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**REQUIREMENTS FOR  
BIOLOGICAL SUBSTANCES**

**7. Requirements for Poliomyelitis Vaccine (Oral)**

**Report of a Study Group**

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**STUDY GROUP ON  
REQUIREMENTS FOR POLIOMYELITIS VACCINE (ORAL)**

*Geneva, 7-12. November 1960*

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## REQUIREMENTS FOR BIOLOGICAL SUBSTANCES

### 7. Requirements for Poliomyelitis Vaccine (Oral) \*

#### Report of a Study Group

The Study Group on Requirements for Poliomyelitis Vaccine (Oral) met in Geneva from 7-12 November 1960.<sup>1</sup>

Dr N. I. Grashchenkov, Assistant Director-General of the World Health Organization, opened the meeting and welcomed the members of the Group. He outlined the task of the Study Group which was to draw up internationally acceptable requirements that should be met in the preparation of oral poliomyelitis vaccine in order to ensure that the product is a safe and immunogenic prophylactic agent. International requirements would provide guidance to those concerned with the preparation and the control of oral poliomyelitis vaccine in different countries and, by promoting uniformity, would facilitate the exchange of vaccines between different countries.

The Assistant Director-General reviewed the difficulties facing the Study Group in its task, owing to the rapid development of virological techniques and of preventive public health measures. In spite of the short time that live oral poliomyelitis vaccine had been in use, a large body of data, both from the laboratory and from the field, would demand the attention of the Study Group.

#### 1. GENERAL CONSIDERATIONS

The Study Group noted the papers presented at the First and the Second International Conferences on Live Poliovirus Vaccines that were held in Washington, D.C., in June 1959 and June 1960, sponsored by the Pan American Health Organization and the World Health Organization.<sup>2</sup> The Study Group also noted the third report of the Expert Committee on

\* Live poliovirus vaccine.

<sup>1</sup> Publication of this report has been delayed pending agreement by the members of the Study Group on various technical amendments proposed by other experts in thirty countries.

<sup>2</sup> Pan American Health Organization, *Scientific Publication*, 1959, No. 44 and 1960, No. 50.

Poliomyelitis<sup>1</sup> in which the Expert Committee recommended that the World Health Organization convene a study group to undertake the task of drafting international requirements for the production and testing of this vaccine.

The Study Group considered the reports<sup>2</sup> of the previous Study Groups on Requirements for Biological Substances that had drafted the International Requirements Nos. 1 to 6, and it agreed that International Requirements for Oral Poliomyelitis Vaccine could be fitted into the framework adopted in these reports. In its discussions, the Study Group also considered the preliminary draft of international requirements for oral poliomyelitis vaccine, prepared by the WHO Secretariat. It surveyed the regulations and requirements<sup>3</sup> for the manufacture and control of oral poliomyelitis vaccine that had been proposed in Canada, Czechoslovakia, Italy, Switzerland, the Union of South Africa, the USSR, the United Kingdom and the United States of America. The Group furthermore considered several working documents and unpublished data submitted by its members, as well as the opinions and data received from Dr H. R. Cox, Dr H. Koprowski, Dr A. B. Sabin, and other experts.<sup>4</sup> The Group acknowledged its appreciation of this assistance, which had greatly facilitated its task.

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1960, 203, 38.

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1959, 178, 179 and 180; 1960, 200.

<sup>3</sup> Canada, *Proposed regulations for live, oral, poliovirus vaccine* — Nagler, F. P., Laboratory of Hygiene, Ottawa, unpublished working document WHO/BS/IR/80; Czechoslovakia, *Proposed regulations for live poliovirus vaccine* — Skovranek, V., Department of Epidemiology, Ministry of Health, Prague, unpublished working document WHO/BS/IR/82; Italy, *Proposed requirements for poliomyelitis vaccine prepared from attenuated virus strains* — Penso, G. & Balducci, D., Istituto Superiore di Sanità, Rome, unpublished working document WHO/BS/IR/90; Switzerland, *Directions for the production and testing of live poliovirus vaccine* — Schär, M., Serum and Vaccine Control Office, Federal Health Department, Berne, unpublished working document WHO/BS/IR/92; Union of South Africa, *Proposed regulations for live attenuated poliovirus vaccine* — Gear, J. H. S., Union Health Department, Pretoria, unpublished working document WHO/BS/IR/81, Rev. 1; Union of Soviet Socialist Republics, *Technical requirements for production, control and release of live, oral poliomyelitis vaccine from Sabin's attenuated strains* — Chumakov, M. P., Institute for Poliomyelitis Research, Academy of Medical Sciences, Moscow, unpublished working document WHO/BS/IR/94; United Kingdom, *Suggested methods for the preparation and testing of live attenuated poliovirus vaccine* — Evans, D. G., British Medical Research Council's Advisory Committee on Safety Tests for Poliomyelitis Vaccine, London, unpublished working document WHO/BS/IR/79, Rev. 1; United States of America, *Recommendations relating to the manufacture of poliovirus vaccine, live, oral* — Murray, R., Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, Bethesda, Md., unpublished working document WHO/BS/IR/78; *Biologic products, additional standards: Poliovirus vaccine, live, oral, notice of proposed rule making*, Public Health Service (42 CFR Part 73), Department of Health, Education and Welfare, Washington, D.C.

<sup>4</sup> Chumakov, M. P., et al., unpublished working document WHO/BS/IR/95; Cox, H. R., unpublished working document WHO/BS/INT/20; United States of America, Division of Biologics Standards, National Institutes of Health, Bethesda, Md., unpublished working document WHO/BS/IR/89; Anderson, Gaylord, W., unpublished working document WHO/BS/INT/22; Koprowski, H., unpublished working document

After a general discussion of preventive measures against poliomyelitis, both by means of inactivated and of oral poliomyelitis vaccines, and after a survey of the methods for the production and control of oral poliomyelitis vaccine currently in use in various countries, the Study Group decided to restrict its recommendations to the preparation of oral poliomyelitis vaccine in monkey kidney tissue cultures, since only vaccines prepared by this method had so far been extensively used in man.

On the basis of the above-mentioned documents and considerations, the Study Group proceeded to prepare the draft of the International Requirements for Oral Poliomyelitis Vaccine which are given in the Annex to the present report.

## 2. PROBLEMS NEEDING FURTHER INVESTIGATION

The Study Group noted the recommendations for further research made by the Expert Committee on Poliomyelitis in its Third Report, particularly the problem of strain identification dealt with in sub-section 6.1 (c) of that report.<sup>1</sup>

### 2.1 International and national reference preparations of poliovirus

The Study Group noted the arrangements that had so far been made by the Expert Committee on Biological Standardization for providing international reference preparations of anti-poliovirus sera and of inactivated poliomyelitis vaccine.

In the control of oral poliomyelitis vaccine, reference preparations of poliovirus are needed both for the control of titration methods, and for comparative purposes in tests for the constancy of neurovirulence and other properties.

The national control authorities in all countries producing oral poliomyelitis vaccine should provide national reference preparations for these purposes. The Study Group referred the problems of establishing international reference preparations of poliovirus strains and vaccines to the attention of the Expert Committee on Biological Standardization.

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WHO/BS/IR/83; Koprowski, H. & Plotkin, S. A., unpublished working document WHO/BS/IR/85; Melnick, J. L. & Penttikokko, U., unpublished working document (received from Murray, R.) WHO/BS/INT/19; Murray, R., unpublished working document WHO/BS/IR/88; Plotkin, S. A. unpublished working document WHO/BS/IR/84; Przesmycki, F. & Dobrowolska, H., unpublished working document WHO/BS/IR/83; Sabin, A. B., unpublished working document WHO/BS/IR/87; Schär, M., unpublished working document WHO/BS/IR/91; Slonim, D., Mareš, I., Dřevo, M., Cinnerová, O., Šturmová, H. & Michl, J., unpublished working document WHO/BS/IR/86.

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1960, **203**, 49.

## 2.2 Collection of vaccine and serum samples

Advances in virological techniques may lead to the development of methods for testing further properties of oral poliomyelitis vaccine strains and for detecting adventitious agents hitherto unrecognized which may have been present in vaccines previously administered to man. It would therefore be desirable to retain, for possible study at a later date, samples of all lots of vaccines produced.

It would also be of value to maintain samples of sera from vaccinated persons for comparative studies in the future.

The Study Group was of the opinion that the establishment of collections of vaccine samples and serum samples should be undertaken at a national level and that the possibility of creating such collections at an international level might well be explored.

## 2.3 Adventitious agents

The frequency with which adventitious agents, particularly the "vacuolating agent" and the so-called "foamy agent", are encountered in the production and control procedures for oral poliomyelitis vaccine, presents problems of great difficulty. If it were possible to manufacture virus suspensions free from such agents by the use of simple cell culture methods, other than primary monkey kidney cell culture, the production of vaccines would be greatly simplified and their cost reduced. The availability of a cell system more suitable than primary monkey cell cultures would be of importance, not only in the manufacture of poliomyelitis vaccine, but also in the development of other live virus vaccines. The continued search for such cell systems should be supported.

The detection of certain agents originating from monkey kidney cell cultures raises the problems of whether their presence is permissible and whether they can be removed. Some workers would permit the presence of agents that are not known to be pathogenic for man. Vacuolating agent has undoubtedly been present in several vaccine lots that have been administered to man. Studies concerning the infectivity of this agent in man have produced evidence suggesting that vacuolating agent, simian virus 40 (SV40), can multiply upon administration to man by the oral route, but clinical symptoms have not been recognized. The Group considered it desirable that all seed lots should be free from adventitious agents.

As regards the removal of contaminating viral agents from oral poliomyelitis vaccine, the Study Group noted encouraging experimental developments in differential inactivation procedures, such as the treatment of bulk suspensions with chloroform-ether or with magnesium chloride prior

to filtration, or the use of photodynamic inactivation methods.<sup>1,2</sup> By photodynamic action in the presence of a dye many viruses, such as B virus, vaccinia virus, Echo-10 virus, vacuolating agent, adenovirus, and several of the so-called simian agents, can be inactivated at a high rate while the loss of titre of poliovirus and other enteroviruses under the same conditions takes place at an almost imperceptible rate. On incubation of bulk suspensions at 50°C for two hours in the presence of 1 molar magnesium chloride, poliovirus remains stable whereas viruses containing deoxyribonucleic acid, such as vacuolating agent (SV40), are inactivated. Studies aimed at the application of such differential inactivation methods on a production scale are of considerable interest.<sup>3</sup>

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<sup>1</sup> Pan American Health Organization, *Scientific publication*, 1960, No. 50; United States of America, Division of Biologics Standards, National Institutes of Health, Bethesda, Md., unpublished working document WHO/BS/IR/89.

<sup>2</sup> Murray, R., unpublished working document WHO/BS/IR/88.

<sup>3</sup> Wallis, C. & Melnick J. L., *Stabilization of poliovirus by magnesium chloride*. *Tax. Rep. Biol. Med.* (in press).



## Annex

### REQUIREMENTS FOR POLIOMYELITIS VACCINE (ORAL) (REQUIREMENTS FOR BIOLOGICAL SUBSTANCES No. 7)

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#### General Considerations

The formulation of international requirements for oral poliomyelitis vaccine is complicated by the following: (a) a number of different attenuated strains of poliovirus have been developed, all of which have been used for human immunization; (b) large-scale production and control methods are based on relatively few years of experience, and (c) oral poliomyelitis vaccine differs from any other live vaccine in several respects, such as the degree of genetic stability of the virus strain used, its capacity to spread from vaccinated to non-vaccinated persons, and the fact that it is prepared in monkey kidney tissue which can harbour simian viruses that are difficult to detect and of unknown pathogenicity for man. Moreover, the major advances in the techniques of virus detection that have coincided with the development of this vaccine have focused attention on the possible presence of viral contaminants not only in oral poliomyelitis vaccine but also in other live viral vaccines.

Immunization campaigns on a large scale have been conducted in recent years by public health authorities in several countries, including Albania, Bulgaria, Czechoslovakia, Germany, Hungary, South Africa and

USSR, using vaccine prepared from strains of types 1, 2 and 3 supplied by Dr A. B. Sabin, and in Poland using vaccines of types 1 and 3, supplied by Dr H. Koprowski. Furthermore, vaccines prepared from these strains, as well as from those developed by Dr H. R. Cox, have been used in large-scale immunization projects in many countries, including Colombia, Congo, Costa Rica, Germany, Malaya, Mexico, Nicaragua, Southern Africa, Uruguay and the United States of America. The evidence thus far obtained in these mass campaigns has indicated that the immunization of man with live poliovirus vaccines by the oral route is an effective and safe procedure.

The reliance on the efficacy and safety of oral poliomyelitis vaccine rests mainly on the experience gained in the immunization of man, and the primary purpose of laboratory tests is, therefore, to ensure that new batches of vaccines do not differ from those that have already been shown to be safe and effective in man.

In spite of these considerations, which necessitate an elaborate control system, oral poliomyelitis vaccine is already in use on an extensive scale in many countries. It is therefore important that international agreement be obtained with respect to the acceptance criteria to which this vaccine should conform. The present formulation of international requirements is based on the methods currently in use for preparation of the vaccine from virus grown in monkey kidney tissue, and revisions in the light of future developments will be necessary.

Each of the following sections constitutes a recommendation. The parts of each section that are printed in large type have been written in the form of requirements so that, if a health administration so desires, these parts as they appear may be included in definitive national requirements. The parts of each section that are printed in small type concern points on which comments seemed desirable. Attention is drawn to the recommendations printed in small type in Part A, sections 3.2.3, 3.2.4, and 3.3.4, concerning tests in human amnion cell cultures for the presence of adventitious viruses. Several workers are of the opinion that such tests in human cell cultures should be made obligatory at some stage during the manufacture of each lot of oral poliomyelitis vaccine. It has not been possible to reach general agreement on this point. In release certificates, issued for international purposes, the national control authority should therefore state whether or not the lot of oral poliomyelitis vaccine has been tested in human cell cultures, apart from meeting the requirements of the present document (see Part B, section 2).

Should individual countries wish to adopt these requirements as the basis of their national regulations concerning oral poliomyelitis vaccine, it is recommended that a clause be included which would permit modifications of manufacturing requirements 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 potency of the vaccine

are at least equal to those provided by the requirements formulated below. 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. Definition

#### 1.1 *International name and proper name*

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

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

#### 1.2 *Descriptive definition*

Vaccinum poliomyelitis perorale is a preparation of live attenuated *Poliovirus hominis* (types 1, 2 or 3), containing any one or any combination of the three types, and satisfying all the requirements formulated in this document.

#### 1.3 *International standards and international reference preparations*

International reference preparations of antipoliomyelitis sera of types 1, 2 and 3 were established by the WHO Expert Committee on Biological Standardization in 1958. Samples of these international reference preparations may be obtained from the International Laboratory for Biological Standards, Statens Seruminstitut, Copenhagen, Denmark. The Expert Committee has taken steps to replace these international reference preparations by international standards for antipoliovirus sera of types 1, 2 and 3. The Expert Committee has also taken steps to provide an international reference preparation of inactivated poliomyelitis vaccine.

Since no international standards or international reference preparations of live, attenuated polioviruses, or of oral poliomyelitis vaccine, are yet available, no requirements based on comparisons with such preparations can at present be formulated.

#### 1.4 *Terminology*

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

*Seed lot* : A quantity of virus processed together and of uniform composition. *Primary* and *secondary seed lots* are not more than four passages removed from an original vaccine.

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

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

The terms *final bulk* and *final lot* are used in the sense defined in Part A, section 2, of Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances).<sup>1</sup>

A *final vaccine lot* is not more than five passages removed from an original vaccine.

## 2. General manufacturing requirements

The general manufacturing requirements contained in Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)<sup>2</sup> shall apply to establishments manufacturing oral poliomyelitis vaccine, with the addition of the following :

Production of oral poliomyelitis vaccine shall take place in completely separate areas, using separate equipment.

Such areas should be so situated and ventilated that the hazard of contamination is reduced to a minimum.

Procedures involving micro-organisms other than the vaccine strains, or the use of tissue culture cell lines other than primary cultures, shall not be conducted in the production areas.

In order to ensure that there will be no risk of contaminating the vaccine at any stage during manufacture by pathogenic micro-organisms that may have previously been present in the production areas, these areas shall be decontaminated before they are used for the manufacture of oral poliomyelitis vaccine.

The production of oral poliomyelitis vaccine shall be conducted by a separate staff, which shall consist of healthy persons, who shall submit to regular medical examination. Steps shall be taken to ensure that all persons in the production areas and monkey quarters are immune against poliomyelitis and do not excrete poliovirus or other micro-organisms of significance to the safety of the vaccine. If persons employed in the production have been working with other infectious agents or with animals,

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1960, 200.

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1959, 178, Annex 1.

they shall not return to the production areas on the same day. This also applies to persons engaged in operating on monkeys.

Suitable laboratory clothing and shoes shall be worn in the production areas.

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

Written descriptions of procedures for the preparation of oral poliomyelitis vaccine 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.

### 3. Production control

#### 3.1 Control of source materials

##### 3.1.1 Virus strains

The strains of poliovirus used in the production of oral poliomyelitis vaccine shall be identified by historical records, which should include information on the origin of the strains, on the methods used in their attenuation, as well as on all other points listed in Part B, section 1.1, of this document, in which recommendations to the national control authorities concerning strain selection are set forth. Only strains approved by the national control authority shall be used.

##### 3.1.2 Monkeys for the production of seed virus and vaccine

Monkeys of a suitable species, in good health, and which have not previously been used for experimental purposes of significance to the safety of the vaccine, shall be used as the source of kidney tissue for the production of seed virus and vaccine. They shall conform to all the requirements given in section 3.2.1 below.

Different species of monkeys of the genera *Macaca* and *Cercopithecus* are currently being used.

##### 3.1.3 Seed lot system

The production of vaccine shall be based on the seed lot system. The seed virus for the production of vaccine shall be original vaccine, or a seed lot used in preparing original vaccine, or a seed lot prepared therefrom. Seed lots shall be prepared in monkey kidney-cell cultures under conditions satisfying the requirements of section 3.2, and shall be stored at a temperature below  $-20^{\circ}\text{C}$ . All seed lots shall meet the criteria of section 3.1.4.

It is recommended that a large primary seed lot be set aside as the basic material to which the manufacturer can always return for the preparation of secondary seed lots. It is desirable to store seed lots at a temperature below  $-60^{\circ}\text{C}$ .

#### 3.1.4 *Tests on seed lots*

The seed lot used for the production of vaccine shall be free from detectable extraneous viruses pathogenic to man, and shall satisfy the requirements of sections 3.3, 3.4, 3.5 and 3.6. Each seed lot shall have been tested in parallel with an original vaccine, according to the requirements of sub-section 3.5.5.

It is desirable that the seed lot be free from detectable adventitious agents.

The manufacturer should obtain a sufficient amount of original vaccine to enable him to perform all tests that are designed to ensure close similarity in laboratory performance between new seed lots and original vaccine.

The test described in sub-section 3.5.5.1 shall also be done using the intraspinal and the intramuscular routes of injection. For the intramuscular test the same virus dilutions, in 5 ml quantities, and the same number of monkeys shall be used as described for the intrathalamic test. For the intraspinal test 5 virus dilutions, spaced tenfold, shall be used and, for each dilution, at least 5 monkeys shall be used, 0.1 ml of the dilution being injected into the grey matter of the lumbar spinal cord enlargement. These tests shall not show any significant differences between a seed lot and original vaccine.

#### 3.2 *Production precautions*

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

##### 3.2.1 *Monkeys used for the production of vaccine*

Monkeys shall be kept in well-constructed animal rooms. The cages shall be covered on all sides except the front.

It is recommended that not more than two monkeys be housed per cage and that the cages be spaced as far as possible, with adequate ventilation.

Monkeys shall be kept in quarantine groups and cage-mates shall not be interchanged. A quarantine group is a colony of selected, healthy monkeys kept in one room, with separate feeding and cleaning facilities, and having no contact with other monkeys during the whole of the quarantine period. The quarantine period shall be at least 6 weeks.

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1959, 178, Annex 1.

The monkeys shall be under veterinary supervision. If disease is observed in any monkey, none of the monkeys from the quarantine group concerned shall be used for vaccine production until the cause of the disease has been resolved and shown not to impair the safety of the vaccine.

It is recommended that strict measures be enforced in order to reduce the risk of dissemination of infections within and between quarantine groups.

Past experience has shown that monkeys may be infected with *Mycobacterium tuberculosis*. Adequate diagnostic measures should therefore be applied, and precautions should be taken to protect the personnel.

When monkeys are killed for removal of kidneys, they shall be thoroughly examined by a qualified person, experienced in the diagnosis of monkey diseases, particularly for evidence of tuberculosis and B-virus infection. If a monkey shows any pathological lesion relevant to the use of its kidneys in the preparation of the vaccine, this monkey shall not be used, and none of the remaining monkeys of the quarantine group concerned shall be used for vaccine production unless it is evident that their use will not impair the safety of the vaccine.

All operations described in this section shall be conducted outside the actual production areas.

### 3.2.2 *Monkey kidney cell cultures for vaccine 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 added serum.

Suitable antibiotics in small concentrations may be used. If penicillin is used its concentrations should not exceed 200 international units per ml. Non-toxic pH indicators may be added, such as phenol-red in a concentration of about 0.002%.

Each group of cell cultures derived from a single monkey is used to produce a single harvest and shall, therefore, be prepared and tested as an individual group.

### 3.2.3 *Tests of cell cultures used for vaccine production*

On the day of inoculation with seed virus, each cell culture shall be examined for degeneration. If this examination shows evidence that a culture is infected with cytopathic virus, or with other microbial agents, the whole group of cultures concerned shall not be used for vaccine production.

A sample of 10 ml of the pooled fluid removed from the cell cultures of the kidneys of each single monkey on the day of inoculation with the seed virus shall be inoculated into monkey kidney-cell cultures prepared from the same species, but not the same animal, as that used for vaccine production, as described in sub-section 3.3.4. Another sample of 10 ml shall be inoculated into rabbit kidney-cell cultures. If by these tests evidence is found of the presence of an adventitious agent in a cell culture, the single harvest from the group of cell cultures concerned shall not be used for vaccine production. If these tests are not done immediately, samples shall be stored as required in section 3.3.2.

Some workers consider that, in addition to these tests, the fluid removed from the cell cultures on the day of inoculation with the seed virus must be tested for the presence of adventitious agents by inoculation of 10-ml samples into human amnion-cell cultures and into kidney-cell cultures from *Cercopithecus* monkeys, and that, if evidence is found of the presence of an adventitious agent by these tests, the single harvest from the group of cell cultures concerned should not be used for vaccine production.

#### 3.2.4 Tests of control cell cultures

On the day of inoculation with the seed virus, cultures prepared from 25 % of the cell suspension obtained from the kidneys of each single monkey shall remain uninoculated, and shall serve as controls. These control cultures shall be incubated under the same conditions as the inoculated cultures for at least 2 additional weeks, and shall be examined during this period for evidence of cytopathic changes. Not more than one fifth of the control cultures shall be discarded for non-specific, accidental reasons.

At the time of harvest, or not more than 7 days after the day of inoculation with the seed virus, 10-ml volumes of the pooled fluid from each group of control cultures shall be subcultured (*a*) into monkey kidney-cell cultures prepared from the same species, but not the same animal, as that used for vaccine production, and (*b*) into rabbit kidney-cell cultures. Uninoculated cell cultures shall be used as controls. All cultures shall be observed for at least 2 weeks. The tests shall be conducted as described in sub-sections 3.3.4 and 3.3.5.

The control cultures shall also be shown, by the addition of guinea-pig red blood cells, to be free from haemadsorption viruses.

If by the tests required in this section evidence is found of the presence in a control culture of any adventitious agent that may be pathogenic to man, the single harvest from the group of cell cultures concerned shall not be used for vaccine production.

At the end of the observation period, the control cell cultures shall be examined for degeneration. If this examination shows evidence that a

culture is infected with cytopathic virus, the whole group of cultures concerned shall not be used for vaccine production.

In several countries it is considered obligatory to make additional tests for adventitious agents in the fluid from the control cultures by inoculation of 10-ml samples into human amnion-cell cultures and into kidney-cell cultures from *Cercopithecus* monkeys; and to repeat these tests in all 4 cell culture systems at the end of the observation period of at least 2 weeks. It would be desirable to exclude, without qualification, all vaccines containing adventitious agents.

### 3.2.5 *Temperature of incubation*

During the period between inoculation and harvest, the cell cultures shall at no time be at a temperature above 35°C.

## 3.3 *Control of single harvests*

### 3.3.1 *Single harvest*

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

### 3.3.2 *Sampling*

Samples for testing single harvests shall be taken immediately on harvesting. If the tests described in sub-sections 3.3.4, 3.3.5 and 3.3.6 are not performed immediately, the samples for these tests shall be kept at a temperature below -60°C.

The volumes of single harvests required to be tested in the sub-sections of section 3.3 are based on the assumption that the virus concentrations in these samples are within the range between  $10^7$  TCID<sub>50</sub> and  $5 \times 10^7$  TCID<sub>50</sub> per ml.<sup>1</sup> If the titre is significantly higher, the volumes to be tested may be reduced accordingly. If the titre is below  $10^7$  TCID<sub>50</sub> per ml, the volumes to be tested shall be increased accordingly.

### 3.3.3 *Sterility tests*

A volume of 10 ml, or at least 0.5% of each single harvest shall be tested for bacterial and mycotic sterility as described in Part A, sections 5.2.1.2 and 5.3.1.2 of the Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances).<sup>2</sup>

### 3.3.4 *Tests of neutralized single harvests*

A sample of at least 5 ml of each single harvest shall be neutralized by specific poliomyelitis antisera prepared in animals other than monkeys.

<sup>1</sup> TCID<sub>50</sub> = tissue culture infective dose 50%.

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1960, **200**.

The immunizing antigens used in preparing such antisera shall be cultured in non-simian cells.

The neutralized suspension shall be tested in monkey kidney-cell cultures prepared from the same species, but not the same animal, as that used for vaccine production.

The neutralized suspension shall be inoculated into bottles of these cell cultures, in such a way that the dilution of the suspension in the nutrient medium does not exceed 1 : 4. The area of the cell sheet shall be at least 3 cm<sup>2</sup> per ml of neutralized suspension. At least one bottle of uninoculated cell culture shall serve as a control and shall be maintained by nutrient medium containing the same concentration of the specific serum used for neutralization. The cultures shall be incubated at a temperature of 37°C and be maintained for a period of at least 2 weeks. If any cytopathic changes occur in any of the cultures the causes of these changes shall be investigated. If the cytopathic changes are shown to be due to unneutralized poliovirus the test shall be repeated. If the cytopathic changes are shown not to be due to virus infection originating from the single harvest, or if there are no cytopathic changes, the single harvest shall have passed this test.

Some workers consider it necessary also to test the neutralized single harvest in kidney-cell cultures of *Cercopithecus* monkeys, as well as in human amnion-cell cultures.

#### 3.3.5 Test in rabbit kidney-cell cultures

A sample of at least 20 ml of each single harvest shall be tested in rabbit kidney-cell cultures. The sample shall be inoculated into bottles of these cell cultures, in such a way that the dilution of the suspension in the nutrient medium does not exceed 1 : 4. The area of the cell sheet shall be at least 3 cm<sup>2</sup> per ml of suspension. At least one bottle of uninoculated cell cultures shall serve as control. The cultures shall be incubated at 37°C and maintained for a period of at least 2 weeks. If any cytopathic changes occur in any of the cultures the cause of these changes shall be investigated and if they are shown not to be due to virus infection originating from the single harvest, or if there are no cytopathic changes, the single harvest shall have passed the test. Serum used in the nutrient medium of rabbit kidney-cell cultures shall have been shown to be free from B virus inhibitors.

If the presence of B virus is demonstrated in this test, or in the test of sub-section 3.4.3, the manufacture of oral poliomyelitis vaccine shall be discontinued. It shall not be resumed until a thorough investigation has been completed and the necessary precautions have been taken, and then only with the approval of the national control authorities.

It would be desirable if the absence of B virus inhibitors from the serum used in the nutrient medium for the above test could be ascertained in a different laboratory, so as to avoid the handling of B virus in the vaccine-producing establishment.

### 3.3.6 *Tests in animals*

The single harvests shall be tested in animals, as described in the sub-sections of section 3.4 unless these tests are performed on the bulk suspension.

### 3.3.7 *Preservatives and stabilizers*

Preservatives or stabilizers that may be added to the single harvests or to the bulk suspension, as well as all operations aimed at the removal or inactivation of adventitious agents, shall have been shown not to impair the safety and effectiveness of the vaccine.

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

## 3.4 *Control of the bulk suspension before filtration*

### 3.4.1 *Bulk suspension*

The bulk suspension shall be prepared by pooling a number of single harvests of the same virus type.

### 3.4.2 *Sampling*

Samples for testing the bulk suspension before filtration shall be taken immediately after the bulk suspension has been prepared and, if not tested immediately, shall be kept at a temperature below  $-60^{\circ}\text{C}$  until the tests for the presence of adventitious micro-organisms described in sub-sections 3.4.3, 3.4.4, 3.4.5 and 3.4.6 are performed. The volumes of bulk suspensions required to be tested in these sub-sections are based on the assumption that the virus concentration in the samples is within the range between  $10^7$  TCID<sub>50</sub> and  $5 \times 10^7$  TCID<sub>50</sub> per ml. If the titre is significantly higher, the total volumes of undiluted bulk suspension to be tested may be reduced accordingly, without reducing the total number of animals required in these tests. If the titre is below  $10^7$  TCID<sub>50</sub> per ml, the total volumes to be tested shall be increased accordingly.

The bulk suspension shall pass all tests given in the sub-sections of section 3.4, with the exception of those tests that have already been performed on proportional samples of each of the single harvests represented in the bulk suspension (see sub-section 3.3.6).

The manufacturer should take care to maintain the bacterial and mycotic sterility of the vaccine during all stages of production, and should verify this by appropriate testing.

### 3.4.3 *Test in rabbits*

A sample of 100 ml of the bulk suspension shall be tested for the presence of B virus by injection into at least 10 rabbits weighing between 1.5 and

2.5 kg. Each rabbit shall receive not more than 10 ml of the bulk suspension, at multiple sites, 1 ml being given intradermally and the remainder subcutaneously. The rabbits shall be observed for at least 4 weeks. At least 80% of the rabbits shall survive the observation period. If any rabbit dies or shows signs of disease the cause shall be investigated. If the cause is not satisfactorily resolved, the test shall be repeated. If the death or the disease is shown not to be due to virus infection originating from the bulk suspension, or if none of the rabbits dies or shows signs of disease, the bulk suspension shall have passed this test. If the death has been shown to be due to B virus, the measures described in sub-section 3.3.5 shall be taken.

#### 3.4.4 *Test in adult mice*

The bulk suspension shall be tested in at least 20 adult mice, weighing between 15 g and 25 g, each of which shall be injected with 0.03 ml intracerebrally and with at least 0.5 ml intraperitoneally. They shall be observed for 3 weeks. At least 80% of the mice shall survive the observation period. If any mouse dies after the first 24 hours, the cause of death shall be investigated. If the cause of death is not satisfactorily resolved, the test shall be repeated. If the death is shown not to be due to virus infection originating from the bulk suspension, or if no mice die, the bulk suspension shall have passed this test.

If the vaccine strain of poliovirus is pathogenic to mice, it may be necessary to neutralize the sample of bulk suspension for this test with antipoliovirus serum.

#### 3.4.5 *Test in suckling mice*

The bulk suspension shall be tested in at least 10 mice, less than 24 hours old, each of which shall be injected with 0.01 ml intracerebrally and with at least 0.1 ml intraperitoneally. The mice shall be observed for 2 weeks. At least 80% of these mice shall survive the observation period. If any mouse dies after the first 24 hours, the cause of death shall be investigated. If the death is shown not to be due to virus infection originating from the bulk suspension, or if no mice die after the first 24 hours, the bulk suspension shall have passed this test.

If the vaccine strain of poliovirus is pathogenic to mice, it may be necessary to neutralize the sample of bulk suspension for this test with antipoliovirus serum.

#### 3.4.6 *Test in guinea-pigs*

The bulk suspension shall be tested in at least 5 guinea-pigs, weighing between 300 g and 500 g, each of which shall be injected with at least 5 ml intraperitoneally. They shall be observed for 6 weeks. At least 80% of the guinea-pigs shall survive the observation period. All animals shall be

examined for evidence of infection with viruses and *Myco. tuberculosis*, either at death, or at the end of the observation period. If evidence of infection with viruses or with *Myco. tuberculosis* originating from the bulk suspension is obtained, the bulk suspension shall be discarded. If the cause of death is not satisfactorily resolved in the case of guinea-pigs that die during the observation period, the test shall be repeated.

Many workers recommend a more sensitive test for the detection of *Myco. tuberculosis*, using the deposit centrifuged from a large volume of bulk suspension and resuspended in a small volume of saline, or supernatant.

During the observation period the temperatures of the guinea-pigs should be taken daily.

Some workers also give the guinea-pigs in this test an intracerebral injection of 0.1 ml of the sample.

### 3.5 *Control of bulk suspension after filtration*

#### 3.5.1 *Filtration of bulk suspension*

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

#### 3.5.2 *Sampling of filtered bulk suspension*

Samples of the filtered bulk suspension shall be taken immediately after filtering and shall be kept at a temperature below  $-20^{\circ}\text{C}$  until the tests described in the following sub-sections are performed.

#### 3.5.3 *Identity test*

The poliovirus type in the filtered bulk suspension shall be serologically identified.

International Reference Preparations of Anti-Poliomyelitis Sera of Types 1, 2 and 3 are available, as described in section 1.3.

#### 3.5.4 *Virus concentration*

The determination of the amount of infective poliovirus per ml of filtered bulk suspension shall be made in cell cultures. This determination shall be made in terms of PFU per ml<sup>1</sup> and/or in terms of TCID<sub>50</sub> per ml, in parallel with the determination of the virus concentration of a known reference preparation of the same poliovirus type. The determination of the number of PFU per ml shall be based on a total count of at least 100 clearly defined plaques on at least 5 different cell sheets. The determination of the number of TCID<sub>50</sub> per ml shall be based on the use of

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<sup>1</sup> PFU = plaque forming unit.

10-fold dilution steps with 10 tubes per dilution, or by any other arrangement of dilutions and tubes yielding equal precision.

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

### 3.5.5 *Tests for constancy of vaccine quality*

The poliovirus in the filtered bulk suspension shall be tested in comparison with the seed virus with regard to certain characteristics, as described in the following sub-sections.

The object of these tests is to ensure that the virus has not undergone changes during its multiplication in vaccine preparation. Since the seed virus may be either original vaccine, or a seed lot that has been tested in comparison with original vaccine, the tests also ensure that the virus in the vaccine prepared has the same characteristics as the virus in original vaccine that has been shown to be safe and effective in man.

The tests in monkeys will also serve to detect contamination with wild polio viruses.

#### 3.5.5.1 *Tests in monkeys*

The pathogenicity of the bulk suspension to *Macaca* or *Cercopithecus* monkeys shall be tested in comparison with that of the seed virus by the intrathalamic route of injection. The monkeys shall have been shown to be free from antibodies to all 3 types of poliovirus. The filtered bulk suspension shall be made up so as to contain at least  $10^7$  TCID<sub>50</sub> per ml. Each of at least 10 monkeys shall be given 0.5 ml of this material injected into the thalamus of each hemisphere and each of at least 10 additional monkeys shall be given a similar injection of the same material diluted 1 : 10.

The seed virus shall likewise be made up so as to contain  $10^7$  TCID<sub>50</sub> per ml and the same procedure as above carried out in 2 further groups of 10 monkeys. All monkeys shall be observed for at least 18 days by qualified persons, and all symptoms suggestive of poliomyelitis or other virus infections recorded. At least 80% of the animals in each group shall survive the observation period. At the end of the observation period, histological examinations shall be performed on the lumbar cord, the cervical cord, the lower medulla, the upper medulla and the mesencephalon of each monkey. Negative histological findings in monkeys showing evidence of faulty injection technique shall not be considered.

The filtered virus suspension shall have passed this test if the clinical and histopathological findings indicate no significant difference in pathogenicity between the vaccine virus and the seed virus.

At the present time there are insufficient experimental data to specify the confidence limits of the test described in this sub-section. The interpretation of the test results, therefore, requires exercise of judgement in the light of available information.

Some workers recommend that, in addition to the above tests, the filtered bulk suspension should be tested in monkeys in comparison with seed virus by the intramuscular and the intraspinal routes, as already outlined in section 3.1.4.

#### 3.5.5.2 *Tests in vitro*

The poliovirus in the filtered bulk suspension shall be tested for its property of reproducing at the temperatures of 36°C and 40°C (rct/40 marker) in comparison with the seed virus and with appropriate reference strains of poliovirus of the same type.

It is desirable for national control laboratories to issue reference viruses for this test.

The incubation temperatures used in this test shall be strictly controlled.

The bulk suspension shall have passed the test if, for both the virus in the bulk suspension and the seed virus, the titre determined at 36°C is at least 100 000 times that determined at 40°C. Unless the titres obtained for the reference viruses show expected values, the test shall be repeated.

It is strongly recommended that the manufacturer should perform at least one other test for a genetic marker, since a genetic change could occur which may not be detected by the rct/40 marker test only. Other marker tests currently used are (a) tests based on the study of the antigenic character of the strain, (b) tests for the sensitivity of reproduction to different concentrations of sodium bicarbonate (d-marker) and (c) tests for the capacity to reproduce in tissues of different origin, such as the so-called monkey-stable line (MS-marker).

### 3.6 *The final bulk*

The final bulk shall be either one filtered bulk suspension or a blend of filtered bulk suspensions, or a dilution thereof. The operations necessary for preparing a final bulk shall be conducted in such a manner as to avoid contamination of the product.

#### 3.6.1 *Sterility test on final bulk*

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

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1960, 200.

### 3.6.2 *Test for absence of vacuolating agent, simian virus 40 (SV40)*

A sample of at least 5 ml shall be taken from the final bulk and shall pass the following test for the absence of vacuolating agent (SV40), unless all components of the final bulk have already passed this test at an earlier stage of the manufacture.

The sample shall either not contain added substances that may interfere with the growth of vacuolating agent (SV40) in tissue culture, or such substances shall have been effectively removed.

The sample shall be neutralized by specific poliomyelitis antisera that have been demonstrated not to contain neutralizing antibody against vacuolating agent (SV40).

The neutralized sample shall be tested in kidney-cell cultures of *Cercopithecus* monkeys, using the technical procedure and the acceptance criteria given in section 3.3.4.

## 4. Filling and containers

The requirements concerning filling and containers given in Part A, section 4, of Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)<sup>1</sup> shall apply to vaccine filled out in the liquid form. Vaccine incorporated into a solid medium, such as sweets or candies, shall be processed in accordance with the regulations governing the production of tablets and capsules in pharmaceutical manufacture.

## 5. Control tests on final lot

Samples shall be taken from each final lot for the tests described in the following sub-sections.

### 5.1 *Identity test*

The poliovirus type or types shall be serologically identified.

### 5.2 *Virus titration*

The determination of the poliovirus titre shall be made as described in section 3.5.4.

### 5.3 *Sterility test*

Samples of each final lot of liquid vaccine shall be tested for bacterial and mycotic sterility according to the requirements given in Part A, section 5,

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1959, 178.

of Requirements for Biological Substances No. 6 (Requirements for the Sterility of Biological Substances).<sup>1</sup>

#### 5.4 *Innocuity tests*

The innocuity of each final lot shall be tested by appropriate tests in mice, guinea-pigs, and rabbits, using parenteral injections.

### 6. Records

The requirements given in Part A, section 6, of Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)<sup>2</sup> shall apply with the addition of the following :

Written records shall be kept of all seed lots, all cell cultures intended for vaccine production, all single harvests, all bulk suspensions and all vaccine in the final containers produced by the manufacturing establishment, including all tests irrespective of their results. The records shall be of a type approved by the national control authorities.

### 7. Samples

The requirements given in Part A, section 7, of Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)<sup>2</sup> shall apply.

### 8. Labelling

The label printed on or affixed to each container, or affixed to the wrapper of the package in which the container of the vaccine is distributed, shall show at least :

- the name and address of the manufacturer ;
- the words "Vaccinum poliomyelitidis perorale, Typus I, II, III" (whichever types apply) and/or the proper name of the product ;
- the number of the final lot ;
- the recommended human dose ;
- the temperature of storage and the expiry date if kept below that temperature ;
- the fact that the vaccine is for oral administration only.

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<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1960, 200.

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1959, 178, Annex I.

The statement of the recommended human dose shall be submitted to the national control authority for approval.

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

- the designation(s) of the strain(s) of poliovirus contained in the vaccine ;
- the fact that the vaccine fulfils the requirements of this document ;
- the fact that the vaccine was prepared in monkey kidney tissue ;
- the nature and amount of stabilizers or preservatives present in the vaccine ;
- the nature and amount of antibiotics used in the preparation of the vaccine ;
- the amount of virus of each type contained in one recommended human dose (this statement shall be based on the virus titration required in section 5.2) ;
- the conditions recommended during storage and shipping, with information on the reduced stability of the vaccine if exposed to higher temperatures than that stated on the label.

#### **9. Distribution and shipping**

The requirements given in Part A, section 9, of the Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)<sup>1</sup> 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 section 8 of this document, shall be based on experimental evidence and shall be submitted for approval to the national control authorities.

##### **10.1 Storage conditions**

Before being distributed by the manufacturing establishment, or before being issued from a depot for the maintenance of reserves of vaccines, all vaccines in their final containers shall be kept continuously at a temperature below  $-20^{\circ}\text{C}$ .

Precautions shall be taken to maintain the vaccine at a temperature below  $-5^{\circ}\text{C}$  during transport.

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1959, 178, Annex 1.

Liquid vaccine, after thawing for intended use, cannot be expected to remain satisfactory for more than 7 days if kept at a temperature between  $+2^{\circ}$  and  $+10^{\circ}\text{C}$ .

Vaccine incorporated into sweets or candies has been shown to remain satisfactory for at least 30 days if stored at  $+4^{\circ}\text{C}$ , and for at least five days if stored at room temperature.

### 10.2 *Expiry date*

The date after which the vaccine may not be used shall be not more than two years after passing the last virus titration as described in section 5.2, provided that the vaccine has been stored continuously at a temperature below  $-20^{\circ}\text{C}$ . After issue by the manufacturer, the expiry date shall not be more than one year from the date of issue, provided that it is stored below  $0^{\circ}\text{C}$ .

## Part B. National Control Requirements

### 1. General

The general requirements for control laboratories given in Part B of Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)<sup>1</sup> shall apply.

The national control authorities should give directions to manufacturers concerning the poliovirus strains to be used in vaccine production and concerning the recommended human dose.

#### 1.1 *Virus strains*<sup>2</sup>

The strains used in the production of oral poliomyelitis vaccine must have been shown to yield vaccines which are immunogenic and free from harmful effects upon oral administration to susceptible children and adults.

A number of attenuated strains<sup>3</sup> have been developed by various authors for this purpose during recent years. Those designated in the table on the following page have been tested in large-scale immunization campaigns in man.

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1959, 178, Annex 1.

<sup>2</sup> A more detailed analysis of the problems of this section will be found in the third report of the Expert Committee on Poliomyelitis (*Wld Hlth Org. techn. Rep. Ser.*, 1960, 203). Many basic data relevant to these problems are contained in *Scientific publication* No. 44 (1959) and No. 50 (1960) published by the Pan American Health Organization.

<sup>3</sup> Arrangements for obtaining these strains should be made by application direct, or through the World Health Organization, to : Dr A. B. Sabin, Children's Hospital, Research Foundation, Cincinnati, Ohio, USA ; or to : Dr H. Koprowski, Wistar Institute, Philadelphia, Pennsylvania, USA.

	A. B. Sabin	H. Koprowski	H. R. Cox
Type 1	L. Sc. 2ab	Wistar-Chat	Lederle SM
Type 2	P712 Ch 2ab	—	Lederle MEF
Type 3	Leon 12 ab	Wistar-Fox	Lederle Fox

National control authorities in countries that may wish to start the production of oral poliomyelitis vaccine are advised to obtain all available data on the properties of the strains that are under consideration. These data should provide information for each strain on (a) experience gained from field investigations and immunization campaigns, both with regard to absence of harmful effects when administered to susceptible children and adults and with regard to the immunizing effectiveness as determined by evidence of virus multiplication in the intestinal tract and of antibody response when administered as a monovalent vaccine, together with evidence of epidemiological effectiveness; (b) genetic stability on human passage as determined by tests of the viruses from vaccinated persons and their contacts; (c) capacity to spread from vaccinated to non-vaccinated persons; (d) the degree of viraemia during the week following oral administration; (e) the neuropathogenicity for monkeys after peripheral injection and after injection directly into the central nervous system; (f) genetic markers.

It is recognized that it may be necessary to accept a particular strain for use on the basis of data that are not equally favourable with respect to all the above-mentioned points. The overriding principle for selection, however, must be unequivocal evidence of safety and effectiveness as demonstrated in man. Furthermore, the strains selected should present properties that can be recognized with a high degree of certainty in laboratory tests so as to allow the enforcement of adequate controls of the vaccine in the laboratory.

### 1.2 *Human dose*

The amount of poliovirus in one human dose depends on the strains used, and on the conditions under which the vaccine is to be given. A decision concerning the doses to be applied in vaccination campaigns will depend on whether the vaccine is being given during an epidemic, and whether it will be administered in repeated monovalent doses, or in the polyvalent

form. It will also depend on the age of the persons vaccinated, and on the widespread occurrence of interfering viruses.

For the strains currently in use, a human dose between 50 000 and one million TCID<sub>50</sub> or PFU has been found adequate and has been shown to produce a demonstrable immune response in more than 90% of susceptible, antibody-negative persons, under optimal conditions.

## **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 whether or not the vaccine lot has been tested in human cell cultures for the presence of adventitious viruses. The certificate shall, furthermore, state the date of the last satisfactory determination of virus concentration, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document shall be attached.

The purpose of the certificate is to facilitate the exchange of 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 are safe and effective.

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