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REQUIREMENTS FOR BIOLOGICAL SUBSTANCES

Manufacturing Establishments and Control Laboratories — Poliomyelitis Vaccine (Inactivated) — Poliomyelitis Vaccine (Oral) — Smallpox Vaccine

Revised 1965

Report of a WHO Expert Group

WORLD HEALTH ORGANIZATION

GENEVA

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NOTE: To reduce document size, Annexes 2 and 3 are not included in this document.

WHO EXPERT GROUP ON REQUIREMENTS FOR BIOLOGICAL SUBSTANCES

Revised 1965

Geneva, 16-22 March 1965

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REQUIREMENTS FOR BIOLOGICAL SUBSTANCES

Report of a WHO Expert Group [1]

A meeting of a WHO Expert Group on Requirements for Biological Substances was held in Geneva from 16 to 22 March 1965. Dr P. Dorolle, Deputy Director-General, on behalf of the Director-General, welcomed the participants and thanked them for their willingness to come to Geneva for this meeting.

Since this was the first meeting of its kind, he explained the nature of the task it would undertake. Over the past six years, a number of international requirements for biological substances of importance in medicine had been formulated by groups of experts and published by WHO, and these requirements had been found useful in many countries. None of them had, however, been revised, and the task before the present meeting was to suggest the revisions to some of the earlier sets of requirements which were considered to be necessary in the light of advances in knowledge and experience. He also requested the views of the participating experts on this procedure for revising the requirements recommended for international use, and invited them to make any suggestions as to the procedures that should be adopted for this work.

INTRODUCTION

One of the purposes of international requirements for biological substances is to facilitate the exchange of these substances between countries. Frequently WHO is involved in the supply of vaccines either on behalf of or as a gift to a developing country, and in such instances a vaccine is considered to be satisfactory if the national control laboratory of the country of origin certifies it to have satisfied both the national and the international requirements. WHO has arranged with certain laboratories to check the properties of some vaccines but this is not so for others and extension of this facility to all vaccines would strengthen the control by WHO of vaccines used in its own international programmes.

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[1] The text of this report was submitted before publication to the eighteenth WHO Expert Committee on Biological Standardization. The Committee agreed that the revisions were in accordance with the advances in knowledge and experience that had been made since the original requirements were formulated. In preparing the report for publication, certain minor editorial amendments suggested by the Committee have been taken into account. In addition, a few editorial footnotes have been introduced to provide supplementary information that has become available since the meeting of the WHO Expert Group. — ED.

In most instances the international requirements are similar to national requirements, but in those cases where a difference exists it is the responsibility of the national control authority to authorize such deviations and changes. It would be of great help to WHO if all deviations from international requirements were notified to them, with a statement of the reasons why the changes had been made.

During the last six years, the following requirements have been formulated by international groups of experts and published in the WHO Technical Report Series.

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

In addition to these meetings of study groups and of the Expert Committee on Biological Standardization for the formulation of requirements, groups of experts also meet to consider problems concerned with the eradication of a particular disease and the recommendations of such groups often have a bearing upon the requirements for the control of the various preparations of biological substances. It is important that such recommendations should be incorporated into the requirements.

The present Group was asked to consider revision of the following :

No.	*Year*	
178	1959	Requirements for Biological Substances : 1. General Requirements for Manufacturing Establishments and Control Laboratories 2. Requirements for Poliomyelitis Vaccine (Inactivated)
180	1959	Requirements for Biological Substances : 5. Requirements for Smallpox Vaccine
237	1962	Requirements for Biological Substances : 7. Requirements for Poliomyelitis Vaccine (Oral).

Publication of a set of amendments would be an impractical way of revising the requirements since the complete set of amended requirements would not be contained in a single document. It was therefore decided to prepare new documents.

No attempt has been made to alter the requirements formulated by the original study groups except where this was indicated. Where necessary new requirements have been added to bring them up to date.

GENERAL CONSIDERATIONS

With the rapid development of techniques required in the production and control of virus vaccines the need for keeping accurate records and for submitting full protocols to the national control authority has been recognized. Moreover, the inspection of production areas and the employment of skilled and experienced scientists, both for producing and for controlling the vaccines, give far greater confidence in the product than can be gained solely by the application of tests to the final material. Indeed, perhaps the greatest safety factor is in consistency of production of successive batches.

The developments in virology have largely been in two directions (*a*) the use of cell cultures known to be free from all detectable extraneous agents and (*b*) the development of more sensitive tests for the detection of contaminant viruses. For example, all national requirements for live measles vaccines produced in chick embryo cultures have demanded either by regulation or implication that the tissue used for the growth of virus shall be produced from embryos derived from a flock known to be free from avian leucosis as well as from all other avian pathogens. This demand has been met by the manufacturers, and vaccines free from all detectable extraneous viruses are freely available. Since live measles vaccine, one of the more recently developed vaccines, is being produced in such tissue it is illogical to continue to allow other vaccines to be produced on tissue known to carry a risk of being contaminated by extraneous viruses. Whilst it is recognized that immediate drastic changes in the requirements may severely interrupt the supply of vaccines, it is recommended that steps should be taken to produce all seed lots and vaccines free from detectable extraneous agents as soon as possible. Cell strains known to be free from such agents are rapidly being made available and manufacturers may wish to gain experience in their handling in the event of such cell strains being approved for vaccine production. Already poliomyelitis vaccine (oral) has been produced on human diploid cell cultures and experimental batches of common cold vaccines are being produced in these cells.[1]

[1] The current revisions of the requirements under consideration have, however, not been extended to include vaccines produced on human diploid cells. No national control laboratory has yet approved such vaccines for general use. If such approval is given and the vaccines become freely available, relevant international requirements will need to be formulated.

Developments in the field of inactivated virus vaccines include the production of purified and concentrated vaccines that give long-lasting immunity. These developments should be encouraged. For some virus vaccines, the technical developments for concentrating and extracting the virus antigen have been achieved. In other cases, the addition of an adjuvant may be necessary but knowledge regarding the safety of some adjuvants is scanty. The whole question of adjuvants in vaccines needs consideration with a view to the formulation of requirements for vaccines containing adjuvants.

In revising these international requirements for biological substances account has been taken of the opinions of consultants, the regulations and requirements for the manufacture and control of biological substances that have been formulated in a number of countries, as well as information from both published and unpublished reports. As far as possible, the views of the participants of the groups of experts who formulated the original requirements were sought. In addition, opinions and data have been received from a number of experts, whose assistance is gratefully acknowledged (see " Acknowledgements ", page 8).

The main modifications to the requirements under current consideration concern the following points:

1. *General requirements for manufacturing establishments and control laboratories*

Requirements have been added specifying that the names of scientists responsible for production should be registered with national control authorities and that separate production processes should be isolated. The need for carefully kept records, for ensuring consistency of production and for submission of protocols is emphasized.

2. *Requirements for poliomyelitis vaccine (inactivated)*

Since the original requirements were written, the simian virus SV40 has been detected in virus harvests grown on monkey kidney tissue, especially on tissue from rhesus monkeys. Steps have been taken to eliminate this agent, both by a recommendation that only monkeys known to be free from this virus shall be used for production of the vaccine and by the introduction of tests for the presence of the virus. Recommendations have also been made to ensure that more potent vaccine is produced. The International Reference Preparation of Poliomyelitis Vaccine (Inactivated) and International Standards for Anti-poliovirus Sera types 1, 2 and 3 are now established and it is desirable that information be obtained on the use of these preparations in the control of poliomyelitis vaccine (inactivated).

3. *Requirements for poliomyelitis vaccine (oral)*

The technique for the detection of simian virus SV40 has required modification and a recommendation that only monkeys known to be free from this virus shall be used for the production of the vaccine has been introduced. A test for mycoplasma has been included and the monkey neurovirulence test has been modified to increase its value. It is suggested that reference preparations for each of the types of poliomyelitis virus be made available for the control of virus titre. Since poliomyelitis vaccine (oral) produced in human diploid cell strains has been given to man without, so far, producing untoward effects, indication of the availability of these cells has been mentioned. However, the requirements are not intended to cover these vaccines.

4. *Requirements for smallpox vaccine*

A WHO Expert Committee on Smallpox [1] drew attention to the need for a smallpox vaccine known to be successful especially for revaccination. Accordingly, the requirements for virus concentration have been revised. The need to use for vaccine production a virus strain known to give adequate immunity without untoward reaction in man has been emphasized. Although it is not possible to produce in the skin of animals vaccine that is intrinsically free from detectable extraneous agents, the total bacterial count permitted has been decreased. Vaccine produced in eggs or tissue culture, however, should use only tissue free from detectable extraneous agents. It has been recommended that dried vaccine known to be stable at ambient temperatures should be used in hot countries and where transportation and refrigeration are difficult. The International Reference Preparation of Smallpox Vaccine has now been established and it is recommended for use in testing vaccine for virus concentration.

FUTURE REVISIONS OF REQUIREMENTS

Advances in knowledge and development of new techniques will call for future revisions of requirements for various biological substances published by WHO if they are to remain useful working documents. This is the first time that any of these requirements has been revised and the Group was of the opinion that such revisions could only be made following discussion by groups of experts in the particular fields to which the requirements refer. Such groups should make use of all available information on the experience gained in various countries in the application of the

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1964, **283**.

requirements since their original publication and should take into account the difficulties encountered and how they may have been resolved.

Once revisions have been made and approved for distribution they should be circulated to national control authorities and other interested institutions as soon as possible.

Revision of national requirements for biological substances generally results in improvement in the quality of the products and it is equally important that revision of international requirements should have a similar effect.

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Annex 1

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

Revised 1965 *

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

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* General Requirements for Manufacturing Establishments and Control Laboratories (Requirements for Biological Substances No. 1) were first published in *Wld Hlth Org. techn. Rep. Ser.*, 1959, **178**. 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. This Annex comprises a revised version of these requirements, incorporating additions and amendments made by the WHO Expert Group on Requirements for Biological Substances which met in March 1965.

imported products, by a control authority may have international as well as national implications.

The most important information concerning the safety of a biological substance is given by consistency of production, which is complementary to the tests applied to the final filled material. The approval of manufacturing methods, the maintenance of accurate records, and the inspection of production by the national control authority play a major part in creating confidence in the safety of the product.

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 included in definitive national requirements. The parts of each section which are printed in small type are comments and recommendations for guidance.

Should individual countries 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 it be demonstrated, to the satisfaction of the national control authority, that such modified requirements ensure a degree of safety and a potency of the products at least equal to those provided by the requirements formulated below. It is desirable that the World Health Organization should then be informed of the action taken.

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

Part A. General Requirements for Manufacturing Establishments

1. Personnel

Manufacturing shall be in charge of 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. The names and qualifications of such specialists, especially those responsible for signing a protocol, shall be registered with the national control authority.

> 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, pharmacology, 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 regular 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. Any persons not concerned with the production process who enter the production area for the purposes of inspection shall be supplied with sterile protective clothing.

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

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 should preferably be equipped with recording thermometers and with appropriately placed alarm signals to ensure that an early repair can be effected in case of breakdown.

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 that in adjacent rooms.

Staff working in these rooms shall be provided with a special changing room and wear sterile gowns.

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 should be 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. Provision shall be made for animal inoculation rooms which shall be separate from the post-mortem rooms.

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

2.6 *Apparatus*

Instruments and apparatus shall be of high precision.

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

3. Production control

3.1 *Production methods*

Production methods shall be approved by the national control authority and written procedures shall be prepared for each product, describing each step in production and testing. Proposals for modifications shall be submitted for approval to the national control authority before their implementation. At any one time, manufacture of each biological product shall take place in a separate area using separate equipment. Only strains of micro-organisms or viruses used for the production of the particular biological product shall be permitted in the manufacturing area.

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.

Adequate staff shall be provided to avoid the necessity for staff to work in any one working day in areas in which different biological products are being manufactured.

Sequential manufacture of different products in the same area shall be allowed provided that the method of sterilization of the area between manufacture of the different products is shown to be satisfactory and has the approval of the national control authority.

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. If the production process or test necessitates the use of animals of a particular species or strain, the animals used shall be approved by the national control authority.

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.

It is desirable to use specific pathogen-free (S.P.F.) animals for both production and testing of certain biological substances.

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. They shall take place in areas that are completely separate from those in which living micro-organisms, including viruses, are handled.

The filling process should be checked at least twice each year at the end of a working day by filling not less than 500 ampoules with a nutrient medium containing no antibiotics or bacteriostatic substances and incubating the complete batch of filled ampoules. Not more than 1% of the ampoules filled in this way should show signs of contamination and all contaminants should be identified.

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

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 maintained 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 repetition of the control tests.

8. Labelling

All products shall be clearly identified by labels. The information given on the label on the container or the label on the package shall be determined by the national control authority.

The label on the container shall show at least :

the name of the product (i.e., the international name and/or the proper name) ;
the name of the manufacturer (and address if required) ;
the number of the final lot ;
the recommended human dose and route of administration ;
the condition of storage and expiry date.

The label on the package shall, in addition to the information shown on the label on the container, show at least :

the nature and amount of any preservative or added substance present in the product ;
a description of any substance likely to cause any adverse reaction ;
any contra-indications to the use of the product.

In addition, the label on the package or the leaflet in the package should indicate the stability under different storage conditions, contain instructions for the use of the product and give information about reactions that may follow administration of the product.

It is desirable to have an indication that the product fulfils the relevant requirements published in the WHO technical report series.

It is also desirable that the labels used remain permanently attached to the containers under all storage conditions and that an area of the container be left uncovered to allow inspection of the contents.

If the final container is not suitable for labelling (for example, a capillary tube), it should be in a labelled package.

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.

No new biological substance shall be released until consistency of production has been established.[1] In routine production, failure of a single batch to meet the requirements for safety shall be considered as a breakdown in production, and consistency shall be re-established to the satisfaction of the national control authority before any further batches are released.

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

The statements concerning storage temperatures and expiry dates appearing on the label and the packing leaflet, as required in Part A, section 8, shall be based on experimental evidence and shall be submitted for approval to the national control authority.

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 administered by or on behalf of the national control authority. In the event of manufacture and national control being done in the same establishment, the control laboratory shall be an independent unit directly responsible to the national control authority.

Authority for taking measures designed to ensure that biological substances used in a country are safe, potent and

[1] In some countries, consistency of production has to be established for the first five production batches.

biologically pure, normally rests upon the health department of the government of that country. This authority must, however, 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.

3. Scope of activities

The system of control shall include licensing of manufacturers and routine inspection of their establishments. In addition, the national control authority shall determine the extent of control testing of individual products.

All 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 of these requirements 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. The manufacturing area and process shall be made accessible to the national control authority for inspection at all reasonable times.

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 should therefore develop their own technique for this purpose.

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. This is best accomplished by breeding and maintenance of animals primarily free from specific pathogens and protected against infections by contaminated food, air and water, or by contact with vectors, animals and man.

The national control authority should investigate the use of specific pathogen-free (S.P.F.) animals for control tests.

Control authorities should be familiar with international standards for the assay of potency and, where national standards exist, they should be calibrated in terms of the international standards.

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 adequately to fulfil the requirements for licensing and inspection of manufacturing establishments 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 to fulfil specified requirements.

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 the administrative aspects of control procedures. In general, this will be best accomplished by direct supervision of junior staff during the actual performance of duties, but such supervision may be supplemented where conditions permit by more formal instruction.

Annex 4

REQUIREMENTS FOR SMALLPOX VACCINE (REQUIREMENTS FOR BIOLOGICAL SUBSTANCES No. 5)

Revised 1965 *

General Considerations

Since the Requirements for Smallpox Vaccine (Requirements for Biological Substances No. 5) [1] were published in 1959, there have been advances in production and control which necessitate modification of the require-

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* Requirements for Smallpox Vaccine (Requirements for Biological Substances No. 5) were first published in *Wld Hlth Org. techn. Rep. Ser.*, 1959, **180**. The original draft of these requirements was prepared by a Study Group on Requirements for Biological Substances which met in Geneva from 3-8 November 1958. The members of this Study Group were: Dr J. Desbordes, Directeur du Contrôle bactériologique, Laboratoire national de la Santé publique, Ministère de la Santé publique et de la Population, Paris, France; Dr D. G. Evans, Director, Department of Biological Standards, National Institute for Medical Research, London, England (Chairman); Dr R. Gispen, Director, National Institute of Public Health, Utrecht, Netherlands; Dr L. Greenberg, Chief, Biologics Control Laboratories, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada; Dr E. Krag Andersen, Statens Seruminstitut, Copenhagen, Denmark; Dr U. Krech, Chief, Virus Department, Serum and Vaccine Institute, Berne, Switzerland (Rapporteur); Dr A. Lafontaine, Directeur, Institut d'Hygiène et d'Epidémiologie, Brussels, Belgium; Dr R. Muckenfuss, Technical Director, Naval Medical Research Institute, Bethesda, Md., USA; Dr C. Puranananda, Director, Queen Saovabha Memorial Institute, Bangkok, Thailand; Dr G. Renoux, Directeur, Institut Pasteur, Tunis, Tunisia; Dr R. Sanjiva Rao, Assistant Director, Virus Research Centre, Poona, India (Vice-Chairman); Dr N. K. Jerne, Chief Medical Officer, Biological Standardization, WHO, acted as Secretary. This Annex comprises a revised version of these requirements, incorporating additions and amendments made by the WHO Expert Group on Requirements for Biological Substances which met in March 1965.

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1959, **180**.

ments. Furthermore, there has been a meeting of a WHO Expert Committee on Smallpox [1] and some of the recommendations of that Committee suggest the need for modification of these requirements.

The recommendation of international requirements for smallpox vaccine is complicated by the fact that a number of different manufacturing and testing procedures are in use in various countries. The procedures differ in the vaccinia virus strain used, the preservatives added, the form in which the vaccine is issued, the methods for testing the potency, and the animal or tissues used for growing the virus.

The origin of the different strains is not known. Some vaccine strains are more pathogenic for man than others and since there is no evidence that a strain producing severe local lesions and marked systemic disturbance confers better protection than strains producing milder clinical reactions, the less pathogenic strains giving satisfactory immunity should be preferred for vaccine production. Further studies should be made of the various strains used and the vaccine obtained by different procedures. The local systemic reactions after vaccination and revaccination in the field should be compared in order to test the validity of the present requirements for virus strains. Studies on the antigenic and immunogenic properties of strains in relation to their reactivity would be useful and the genetic characters of these strains should be investigated.

Smallpox vaccines prepared in the skin of living animals have been in world-wide use for generations and considerable evidence has been accumulated on the protective value of vaccination with this type of vaccine. Adequate precautions must be taken, however, to ensure that no infectious agent transmissible to man exists in the animal. More recently, vaccine prepared by growing the virus in the developing chick embryo has come into use, but although this type of vaccine has certain advantages, there is, as yet, only limited information on its efficacy and safety in the field and on the duration of the immunity which it induces. As a result of the advances in tissue-culture techniques, an increasing interest has now developed in the use of such cultures for production of vaccine. Further investigations of smallpox vaccine prepared in this way are highly desirable in order that information may be collected over a sufficient period to permit a final evaluation of its efficacy and safety.

It is recognized that prolonged repeated passage of a virus strain in tissue culture or on the chorio-allantoic membrane may reduce its immunogenic property. The seed lot used for inoculating the tissue cultures or eggs for vaccine production should not be more than five passages removed from the animal host to which the virus was adapted. When eggs or tissue derived from chick embryos are used, the eggs must be obtained from a flock free from avian leucosis viruses. Similarly, tissue cultures should be

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1964, **283**.

free from known adventitious agents. It has been reported that smallpox vaccine prepared in human diploid cell strains has been used on an experimental scale.

Some observations suggest that vaccines of relatively low potency, though adequate for primary vaccination, may be inadequate for revaccination. Evidence of consistent successful revaccination in comparison with a suitable reference vaccine should be obtained before a vaccine produced on a new tissue is accepted. In the present requirements for virus concentration, full stress has been laid on the enumeration of pock-forming units in the chorio-allantoic membranes of chick embryos, as it is only with this test that the results have been correlated with the results of vaccination and revaccination in man.

Liquid vaccines deteriorate rapidly at moderately high atmospheric temperatures and on exposure to sunlight. Since a more stable product can be obtained by freeze-drying, the production of vaccine in a dried form should be preferred for hot countries and where transport or refrigeration is difficult. The use of freeze-dried smallpox vaccine is particularly important in extensive vaccination programmes involving several countries and every effort should be made to develop methods for producing large quantities of freeze-dried vaccine that conform to the requirements recommended for international use.

The present requirements have been formulated principally to cover vaccines intended for administration by the multiple pressure or single scratch method of vaccination.

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 included in definitive national requirements. The parts of each section which are printed in small type are comments and recommendations for guidance.

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

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

Part A. Manufacturing Requirements

1. Definitions

1.1 *International name and proper name*

The international name shall be " Vaccinum variolae ". 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 variolae is a fluid or dried preparation of vaccinia virus grown in the skin of living animals or in the membranes of the chick embryo or in *in vitro* cultures of suitable tissues. The preparation shall satisfy all the requirements formulated below.

1.3 *International standard and reference preparations*

The International Reference Preparation of Smallpox Vaccine (established in 1962) is dispensed in ampoules containing 14 mg of freeze-dried smallpox vaccine.

This reference preparation is in the custody of the International Laboratory for Biological Standards, Statens Serum-institut, Copenhagen. Samples are distributed free of charge on request to national control laboratories. The international reference preparation is intended for the calibration of national reference preparations for use in the manufacture and laboratory control of smallpox vaccine.

The provision of an international standard for anti-smallpox serum is at present the subject of an international collaborative study sponsored by the World Health Organization. Such a standard can be used for the assay of variola and vaccinia antibodies.[1]

1.4 *Terminology*

Primary seed lot : A quantity of virus adapted to, and grown on the skin of a living animal, which has been processed together and has a uniform composition.

Secondary seed lot : A quantity of virus grown in the skin of living animals or in the chorio-allantoic membranes of chick embryos or in tissue cultures, which is uniform with respect to composition and is not more than 5 passages removed from a primary seed lot.

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[1] The International Standard for Anti-Smallpox Serum was established by the eighteenth WHO Expert Committee on Biological Standardization (*Wld Hlth Org. techn. Rep. Ser.*, to be published) — ED.

Single harvest : A quantity of material harvested from one animal or a quantity of material harvested from a group of chick embryos or tissue cultures inoculated, incubated and harvested together.

Bulk material : The material at any stage after harvesting and before filling into final containers. Bulk material may be prepared from one or a number of single harvests.

Final bulk : A quantity of vaccine after completion of preparations for filling and present in the container from which the final containers are filled.

Filling lot (final lot) : A collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling or drying. A filling lot must, therefore, have been filled in one working session and (if applicable) have been dried together.

Pock-forming unit : The smallest quantity of virus suspension that will produce a single pock on the chick chorio-allantoic membrane.

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

2. General manufacturing requirements

The general requirements for manufacturing establishments contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] shall apply to establishments manufacturing smallpox vaccine.

3. Production control

3.1 *Control of source materials*

3.1.1. *Virus strains*

The strains of virus used in the production of all seed lots shall be identified by historical records. They shall have been shown to the satisfaction of the national control authority to yield immunogenic vaccines which produce typical vaccinal lesions in the skin of man followed by insusceptibility to subsequent challenge by revaccination with a strain of virus known to protect man against variola. The strains shall produce a characteristic vesicular eruption in the skin of rabbits and reproducible characteristic pock lesions in the membranes of chick embryos. In addition, the vaccine strains shall be characterized by serological tests and animal inoculation.

Records shall be maintained of all tests made periodically for verification of strain characters.

The strain used for vaccine production should be one that has never shown a greater tendency to produce generalized

[1] See Annex 1 of this report.

lesions or lesions of the nervous system in either man or animals than other strains of vaccinia virus which have been in general use for many years and have been found to be satisfactory without producing severe local lesions and marked systemic disturbance. Strains of so-called " neurovaccine " should be excluded.

3.1.2 *Animals or tissues for the production of seed virus and vaccine*

Only healthy animals or tissues from healthy animals, susceptible to ectodermal inoculations with vaccinia virus, or chick embryos obtained from healthy flocks shall be used for vaccine production. They shall conform to all the requirements given in Part A, section 3.2 of these requirements. If cell cultures are used for vaccine production they shall be shown to be free from detectable adventitious agents.

Different species of animals may be used for vaccine production or for preparing seed virus. Calves, sheep, buffaloes, donkeys and rabbits are used successfully in different countries.

The chorio-allantoic membrane of the developing chick embryo and tissues from the embryos or young animals of susceptible species have also been found suitable for virus propagation.

3.1.3 *Seed lot system*

A primary seed lot shall be used as original material for the preparation of a secondary seed lot. The secondary seed lot shall be not more than five passages removed from a primary seed lot. If vaccine is produced in the skin of a living animal the secondary seed lot shall be prepared from the primary seed lot without passage in chick embryos or tissue cultures. Vaccines shall be prepared from a seed lot without intervening passage.

Seed lots should be maintained either in dried, frozen, or glycerinated form. If a glycerinated seed lot is used it should be kept continuously at a temperature below 0°C.

3.1.4 *Tests on seed lots for the presence of extraneous micro-organisms*

The seed lot, in the dilution used as inoculum for the production of vaccine in the skin of animals, shall satisfy the requirements of Part A, section 3.3.4 of these requirements.

The seed lot used for the production of vaccine in chick embryos or in tissue cultures shall, after rehydration if applicable, satisfy the requirements of Part A, section 3.3.5.

3.2 *Production precautions*

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

Laboratories) [1] shall apply to the manufacture of smallpox vaccine with the addition of the following:

3.2.1 *Vaccines produced in the skin of living animals*

The animals shall be freed of ectoparasites, and each animal shall be kept in quarantine under veterinary supervision for at least two weeks prior to the inoculation of the seed virus. Before inoculation the animals shall be cleaned, and thereafter kept in scrupulously clean stalls until the vaccinal material is harvested.

> The use of bedding, unless sterilized and changed frequently, should be avoided. The stalls, including feed boxes, should be designed so as to make cleaning easy, and dust-producing food should be avoided.

During a period of five days before inoculation and during incubation the animals shall remain under veterinary supervision, they shall remain free from any sign of disease, and daily rectal temperatures shall be recorded. If any abnormal rise in temperature occurs, or if any clinical sign of disease is observed, the production of vaccine from the group of animals concerned shall be suspended until the cause of these irregularities has been resolved. The prophylactic and diagnostic procedures adopted to exclude the presence of infectious disease shall be submitted for approval to the national control authority.

> According to the species of animal used and the diseases to which that animal is liable in the country where the vaccine is being produced, the prophylactic and diagnostic procedures to be used will vary. They must exclude the possibility of transmitting diseases within the country where the vaccine is prepared, but consideration should also be given to the danger of spreading diseases to other countries or continents to which the vaccine may be shipped.
>
> Special attention should always be given to foot-and-mouth disease, brucellosis, Q fever, tuberculosis, and dermatomycosis, but in some areas it will be necessary to consider diseases such as contagious pustular dermatitis (orf), pulpy kidney disease, sheep pox, anthrax, rinderpest, haemorrhagic septicaemia, Rift Valley fever, and many others.

The inoculation of seed virus shall be made on such parts of the animal as are not liable to be soiled by urine and faeces. The surface used for inoculation shall be so shaved and cleaned as to procure the nearest possible approach to surgical asepsis. If any antiseptic substance deleterious to the virus is used in the cleaning process it shall be removed by thorough rinsing with sterile water prior to inoculation. During inoculation, the exposed

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[1] See Annex 1 of this report.

surface of the animal not used for inoculation shall be covered with sterile covering.

Many workers prefer to inoculate the ventral surface of female animals. If male animals are used this area is more liable to soiling by urine and faeces than the flank, which may be equally susceptible to vaccinia virus and easier to keep clean, especially since the animal tends to rest on the uninoculated side.

It is recommended that narcotic or anaesthetic drugs be used to save the animal from unnecessary discomfort and pain during the process of shaving, cleaning and inoculation.

After inoculation the area may be covered with suitable antibiotics.

Before the collection of the vaccinal material, any antibiotic shall be removed and the inoculated area shall be subjected to a repetition of the cleaning process. The uninoculated surfaces shall be covered with sterile covering.

Before harvesting, the animal shall be killed painlessly. The animals shall be exsanguinated before harvesting to avoid heavy admixture of the vaccinal material with blood.

The vaccinal material from each animal shall be collected separately with aseptic precautions.

All animals used in the production of vaccine shall be examined by autopsy. If evidence of any generalized or systemic disease other than vaccinia is found, the vaccinal material from that animal shall be discarded. If the disease is considered to be a communicable one, the harvest from the entire group of animals exposed shall be discarded.

3.2.2 *Vaccines produced in the chick embryo*

Only eggs from flocks known to be free from disease, including avian leucosis, shall be used.

In particular, it is desirable that the eggs should be derived from flocks free from *Salmonella pullorum*, *Mycobacterium tuberculosis*, Rous virus, mycoplasma and other agents pathogenic for chickens.

Living embryos after incubation for a suitable period shall be inoculated with seed virus which satisfies the requirements of Part A, sections 3.1.3 and 3.1.4 of these requirements. After further incubation for a suitable period, the vaccinal material shall be harvested with aseptic precautions.

3.2.3 *Vaccines produced in tissue culture*

Only primary tissue cultures from animals known to be free from disease shall be used. The virus shall be grown and harvested with aseptic precautions. No material of human origin shall be added to the cultures at any stage.

Suitable antibiotics in minimum concentrations required for sterility may be used but the use of penicillin and streptomycin should be prohibited.

3.3 *Control of the bulk material*

3.3.1 *Initial treatment*

The vaccinal material harvested from the skin of each animal shall be subjected to a treatment designed to reduce its content of living extraneous micro-organisms, if this is necessary, to satisfy the requirements of Part A, section 3.3.4 of these requirements. No antibiotics shall be added to the bulk material.

If the vaccine is intended for issue in the liquid form, this treatment may consist of the addition of glycerol or other suitable diluent, with or without an antibacterial substance, and temporary storage at a suitable temperature.

If the vaccine is intended for issue in the dried form, the treatment may consist of the addition of a suitable antibacterial substance and/or of the removal of micro-organisms by centrifugation.

Vaccinal material collected from chick embryos or tissue cultures does not need such treatment, but glycerol and/or an antibacterial substance should be added as a precaution against later contamination.

It is recognized that antibiotics are used in the preparation of vaccines from tissue cultures, but in general the addition of antibiotics to smallpox vaccine should be discouraged.

3.3.2 *Final bulk*

After the initial treatment, vaccine intended for issue in the liquid form may be made up by dilution of bulk material with glycerol and/or another suitable diluent.

Vaccine intended for issue in the dried form may be subjected to additional processes before dilution of the bulk material.

Before making up a final bulk, it is advisable to do preliminary tests on the single harvests for potency and for the presence of living extraneous micro-organisms.

3.3.3 *Tests for virus concentration on the final bulk*

The final bulk shall pass the test for virus concentration described in Part A, section 5.2.1.

3.3.4 *Tests for the presence of living extraneous micro-organisms in the final bulk prepared in the skin of living animals*

The final bulk shall pass the following tests for the presence of living extraneous micro-organisms, unless these tests have already been passed by each of the single harvests represented in the final bulk.

3.3.4.1 *Tests for total bacterial content*

Suitable dilutions of the final bulk shall be made in a suitable diluent not deleterious to living bacteria. At least three 1-ml samples of each dilution shall be cultured on nutrient-broth-agar plates. The plates shall be incubated for 72 hours between 15°C and 22°C and for a further period of 48 hours between 35°C and 37°C. From the number of colonies appearing on the plates the number of living bacteria in 1 ml of the final bulk shall be calculated. If this number exceeds 500, the final bulk shall be subjected to further treatment or be discarded.

Suitable control plates containing higher dilutions of the final bulk shall be included in this test in order to make sure that the number of colonies appearing on the test plates has not been influenced by the inhibitory action of any preservative present in the final bulk.

3.3.4.2 *Test for the presence of* Escherichia coli

At least three 1-ml samples of a 1 : 100 dilution of the final bulk shall be cultured on plates of a medium suitable for differentiating *E. coli* from other bacteria. The plates shall be incubated for 48 hours at 35°C to 37°C. If *E. coli* is detected, the final bulk shall be subjected to further treatment or be discarded.

The presence of *E. coli* in this test might indicate a heavy faecal contamination.

3.3.4.3 *Test for the presence of haemolytic streptococci, coagulase-positive staphylococci, or any other pathogenic micro-organisms which are known to be harmful if introduced into the human body by the process of vaccination*

At least three 1-ml samples of a 1 : 100 dilution of the final bulk shall be cultured on plates of blood agar. The plates shall be incubated for 48 hours at 35°C to 37°C and the colonies appearing shall be examined.

If any of the organisms mentioned are detected, the final bulk shall be subjected to further treatment or be discarded.

In some countries culture of the final bulk in salt meat broth is made for the purpose of detecting staphylococci.

3.3.4.4 *Test for the presence of* Bacillus anthracis

Any colony seen on any of the plates used in the tests described in Part A, sections 3.3.4.1, 3.3.4.2 and 3.3.4.3 which morphologically resembles *B. anthracis* shall be examined. If the organisms contained in the colony are non-motile, further tests for the cultural character of *B. anthracis* shall be made, including pathogenicity tests in suitable animals. If *B. anthracis* is found to be present, the final bulk shall be discarded.

In countries where anthrax presents a serious risk, this test should be based on a larger number of colonies.

3.3.4.5 *Test for the presence of* Clostridium tetani *and other pathogenic spore-forming anaerobes*

A total volume of not less than 1 ml of the final bulk, taken preferably from the depth of the bulk and not from the upper surface, shall be distributed in equal amounts into ten tubes, each containing not less than 10 ml of a medium suitable for the growth of anaerobic micro-organisms. The tubes shall be held at 65°C for one hour in order to reduce the content of non-spore-forming organisms, after which they shall be incubated for at least one week between 35°C and 37°C.

From every tube showing growth, subcultures shall be made on to plates of a suitable medium which shall be incubated anaerobically at the same temperature. All anaerobic colonies shall be examined and identified and if *Cl. tetani* or other pathogenic spore-forming anaerobes are present the final bulk shall be discarded.

Organisms resembling pathogenic *Clostridia* found in the tube culture from which the subculture was made may be tested for pathogenicity by inoculation into animals as follows: Groups of not less than two guinea-pigs and five mice are used for each tube culture to be tested. 0.5 ml of the cultures is mixed with 0.1 ml of a freshly prepared 4% solution of calcium chloride and injected intramuscularly into each of the guinea-pigs; 0.2 ml of the cultures mixed with 0.1 ml of this calcium chloride solution are injected intramuscularly into each of the mice. The animals are observed for one week. If any animal develops symptoms of tetanus, or if any animal dies as a result of infection with spore-forming anaerobes, the final bulk should be discarded.

If other methods are used for this test, they should have been demonstrated, to the satisfaction of the national control authorities, to be at least equally effective for detecting the presence of *Cl. tetani* and other pathogenic spore-forming anaerobes.

3.3.5 *Test for bacteriological sterility of the final bulk prepared in chick embryos, or in tissue cultures*

Each final bulk shall be tested for bacterial sterility according to the requirements given in Part A, section 5 of Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances).[1]

If growth appears in any of the cultures the final bulk shall be discarded or the test repeated. The final bulk shall be discarded if the same type of organism appears in more than one test, but no final bulk shall be passed unless the final test shows no growth throughout.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1960, **200**, 13.

4. Filling and containers

The requirements concerning filling and containers given in Part A, section 4 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] shall apply with the addition of the following:

All containers of the final vaccine shall be shown to be sterile before filling and shall be made of a material demonstrated, to the satisfaction of the national control authority, to have no deleterious effect on the vaccine.

In some countries, containers of liquid vaccine may not be hermetically closed; if this is permitted the form of closure shall be submitted to the national control authority for approval.

Containers of dried vaccine shall be hermetically sealed under vacuum or after filling with pure, dry, oxygen-free nitrogen or any other gas not deleterious to the vaccine.

All hermetically sealed containers shall be tested for leaks after sealing. All defective containers shall be discarded.

Single- and multiple-dose containers may be used. Each container of dry vaccine should be issued together with an ampoule of sterile reconstituting fluid. This fluid may contain glycerol and/or some suitable antiseptic substance. The containers should be issued in a form that renders the process of reconstitution as simple as possible.

5. Control tests on final product

5.1 *Identity test*

An identity test shall be performed on at least one labelled container from each filling lot by appropriate methods.

The test for virus concentration as described in Part A, section 5.2.1 of these requirements may serve as an identity test.

A test may also be made in the scarified skin of rabbits. Suitable dilutions of vaccine are applied on scarified areas of skin. After four to seven days the vaccine should produce lesions characteristic of vaccinia.

5.2 *Tests for virus concentration on vaccine in final containers*

A test for virus concentration shall be made on each filling lot in accordance with the requirements described in Part A, section 5.2.1 of these requirements. Dried vaccine shall be reconstituted to the form in which it is to be used for human inoculation before the test is made.

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[1] See Annex 1 of this report.

Tests should be done in parallel with a reference vaccine which has been calibrated against the international reference preparation of smallpox vaccine.[1]

5.2.1 *Test for virus concentration in membranes of chick embryos*

At least ten chick embryos, each of about 12 days' incubation, shall be divided into two equal groups. To the chorio-allantoic membrane of each embryo of the first group, 0.1 ml or 0.2 ml of a suitable dilution of the vaccine shall be applied. To the membrane of each of the second group of embryos 0.1 ml or 0.2 ml of another suitable dilution of the vaccine shall be applied. After the optimal time of incubation the total number of discrete specific lesions shall be counted on the membrane of each embryo. The dilutions shall be so chosen that the membranes of at least one of the groups yield countable numbers of lesions exceeding ten per membrane. From the number of lesions counted in this group and from the dilution and volumes used, the number of pock-forming units in one ml of the undiluted vaccine shall be calculated. This number shall exceed 1×10^8.

It has been shown that the severity of systemic reactions after successful vaccinations in young adults is related to the virus strain rather than to the number of pock-forming units present in the dose of vaccine applied.

5.2.2 *Other tests*

Tests for virus concentration in the scarified skin of rabbits may also be used provided it has been shown that the results correlate with those obtained using the membranes of chick embryos.

In some countries tests are made in tissue culture either by counting the number of plaque-forming units per ml of vaccine or by determining the dilution of vaccine which will produce cytopathic effects in 50% of tissue cultures. More experience should be gained in the use of these tests and in the relationship of the results to those obtained in the test performed in chick embryos before these tests alone can be used for determining virus concentration.

5.3 *Tests for the presence of extraneous living micro-organisms in the vaccine in final containers*

Not less than four final containers (or not less than 10 if single-dose containers) giving a total pooled volume of not less than 0.5 ml shall be taken at random from each filling lot in such a manner that all stages of the filling from the bulk container shall be represented. Dried vaccine shall be

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[1] The purpose of using a reference vaccine is to ensure that the system used has adequate sensitivity. The International Reference Preparation of Smallpox Vaccine when reconstituted with 1 ml of fluid per ampoule has been shown in a number of different laboratories to have approximately 1×10^8 pock-forming units per ml. — ED.

reconstituted to the form in which it is to be used in human inoculation. The vaccine thus collected shall pass the test described in Part A, section 3.3.4.1 or 3.3.5 of these requirements, whichever is applicable.

5.4 *Innocuity test*

Each filling lot shall be tested for abnormal toxicity by appropriate tests involving injection into rabbits. The tests shall be approved by the national control authority.

Mice and guinea-pigs may also be used for this test.

5.5 *Heat-resistance test on dried vaccine*

At least one container of dried vaccine from each filling lot shall be incubated at a temperature of not less than 37°C for not less than 4 weeks and tested for virus concentration. The vaccine passes the test if the requirements described in Part A, section 5.2.1 are fulfilled and at least one tenth of the virus concentration is retained.

In some countries a more rapid stability test is made by heating the vaccine to 100°C for 1 hour. The vaccine passes the test if at least one tenth of the virus concentration is retained.

5.6 *Preservatives and other substances added*

No antibiotics shall be added to smallpox vaccine.

If the liquid vaccine or reconstituted dried vaccine contains preservatives or other added substances such substances shall have been shown, to the satisfaction of the national control authority, to have no deleterious effect on the product in the amounts present and to cause no untoward reactions in vaccinated subjects. If phenol is present, its concentration shall not exceed 0.5%. Further, the substance used shall fulfil the requirements of the International Pharmacopoeia or a pharmacopoeia approved by the national control authority.

6. Records

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

7. Samples

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

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[1] See Annex 1 of this report.

8. Labelling

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

The leaflet accompanying the package shall include the following information:

the tissue or animal in which the vaccine was prepared;

any antibiotics used in the preparation of the vaccine (except such antibiotics as may have been applied to the skin of inoculated animals and removed before harvesting);

if the vaccine is in the dried form, a statement that, after rehydration of the dried vaccine, the vaccine shall be used within 24 hours or within 7 days if it can be stored under conditions in which potency and sterility can be maintained.

Instructions for the use of dried vaccine when issued in a container hermetically sealed under vacuum should specify the precautions to be taken when opening a container in order to avoid dispersion of the vaccine into the surroundings.

9. Distribution and shipping

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

10. Storage and expiry date

The statements concerning storage temperatures and expiry dates appearing on the label and the leaflet, as required in Part A, section 8 of these requirements, shall be based on experimental evidence and shall be submitted for approval to the national control authority.

10.1 *Storage conditions*

Before being distributed by the manufacturing establishment, or before being issued from a depot for the maintenance of reserves of vaccine, all liquid vaccines in their final containers shall be kept constantly at a temperature below −10°C, and all dried vaccines in their final containers at a temperature below +10°C.

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[1] See Annex 1 of this report.

10.2 *Expiry date*

The date after which liquid vaccine may not be used shall be not more than 12 months after passing the last test for virus concentration. The date after which dried vaccine may not be used shall be not more than 36 months after passing the last test for virus concentration. The expiry date shall not, however, be more than three months for liquid vaccine or more than twelve months for dried vaccine from the date on which the vaccine was issued by the manufacturer or from a depot.

Part B. National Control Requirements

1. General

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

2. Release and certification

A vaccine lot shall be released only if it fulfils Part A of the present requirements.

A statement signed by the appropriate official of the national control laboratory shall be provided at the request of the manufacturing establishment and shall certify whether the lot of vaccine in question meets all national requirements as well as Part A of the present requirements. 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.

3. Efficacy and safety of the vaccine in the field

The appropriate health authorities should satisfy themselves, on the basis of vaccination results, that the vaccine lots released give close to 100% " takes " in susceptible children and do not give rise to complications.

It is also important that similar studies should be made periodically to determine the success rate in revaccination.

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[1] See Annex 1 of this report.