Annex 4

REQUIREMENTS FOR TYPHOID VACCINE
(REQUIREMENTS FOR BIOLOGICAL SUBSTANCES No. 15)¹

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Introduction

The twelfth WHO Expert Committee on Biological Standardization ² in 1958 expressed the opinion that there was an urgent need for international recommendations for the control of typhoid vaccine and that even on the basis of knowledge then available, requirements for typhoid vaccine would serve a useful purpose. This opinion was reiterated by the four-

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teenth Expert Committee\(^1\) in 1960. In 1965 the eighteenth Expert Committee\(^2\) considered that although the development of satisfactory laboratory assay methods for potency had not yet been achieved, it would nevertheless be possible to specify a number of characteristics of the typhoid vaccines used in certain controlled field trials (see page 18) and to formulate international requirements embodying specifications that would be useful in the production and control of satisfactory typhoid vaccines.

Having regard to these considerations, the following international requirements for typhoid vaccine have been formulated and fitted into the framework adopted in the Requirements for Biological Substances Nos. 1 to 12 already published by WHO.\(^3\) In drafting these requirements, account has been taken of the opinions of consultants, the regulations and requirements for the manufacture and control of typhoid vaccine that have been formulated in a number of countries, as well as information from both published and unpublished reports. In addition, opinions and data have been received from a number of experts, whose assistance is gratefully acknowledged below.

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\(^3\) For a list of references see *Wld Hlth Org. techu. Rep. Ser.*, 1967, 361, 76.
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General Considerations

Over the years many observations have been published suggesting that typhoid vaccine was of value in the prevention of typhoid fever, while other observations have suggested that it was of little or no value. However, there was no unassailable evidence on this question until a little over a decade ago, when controlled field trials were instituted to evaluate the efficacy of typhoid vaccines in protecting man against typhoid fever. The first trials were begun in 1954 in Yugoslavia and others were subsequently carried out in British Guiana, Poland, Yugoslavia and the USSR. Many of these trials were sponsored or assisted by WHO. These trials showed that vaccines giving good protection against the disease could be prepared and that protection in some cases persisted for at least three years and possibly longer, although at a diminishing level. The vaccines that gave good protection were prepared from one particular strain (Ty 2) by killing the organisms with (a) acetone or (b) heat and phenol or (c) formalin. By contrast, vaccine prepared by other methods failed to give satisfactory

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protection. Experimental evidence of the efficacy of typhoid vaccines is also available. Thus a typhoid-like disease in chimpanzees was largely prevented by vaccination with acetone-killed or heat-phenol-killed vaccines that have also given protection against deliberate infection in human volunteers.

The seventh WHO Expert Committee on Biological Standardization in 1953, considering the need for establishing an international standard for typhoid vaccine, agreed that such a standard should be made of material that had been tested for immunizing efficacy in the field, and recommended that in any such field trial that might be made, large samples of the vaccines used should be held in reserve for possible future use for reference purposes. This Committee and a number of subsequent Expert Committees on Biological Standardization expressed the belief that the carrying out of extensive controlled field trials presented a unique opportunity for comparing the results of laboratory tests with the results obtained with batches of vaccine in the field. Samples of vaccines used in the field were, therefore, reserved for laboratory studies. Several such studies, which included a freeze-dried heat-phenol-killed vaccine and a dried acetone-killed vaccine, were made using 14 different types of assay. By 1962, the fifteenth WHO Expert Committee on Biological Standardization noted that none of the laboratory tests with these vaccines, or with other vaccines used in the field, showed satisfactory correlation with their effectiveness in man. However, the Committee established quantities of these two preparations of dried typhoid vaccine as the International Reference Preparation of Typhoid Vaccine (acetone-inactivated) and the International Reference Preparation of Typhoid Vaccine (heat-phenol-inactivated). The Committee also decided that the attempts to develop a reliable laboratory method for assay that would directly reflect the protective value of typhoid vaccine in man should be continued. Such collaborative studies, sponsored by WHO, were arranged in 1964 in five laboratories in a number of countries and are still in progress.

The present requirements are applicable only to acetone-killed vaccine, heat-phenol-killed vaccine and formalin-killed vaccine of the kind used in the controlled field trials, since these vaccines fulfil a number of conditions. They have been shown to be effective in man in controlled field trials. The seed strain used in preparing the vaccines is preserved in the lyophilized state and is available on request from WHO; full documentation has been

published of the methods used in preparing the vaccines ¹ and some chemical and physical properties of the vaccines have been characterized.⁹ In the case of two of the vaccines, namely the acetone-killed and the heat-phenol-killed, the vaccines have been tested under different epidemiological conditions in a number of different areas. Furthermore, large quantities of these two vaccines have been set aside in a stable dry form as International Reference Preparations that are available for laboratory studies.

As it has not been possible in these requirements to include an acceptable test for potency of typhoid vaccine, the requirements have been framed applying the seed strain system of manufacture.

Studies of paratyphoid vaccines have been under consideration by WHO for some time.³ In the absence of clear evidence of the efficacy of these vaccines there is at present no basis for the formulation of requirements for them.

The present requirements relate to vaccines intended for subcutaneous injection. Interest has recently arisen in typhoid vaccines intended for oral administration but little evidence of their efficacy is available and therefore they have not been included in these requirements.

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

Should individual countries wish to adopt these requirements as the basis for their national regulations concerning typhoid vaccine, it is recommended that a clause be included that 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 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.

¹ Division of Immunology, Walter Reed Army Institute of Research (1964) Bull. Wild Hith Org., 30, 635.
² Walter Reed Army Institute of Research and Statens Seruminstitut, Copenhagen (1964) Bull. Wild Hith Org., 30, 647.
Part A. Manufacturing Requirements

1. Definition

1.1 International name and proper name

The international name shall be *Vaccinum febris typhoidi* followed, in parentheses, by the method of inactivation used, thus: *Acetono-inactivatum*, *Calore-phenolo-inactivatum* or *Formalino-inactivatum*, as the case may be. 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 febris typhoidi* shall consist of *Salmonella typhi* organisms in an aqueous suspension or as a dried powder prepared from a specified strain and killed by one of three specified methods. The preparation shall satisfy all the requirements formulated below.

*Vaccinum febris typhoidi*, whatever the method of inactivation used, is referred to in this document as "typhoid vaccine" (see p. 18).

1.3 International standards or reference preparations and international units

The International Reference Preparation of Typhoid Vaccine (Acetone-inactivated), established in 1962, is dispensed in ampoules containing 11 mg of dried vaccine. The International Reference Preparation of Typhoid Vaccine (Heat-phenol-inactivated), established in 1962, is dispensed in ampoules containing 34 mg of freeze-dried vaccine. These reference preparations are part of two batches of typhoid vaccine that have been extensively tested in controlled field trials and each of which has been shown to be protective in man.

Since no adequate laboratory test for potency of typhoid vaccine has yet been developed, no requirements for potency based on the international reference preparations can be formulated.

The International Opacity Reference Preparation was established in 1965. It is dispensed in ampoules containing 15 ml of a suspension of Pyrex-glass particles in water (10 IU of opacity per ml).

The above International Reference Preparations of Typhoid Vaccines and the International Opacity Reference Preparation are in the custody of the International Laboratory for Biological Standardization, Statens Seruminstitut, Copenhagen. Samples
are distributed free of charge on request to national control laboratories. The preparations of typhoid vaccine are specifically for use in the development of assay methods that would directly reflect the protective value of typhoid vaccine in man.

In 1952 the International Reference Preparation of Antityphoid Serum was established. The preparation is dispensed in ampoules containing 5 ml of hyperimmune horse serum, dried.1

The tenth WHO Expert Committee on Biological Standardization in 1956 considered the question of the provision of international standards for typhoid agglutinating sera, but the eleventh Committee in 1957 decided that, owing to the divergent views on the use of such sera, the question should be held in abeyance.2

1.4 Terminology

Seed lot. A quantity of Salmonella typhi organisms of a specific strain processed together and of uniform composition. A seed lot shall be maintained in the dried form.

Single harvest. A suspension of bacteria harvested on the same day from one batch of cultures. Single harvests shall not be more than three passages removed from the seed lot.

Final bulk. The finished vaccine prepared from the killed bacteria in a single harvest or in a pool of a number of single harvests 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.

2. General manufacturing requirements

The general manufacturing requirements contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)3 shall apply.

Staff working in the production of typhoid vaccine should be vaccinated against typhoid at appropriate intervals and should be regularly examined to ensure that they are not carriers of S. typhi.

1 This serum was introduced for use in the preparation of therapeutic antityphoid sera and was intended for the titration of the Vi and O antibodies in such sera and not for the control of typhoid vaccine.


3. Production control

3.1 Control of source materials

3.1.1 Strain of Salmonella typhi

The strain of *S. typhi* used for preparing vaccine shall be strain Ty 2, which has been used in preparing vaccines shown to be safe and effective in man. The culture used shall be identified by a record of its history, including the source from which it was obtained, and particulars of all tests made periodically for verification of strain characters. The culture used shall have the following characteristics: (a) stained smears made from a culture shall be typical of *S. typhi*; (b) the great majority of the colonies of a culture shall show the typical opalescence of a Vi-rich strain of *S. typhi*; (c) a live suspension of a culture in saline, when titrated with potent anti Vi, anti H and anti O sera of known agglutinating titres, shall agglutinate to titre with the anti H and anti Vi sera, but show little or no agglutination with the anti O serum. The suspension after boiling, however, shall agglutinate to titre with anti O serum.

The use of particular calibrated agglutinating sera for these tests and exact ranges of titres cannot be specified since relevant international reference sera have not been established and methods of testing differ in different laboratories. The immune sera used by a particular manufacturer should be approved by the national control authority (see Part B, section 1).

(d) A saline suspension of a young culture when injected intraperitoneally into mice of a susceptible strain and 15-20 g weight, shall, in a dose of not more than $50 \times 10^6$ organisms, kill a least 50% of the animals.

3.1.2 Seed lot system

The production of typhoid vaccine shall be based on a seed lot system.

It is recommended that a large seed lot be set aside as the basic material from which the manufacturer can prepare vaccine and to which he can return for the preparation of further seed lots.

Cultures of a seed lot shall have the same characteristics as are required of cultures of the strains from which the seed lot was derived (Part A, section 3.1.1). The seed lot shall be prepared according to the requirements of Part A, section 3.2.

The seed lot shall be maintained in a freeze-dried state.

A suitable menstruum for drying a seed lot is one containing dextran, sucrose and glutamate.

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1 The strain is obtainable on application to the World Health Organization, Avenue Appia, 1211 Geneva, Switzerland.
2 *Bull. Wld Hlth Org.*, 1964, 30, 635.
Samples of the seed lot shall be demonstrated by appropriate cultural methods to be free from bacterial contamination.

3.2 Production precautions

The general production precautions, as formulated in the requirements of Part A, section 3, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)\(^1\) shall apply.

3.2.1 Culture medium for vaccine production

The culture medium used for vaccine production shall be free from ingredients that may cause toxic or allergic reactions in man.

Both liquid and solid media have been used for the preparation of typhoid vaccines shown to be effective in man.

3.2.2 Temperature and time of incubation

The cultures shall be incubated at 36°C ± 1°C for a period not exceeding 24 hours. Manufacturers should determine the optimal time of incubation for their own particular production system.

3.3 Control of single harvests

3.3.1 Purity control

Samples of single harvests taken before killing shall be tested for bacterial contamination by microscopic examination of stained smears and by inoculation into appropriate media; single harvests shall not be used for further processing unless they have been shown to be free from contaminating organisms.

3.3.2 Characteristics of organisms in single harvests

When cultured on solid medium, the organisms present in single harvests shall have the morphological characteristics and the characteristic appearance of colonies required of the strain used for vaccine production given in Part A, section 3.1.1.

3.3.3 Test for total bacterial content

The total bacterial content of each single harvest shall be determined by a method approved by the national control authority.

The estimation of total bacterial content may be made by a nitrogen determination or an opacity method that has been calibrated in relation to total bacterial content. If an opacity method is used, the International Reference Preparation, or

3.3.4 Treatment of single harvests

As soon as practicable after harvesting, but in any case not later than the day of harvesting, the organisms shall be killed by one of the following methods: (a) acetone treatment, (b) heat-phenol treatment, (c) formalin treatment. The acetone, phenol and formalin used shall fulfill the requirements of the International Pharmacopoeia or a pharmacopoeia approved by the national control authority.

A satisfactory method of acetone treatment is as follows: A single harvest is slowly instilled into sterile acetone in a proportion of one volume of single harvest to three volumes of acetone. The mixture is held at room temperature (18°C to 25°C) for 12 to 24 hours, after which the supernatant fluid is drawn off. The sediment is then similarly treated three times more in succession with three volumes of acetone. The first two treatments are for 12-24 hours at room temperature and the third treatment is for 24 hours at 37°C followed by storage at 5°C until the results of sterility testing are available. When satisfactory results of sterility testing are obtained, the supernatant acetone is drawn off and the killed organisms kept in a sealed container between 2°C and 10°C until further processing.

A satisfactory method of heat-phenol treatment is as follows: Flasks containing a single harvest are immersed above the level of their contents in a water-bath and held at 56°C for one hour after the contents have reached this temperature. During this treatment the contents of the flasks are agitated to ensure a uniform temperature throughout the flasks. The temperature of the contents of the flasks is controlled so as to ensure that it does not deviate by more than 1°C from 56°C during this period of treatment. Immediately after heating is completed, phenol is added in a quantity sufficient to make a final concentration of 0.5% phenol. The flasks are then held at 18°C to 25°C until the results of sterility testing are available. When satisfactory results of sterility testing are obtained, the killed organisms are then stored at between 2°C and 10°C until further processing.

A satisfactory method of formalin treatment is as follows: Formalin is added to a single harvest to give 1% formalin in a bacterial concentration of 60 000 × 10⁵ organisms/ml. This suspension is held for 24 hours at 36°C ± 1°C, and then stored between 2°C and 10°C until further processing. It is advisable that the period of storage should not exceed 48 hours.

3.4 Control of final bulk

3.4.1 Preparation

The final bulk shall be prepared either from a killed single harvest or by pooling a number of killed single harvests. The material shall be
diluted with a suitable liquid so that in the form in which the vaccine is injected and in the volume that is recommended as a single human dose, the product contains the desired concentration of bacteria. The concentration of bacteria shall be calculated from the results of the tests made on the single harvests (Part A, section 3.3.3).

For the acetone-killed vaccine, used in the successful field trials, the suspending medium was a volatile organic solvent mixture of the same density as the suspended organisms.

For the heat-phenol killed vaccines and the formalin-killed vaccine used in the successful field trials, the diluent was buffered isotonic saline containing phenol in a concentration such that the percentage of phenol in the final bulk was between 0.25 and 0.5.

In the field trials (see page 18) effective vaccination was obtained by giving vaccines containing between 500 and 1000 million organisms per single human dose.

If bulk material is stored before the final bulk stage, it shall be held at a temperature between 2°C and 10°C.¹

3.4.2 Sterility test

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).²

The test on the acetone-treated vaccine should be made after removal of the acetone.

4. Filling and drying

4.1 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)³ shall apply.

Single- and multiple-dose containers may be used.
Containers of dry vaccine should be issued in a form that renders the process of reconstitution as simple as possible.

4.2 Drying

After filling the acetone-killed vaccine, the residual acetone and the

¹ No information is available on storage as a suspension in a volatile organic solvent mixture.
organic diluents used in preparing the final bulk shall be removed by a suitable method.

Heat-phenol-killed and formalin-killed vaccines may be freeze-dried.

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.

The antigenicity test described in Part A, section 5.4, may serve for this purpose.

5.2 Sterility tests

Each filling lot shall be tested for 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).\(^1\)

5.3 Innocuity test

Each filling lot shall be tested for abnormal toxicity by appropriate tests involving parenteral injections into guinea-pigs and mice. The tests shall be those approved by the national control authority.

The tests may be made by the subcutaneous or intramuscular injection of 0.5 ml into each of at least two mice weighing approximately 20 g each and 5.0 ml similarly injected into each of at least two guinea-pigs weighing approximately 350 g each. The animals should be observed for seven days and the injection should cause neither significant symptoms nor death during this period.

5.4 Antigenicity tests

Each filling lot shall be tested for specific antigenic characteristics by two tests, one being a test for protection of mice against challenge with a virulent strain of \textit{S. typhi}, and the other a test for anti \(\text{O}\), anti \(\text{H}\) and anti \(\text{Vi}\) agglutinin-production in rabbits. The tests used shall be those approved by the national control authority.

Until a potency test of proven significance has been developed, no specific requirements for potency can be formulated. It is advisable, however, that typhoid vaccines should be tested for specific antigenic activity.

5.5 *Inspection of final containers*

Each container in each filling lot shall be inspected visually, and those that show abnormalities shall be discarded.

5.6 *Test for residual moisture*

In the case of dried vaccine a test shall be performed on at least one container from each filling lot to determine the amount of moisture in the product. The filling lot passes the test if the moisture content is not more than 5%.

6. *Records*

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

7. *Samples*

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

8. *Labelling*

The requirements given in Part A, section 8, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)³ shall apply, with the addition of the following:

The label printed on or affixed to each container shall show:

the volume and nature of the reconstituting fluid for dried vaccine.⁴

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

a statement that after dry vaccine has been reconstituted it should be used within eight hours.

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.

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² In the field trials the fluid used for reconstituting the vaccine was a buffered salt solution containing 0.5% of phenol.
10. Storage and expiry date

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

10.1 Storage conditions

The manufacturer shall recommend such conditions of storage and shipping as will ensure that the vaccine conforms to these requirements until the expiry date as stated on the label.

Acetone-killed dried vaccine and other dried vaccines should be stored at temperatures below 25°C.

Liquid vaccines should be stored between 2°C and 10°C.

10.2 Expiry date

The expiry date for dried vaccines shall be not more than five years from the date of harvest, and for liquid vaccines not more than two years from the date of harvest. For both types of vaccine the expiry date shall be not more than 18 months from the date of issue.

Part B. National Control Requirements

1. General

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

In addition, the national control authority shall give directions to manufacturers concerning the agglutinating sera used in tests of serological properties (Part A, section 3.1.1) and also concerning the antigenicity tests to be used for the final vaccine (Part A, section 5.4).

2. Release and certification

A vaccine lot shall be released only if it fulfils all requirements set forth in Part A of the present document.

A statement signed by the appropriate official of the national control laboratory shall be provided at the request of the manufacturing establish-

ment 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 antigenicity 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 typhoid vaccine between countries.

3. Safety of the vaccine in man

There is no generally accepted laboratory test that will ensure that a vaccine lot will not cause undue reactions in man. Therefore, the national control authorities should satisfy themselves from time to time that vaccine lots prepared according to the methods given in these requirements give no undue reactions in man. Such tests should be made on groups of not less than 10 persons for each vaccine lot investigated. In addition, when a manufacturer prepares typhoid vaccine for the first time or modifies his method of production before the vaccine is released, the national control authorities should satisfy themselves by vaccinating groups of not less than 10 persons for each vaccine lot, that the first five lots of vaccine give no undue reactions in man.