the sensitivity of each tissue-culture batch be tested by using it for a titration of a reference poliovirus preparation (cf., section 3.3.2) and that the tissue cultures be challenged at the end of the test with a small dose of the reference virus.

Cultures can be maintained in good condition for long periods of time by the addition to the medium of serum, albumin preparations, amniotic fluid, etc. Any such additional components of the medium should have first been shown to be free from virus inhibitors and antibodies.

Maintenance of the cultures in good condition may require frequent changes of culture medium. However, it should be borne in mind that by early changes of fluid unadsorbed virus might be removed and the validity of the test thus impaired.

3.4 *Control on trivalent bulk product*

3.4.1 *Test in tissue cultures for infective poliovirus*

A sample of at least 1500 ml shall be effectively tested for the absence of infective virus by the procedure given in section 3.3.6. If active poliovirus is isolated this trivalent bulk product shall not be used.

3.4.2 *Monkey safety test*

See section 5.3.1.

3.4.3 *Preservatives and other substances added*

Preservatives or other substances that might be added to or combined with the vaccine shall have been shown to have no deleterious effect on the product.

4. **Filling and containers**

The requirements concerning filling and containers given in Part A, section 4 of the General Requirements for Manufacturing Establishments and Control Laboratories¹ shall apply with the addition of the following:

All filling operations shall take place in areas that are completely separate from those where work with active virus is performed. The

¹ See *Wld Hlth Org. techn. Rep. Ser.*, 1959, 178, Annex 1.

containers shall be of colourless and fully transparent glass so as to permit the detection of visible changes in the vaccine.

Single- and multiple-dose containers may be used.

5. Control tests on final product

5.1 Identity test

A specific identity test shall be done on samples of vaccine from the final containers.

The potency test as described in section 5.4 may serve as the identity test.

5.2 Sterility tests

The vaccine in the final containers shall be sterile.

Samples from final containers selected at random from each filling of each lot shall be tested for sterility. The random sampling shall be made in such a manner that all stages of the filling from the bulk container will have an equal chance of being represented. Not less than ten samples from each filling shall be tested. If the volume of the final container is 1.0 ml or less, the entire contents of the containers shall be cultured. If the volume of the final container is greater than 1.0 ml the volume cultured shall be not less than 1.0 ml.

The vaccine samples shall be inoculated into fluid thioglycollate medium or any other medium equally effective for the growth of aerobes and anaerobes and into Sabouraud medium or any other medium equally effective for the growth of yeasts and fungi. The final dilution of the vaccine in the sterility media shall be such that the preservative no longer exerts bacteriostatic activity. The media inoculated for the detection of bacteria shall be incubated at 30°-32°C for not less than one week or they shall be divided into two portions one being incubated at 35°-37°C and the other at 15°-22°C for not less than one week. The media inoculated for the detection of yeasts and fungi shall be incubated at 20°-25°C for not less than two weeks. Other methods may be used if they have been demonstrated to be at least equally effective.

General requirements for sterility of biological preparations will be formulated as Requirements for Biological Substances No. 6.

5.3 Safety tests

5.3.1 Monkey safety test

A test shall be made in *Macaca* or equally susceptible monkeys for the absence of infective poliovirus in vaccine from the final containers, or in the trivalent bulk product.

It is highly desirable that the monkey safety test be performed on vaccine from final containers. Circumstances may exist,

however, in which the performance of this test on vaccine from final containers is not desirable, e.g., in the case of vaccine to which adjuvants have been added, as this may reduce the infectivity of any active virus present and thus diminish the sensitivity of the test. In such circumstances, a monkey safety test on the bulk trivalent pool would be acceptable, providing that special care is taken to exclude contamination with poliovirus of the vaccine in the final container during the late stages of manufacture.

A total of not less than 20 monkeys in overt good health shall be used.

It is recommended that monkeys be conditioned for at least four weeks before they are subjected to the safety test procedure.

A pre-injection serum sample from each animal must contain no neutralizing antibody against any of the three poliovirus types in a dilution of 1:4 when tested against not more than 1000 TCID₅₀ of virus.

Vaccine shall be injected, under deep anaesthesia, by combined intracerebral, intraspinal and intramuscular routes into monkeys. The intracerebral injection shall consist of 0.5 ml into the thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 ml into the lumbar spinal cord enlargement and may be divided between more than one site. The intramuscular injection shall consist of 1.0 ml into the right leg. At the time of inoculation of vaccine, an intramuscular injection of 200 mg of cortisone acetate shall be given and an intramuscular injection of 300 000 units of procaine penicillin. The cortisone acetate may be given in divided doses over a period of several days, starting two days before the inoculation of vaccine. The monkeys shall be observed for 17-19 days and symptoms suggestive of poliomyelitis be recorded. Provided that at least 60% of the animals survive the first 48 hours after injection, those animals that do not survive this 48-hour period may be replaced by an equal number of test animals. If less than 60% of the original test animals survive the first 48 hours, or if the number of animals that survive the entire test period without significant weight loss is less than 80% of the initial number, the test must be repeated.

At the end of the observation period samples of nervous tissue shall be taken for virus recovery and identification. Histological sections from both spinal cord enlargements shall be examined.

Doubtful histopathological findings necessitate (a) examination of samples of sections from several regions of the brain and spinal cord, and (b) attempts at virus recovery from the nervous tissues previously removed from the animal.

Evidence of intraspinal trauma due to injection must be observed in at least 80% of the animals.

The monkey safety test shall be considered negative if the histological and other studies leave no doubt that poliomyelitis infection did not occur. Otherwise, this vaccine shall not be used.

Doubts have been expressed as to whether the monkey safety test is necessary in addition to the tissue-culture test. Experience, however, would indicate that the monkey test is an essential part of the safety-testing procedures, since it was in monkeys that virus was isolated from vaccine suspected of causing infection, whereas in tissue culture isolations were made only with difficulty.

Laboratories using poliovirus strains of low pathogenicity for vaccine preparation have found that *Macaca irus* tends to be more susceptible than *Macaca mulatta* to such strains.

5.3.2 *Innocuity test*

Absence of toxicity of the vaccine in the final containers shall be demonstrated by tests in guinea-pigs and mice.

5.4 *Potency tests*

The final product shall pass a potency test approved by the national control authority.

Pending the establishment of a stable international reference vaccine and an acceptable level of potency that can be internationally recommended the immunizing power of the final product shall be tested by a method involving injection of vaccine into groups of animals and the determination of the antibody response. The method shall be so designed that numerical values for the immunizing effect against each of the three types of poliovirus can be calculated.

The values obtained should not be significantly inferior to the average values obtained by the same method using a vaccine that has previously been shown to induce antibody formation after two inoculations in a high proportion of children initially devoid of detectable antibodies.

A large body of data has been accumulated on different antigenicity tests using monkeys, guinea-pigs, chicks and rats. Some of these data show a correlation between the results obtained in laboratory animals and the capacity of a vaccine to induce antibodies in man.

The two methods most extensively used are : (a) a monkey potency test involving three inoculations of undiluted vaccine into 12 or more monkeys and the determination of the antibody titres obtained in comparison with known reference sera ; and (b) a guinea-pig potency test involving two inoculations of each of three vaccine dilutions into groups of ten or more guinea-pigs, and a determination of the fraction of these animals surpassing a chosen antibody level.

Similar tests in guinea-pigs and in chicks, as well as *in vitro* tests for the presence of antigen, are being used and studied in several laboratories.

At present, acceptance criteria based on the outcome of the various tests available cannot be validly compared. This can only be achieved on the basis of comparisons of the relative potencies, obtained using different methods and assay designs which include a common stable reference antigen, with the performance of the vaccines in the field.

5.5 *Total nitrogen content*

Poliomyelitis vaccine shall not contain more than 0.35 mg of total nitrogen per ml.

6. **Information required by national control laboratory**

Protocols showing the history of each lot of vaccine and the results of all prescribed tests in the degree of detail required by the national control laboratory shall be submitted for approval.

In addition, information shall be provided to the national control laboratory concerning the results of tests for the absence of virus in any pool that has been processed, irrespective of whether the material was incorporated into a finished vaccine or not.

7. **Samples required by national control laboratory**

For each lot of vaccine, samples shall be submitted to the national control laboratory in sufficient volumes to permit a repetition of all tests. One sample shall be taken from the bulk vaccine at the latest possible stage of production, but before final preservatives, adjuvants, or other substances are added. The formaldehyde in such samples shall be neutralized at the time of sampling by the addition of bisulfite. Another sample shall be taken of the finished product in the final containers, and shall be representative of each separate run of fillings. All samples shall be stored at a temperature between 0° and 10°C.

In the case of poliomyelitis vaccine, the samples required are relatively large in size, as much as 2500 ml of the trivalent bulk vaccine and 200 ml in final containers from each lot being needed to perform the tests for potency and safety.

Accidents, contaminations, technical difficulties, etc., may necessitate repetition of any of the required tests. Manufacturers should therefore follow the practice of retaining additional samples.

8. **Labelling**

The following information shall appear on the label affixed to each container :

the name and address of the manufacturer ;
the international name and/or the proper name of the product ;
the number of the lot, and the number under which the lot was released ;
the date after which the preparation may not be used.

Moreover, this label, or the label of the carton enclosing one or more final containers, or the leaflet accompanying the containers shall contain the following additional information :

the fact that the vaccine fulfils the requirements of this document ;
the nature of the preparation and the method used for killing the virus ;
the nature and quantity of the preservative, if any, and the remaining amount of formaldehyde, if left un-neutralized ;
the nature and quantities of antibiotics used in the preparation of the vaccine ;
a statement that the storage temperature should always remain between 0°C and 10°C, but that, during normal distribution, a total period not exceeding seven days at ambient temperatures may be permitted ;
the recommended individual dose, and the mode of administration.

The above requirements for labelling have been drafted pending the formulation of general requirements on labelling applicable to all biological products.

9. Distribution and shipping

Part A, section 9 of the General Requirements for Manufacturing Establishments and Control Laboratories¹ shall apply.

10. Storage and expiry date

10.1 *Storage conditions*

Poliomyelitis vaccine shall be stored at all times at a temperature between 0° and 10°C.

10.2 *Expiry date*

The expiry date shall not be more than 12 months after the date of the last satisfactory potency test, the date of a potency test being that on which the test animals were inoculated with the vaccine. The expiry date shall not, however, be more than 6 months from the date at which the vaccine was issued by the manufacturer or, if the national control authority so decides, not more than 12 months after the date of release.

¹ See *Wld Hlth Org. techn. Rep. Ser.*, 1959, 178. Annex 1.

Part B. National Control Requirements

1. General

The general requirements for control laboratories contained in Requirements for Biological Substances No. 1, Part B,¹ shall apply.

2. Tests to be applied by national control laboratory in the country in which the vaccine is produced

Section 3.2 of Requirements for Biological Substances No. 1, Part B, shall apply.

3. Release and certification

No lot of poliomyelitis vaccine shall be released which does not fulfil the requirements set forth in Part A.

A statement signed by the appropriate official in charge of the national control laboratory shall be provided at the request of the producing laboratory and shall certify whether or not the lot of vaccine in question meets all national requirements as well as the requirements set forth in this document. The certificate shall also state the date of the last satisfactory potency test, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document shall be attached.

The purpose of the certificate is to facilitate the exchange of biological substances between countries.

¹ See *Wld Hlth Org. techn. Rep. Ser.*, 1959, **178**, Annex 1.

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