Annex 2

REQUIREMENTS FOR LOUSE-BORNE HUMAN TYPHUS VACCINE (LIVE)

(Requirements for Biological Substances No. 33)

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INTRODUCTION

Four major groups of rickettsiae continue to be the cause of vexing human health problems throughout the world. They include the typhus group of diseases (louse-borne and flea-borne typhus), scrub typhus (tsutsugamushi disease), tick-borne typhus (the spotted fever group), and Q fever. All of these disease agents, except that of louse-borne typhus, are maintained in nature by transmission cycles in which man is not an essential element in the chain of infection, and all except Q fever are dependent on arthropod transmission for their maintenance in nature. These facts are evident in several generalizations:

(a) man, except in louse-borne typhus, is an accidental host of rickettsiae;
(b) factors affecting man’s biotic environment are influential in the occurrence of rickettsioses as human health problems;
(c) the major mode of transmission of Coxiella burnetii (the causative agent of Q fever) to man and its pathophysiology differ from those of other rickettsiae.

Of all the rickettsioses, louse-borne typhus is the only disease that has potential for explosive epidemics in man. The human body louse, Pediculus humanus humanus, the vector of Rickettsia prowazekii, thrives under conditions where socioeconomic conditions make its control difficult.

Louse-borne typhus continues to be a problem, particularly in the highlands of some countries of Africa and Central and South America. Yet, accurate statistics of its incidence and mortality rates in these areas are lacking. Over the last seven years in Africa, the greatest number of cases were reported from Ethiopia, Burundi, and Rwanda. While the annual number of cases of louse-borne typhus from the last two countries has declined, the number from Ethiopia has continued to range from 7000 to 17000 cases per year. These figures, however, are based on clinical suspicion, with little or no laboratory confirmation. Nevertheless, they do indicate that louse-borne typhus is a major communicable disease in some highland areas of Africa, and that better diagnostic and control measures are required.

In South America, louse-borne typhus is primarily a problem in the Andean highlands. The available data do not reflect the magnitude of the problem in these areas because of the remoteness of the populations affected and the dearth of medical resources, but
specific surveys indicate a likelihood of endemcity of louse-borne typhus among indigenous populations; there are occasional small village outbreaks or household occurrences, and acquisition of antibodies occurs at an early age.

Data on the occurrence of louse-borne typhus elsewhere in the world are almost totally lacking. Louse-borne typhus occurs in the Himalayas, and it is possible that it occurs also in other highlands or cold areas of Asia. Typhus is present in Europe today primarily as Brill-Zinsser disease. In the east of the USA, typhus occurs as a zoonosis in flying squirrels and is occasionally transmitted to man.

GENERAL CONSIDERATIONS

In the face of outbreaks in endemic areas, a louse-borne typhus vaccine has been shown to be a potentially practical means of control. The continued use of insecticide for delousing has not been effective and chemoprophylaxis has serious limitations as a general method. However, in the face of outbreaks, the vaccine could be used in conjunction with insecticides.

A live attenuated vaccine made from the E strain has been tried under field conditions, and recent field trials, specifically designed to test for possible side-reactions, have revealed a much lower rate of late reactions than have been described previously. The efficacy of the live vaccine has been demonstrated in a controlled field trial in Burundi.

A vaccine used in the USSR contains both live rickettsiae (E strain) and soluble antigen and although the vaccine gives a serological response in over 95% of vaccinees, it awaits trials under field conditions. The potency also to be established and further research in this area is needed.

Killed vaccines give limited protection but may modify the disease. Further studies are needed for the development of killed vaccines.

Further research is needed for the establishment of a universally accepted reference preparation and the possibility of developing other live, attenuated rickettsial vaccines should be considered. Research on the production and use of purified protective antigens is needed.
The present requirements have been formulated because of the importance of having a vaccine available for more widespread field evaluation in different countries.

Each of the following sections constitutes a recommendation. The parts of each section that are printed in normal type have been written in the form of requirements, so that, if a health administration so desires, these parts 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 of their national regulations concerning louse-borne typhus vaccine, it is recommended that a clause should be included permitting modifications of manufacturing requirements on the condition that it can be demonstrated to the satisfaction of the national control authority that such modified requirements ensure that the degree of safety and the 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 and/or used.

PART A:
MANUFACTURING REQUIREMENTS

1. DEFINITIONS

1.1 International name and proper name

The international name shall be “Vaccinum typhi humani vivum”. 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 typhi humani vivum” shall consist of a freeze-dried preparation of viable, attenuated Rickettsia prowazekii. The preparation shall satisfy all the requirements formulated below.
1.3 International standards or reference preparations and international units

The establishment of an International Reference Preparation of Typhus Vaccine (Live) is necessary for the evaluation of the sensitivity of titration methods and as a basis for comparison of attenuation.

1.4 Terminology

*Primary seed lot*: A quantity of rickettsial suspension that has been processed together and has a uniform composition. It is used for the preparation of secondary seed lots.

*Secondary seed lot*: A quantity of rickettsial suspension that has been processed together, is uniform with respect to composition, and is only one passage from a primary seed lot. Material is drawn from secondary seed lots for inoculating embryonated eggs or cell cultures for the preparation of vaccine.

*Single harvest*: A quantity of rickettsial suspension harvested from tissues that were inoculated, incubated, and processed together.

*Bulk suspension*: The material prepared from one or more single harvests and before filling into final containers.

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

*Reconstituted vaccine*: Rehydrated vaccine ready for administration.

*Egg infective dose 50% (EID<sub>50</sub>)*: The quantity of rickettsial suspension estimated to produce death in 50% of the inoculated chicken embryos.

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

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) (WHO Technical Report Series, No. 323,
1966, p. 11) shall apply to establishments manufacturing typhus vaccine, with the addition of the following:

Production areas shall be decontaminated before they are used for the manufacture of typhus vaccine. No other infectious agents shall be introduced into the area during the period of production. Because of the pathogenicity of the organism all precautions should be taken to prevent the spread of *R. prowazekii* within the production area and to prevent any escape outside this area.

The production of typhus vaccine shall be conducted by a separate staff of healthy persons who shall be examined medically at regular intervals, and who, during the period of production, shall not work on other infectious agents. Steps shall be taken to ensure that all persons in the production areas are immune to human typhus, and that they do not excrete any microorganisms of significance to the safety of the vaccine.

Visitors and persons not directly concerned with the production process shall not be permitted to enter the production areas.

3. PRODUCTION CONTROL

3.1 Control of source materials

3.1.1 *Rickettsial strains*

The rickettsial strains used in the production of vaccine shall be identified by historical records, and shall have been shown, to the satisfaction of the national control authorities, to be safe and immunogenic.

A strain that has been shown to be a suitable candidate is the E strain of *R. prowazekii*.

3.1.2 *Tissues and cell cultures for production of rickettsiae*

Rickettsiae for the preparation of primary and secondary seed lots and of all vaccine lots shall be grown in the tissues of chick embryos obtained from a healthy flock. Monitoring of the flock or embryos shall include at least tests for exclusion of infection by *Salmonella* species, *Mycobacterium avium*, fowlpox, Rous sarcoma virus, avian leukosis viruses, mycoplasma, and other agents pathogenic for chickens.

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At present the cell cultures give poor virus yield and are not an economic proposition, but the use of such cells may be permitted.

3.1.3 Seed lot system

The production of vaccine shall be based on the primary and secondary seed lot system. All seed lots shall be stored under conditions optimum for the stability of the strain of *Rickettsia* at a temperature of $-70^\circ\text{C}$ or below.

Since the vaccine is freeze-dried, the seed lot should also be stored in the freeze-dried form.

3.1.3.1 Test for attenuation. Each seed lot shall be shown to be attenuated by tests approved by the national control authority.

A suitable test is the inoculation of mice to compare the virulence with that of a strain known to be virulent in man.

3.1.3.2 Test for freedom from extraneous agents. Each seed lot shall be shown by appropriate tests to be free from all extraneous viable microbial agents, and shall be tested according to, and shall satisfy the requirements of, Part A, section 3.3.

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) (WHO Technical Report Series, No. 323, 1966, Annex 1) shall apply to the manufacture of typhus vaccine with the addition of the following.

3.2.1 Tests on control tissues

If the monitoring of the flocks is not under the direct responsibility of the manufacturer, the following controls on the tissues shall be included.

Ten uninoculated embryonated eggs from the batch used for vaccine production shall be incubated under the same conditions as
the inoculated eggs. At the time of virus harvest, the uninoculated eggs shall be processed in the same manner as the infected embryos and the homogenate of the yolk sacs from the control embryos shall be shown to be free from *Salmonella* species, *Mycobacterium avium*, and fowlpox virus by tests approved by the national control authority.

There are other pathogens for flocks of chickens, but control of the flock should eliminate these.

3.2.2 *Test for avian leukosis viruses*

A sample of amniotic fluids from the control embryonated eggs shall be tested for avian leukosis virus using a method approved by the national control authority.

A certificate of freedom from avian leukosis virus provided by the supplier of the fertile eggs may satisfy these requirements.

3.2.3 *Addition of stabilizers and preservatives*

No human protein or penicillin shall be added to the rickettsial suspension at any stage during production. If stabilizing agents are added they shall be shown, to the satisfaction of the national control authorities, to have no antigenic or sensitizing properties for man.

3.2.4 *Harvesting*

Harvesting of the yolk sac of living infected embryos shall be carried out by a method approved by the national control authority. No antibiotics shall be added at the time of harvesting. The yolk sacs are homogenized and centrifuged at a speed at which the solid particles are deposited and the fat forms the upper layer. The middle of the three layers is the rickettsial harvest.

Each single harvest shall be tested for sterility; the rickettsial content shall be tested according to the two tests described below (sections 3.2.4.1 and 3.2.4.2). Each single harvest shall also be tested according to the provisions of Part A, section 3.3, unless those tests are made on the rickettsial pool.

The samples of single harvests shall be taken for testing at the time of harvesting, and, if not tested immediately, the samples shall be kept at a temperature of $-70^\circ$C or below until tested.
3.2.4.1 Sterility tests. A volume of at least 10 ml of each single harvest shall be tested for bacterial and mycotic sterility according to the requirements given in Part A, section 5 of the revised Requirements for Biological Substances No. 6 (General Requirements for Sterility of Biological Substances)¹ as well as for mycoplasma by a method approved by the national control authority.

Tests for mycoplasma should be done using both solid and liquid media which have been shown to be capable of growing mycoplasma.

3.2.4.2 Rickettsial titration. The live rickettsial content of each single harvest shall be determined by cell-culture titration (PFU) using a reference preparation for comparison (see Part A, section 1.3).

Chick embryo fibroblasts are suitable cultures for this test.

3.3 Control of rickettsial pools

After harvesting the typical chick embryos the yolk sacs shall be homogenized and the pool shall be kept at −70°C or colder until further processing.

The rickettsial pool shall be prepared from one single harvest or from a pool of single harvests and shall be submitted to the following tests unless these tests have been done on each single harvest, with the exception that even in that event, sterility tests according to Part A, section 3.3.1, shall also be done on the rickettsial pool.

3.3.1 Sterility tests

A volume of at least 10 ml of each rickettsial pool shall be tested for bacterial and mycotic sterility according to the procedures given in Part A, section 5 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (WHO Technical Report Series, No. 530, 1973, p. 48), as well as for mycoplasma by a method approved by the national control authority.

Tests for mycoplasma should be done using both solid and liquid media that have been shown to be capable of growing mycoplasma.
3.3.2 Test for Mycobacterium tuberculosis

A volume of at least 10 ml of each rickettsial pool shall be tested for the presence of Mycobacterium tuberculosis, M. bovis, and M. avium by culture methods appropriate for detection of the organisms most likely to be found in the fertile eggs used.

In some countries a certificate of freedom from avian tuberculosis provided by the producer of fertile eggs may satisfy the requirement.

3.3.3 Tests in tissue cultures

After the addition of antibiotics to suppress the growth of the rickettsiae, a volume of each rickettsial pool equivalent to at least 50 human doses of vaccine or 5 ml, whichever represents the greater volume, shall be tested for adventitious agents by inoculation into primary monkey kidney tissue cultures. Similar volumes of the rickettsial pool shall likewise be tested in human cell cultures and in cell cultures of the type used in the preparation of the rickettsial pool. The cell cultures shall be observed for at least 14 days.

The rickettsial pool passes the tests if none of the cell cultures shows evidence of the presence of any adventitious agents attributable to the rickettsial pool.

3.3.4 Test in embryonated eggs

After the addition of antibiotics to suppress the growth of the rickettsiae, a volume of each rickettsial pool equivalent to at least 50 human doses of vaccine or 5 ml, whichever represents the greater volume, shall be tested in a group of embryos of fertilized chicken eggs by the allantoic route of inoculation, and a similar sample in a separate group of eggs by the yolk sac route of inoculation, using 0.5 ml of inoculum per egg.

The rickettsial pool passes the test if there is no evidence of the presence of any adventitious agents attributable to the rickettsial pool.

3.3.5 Test for avian leukosis viruses

A volume of each rickettsial pool equivalent to at least 50 human doses of vaccine or 5 ml, whichever represents the greater volume, or proportionate amounts drawn from individual harvests totalling
such a volume, shall be tested for avian leukemia viruses by a method approved by the national control authority.

A procedure for detecting resistance-inducing factor (RIF) is satisfactory for testing for avian leukemia viruses; the COFAL test may also be used. In some countries in which RIF-free eggs are used it is permitted to omit this test.

The rickettsial pool passes the test if there is no evidence of the presence of avian leukemia viruses.

3.3.6 *Test for viable units of rickettsiae (potency test)*

Each rickettsial pool shall be tested for the number of rickettsial organisms by a test approved by the national control authority. The number of viable units in the human dose shall be determined by the national control authority.

A test shown to be suitable is by plaque count in chick embryo cell cultures.

3.3.7 *Test for virulence for mice*

Each rickettsial pool shall be tested for virulence for mice compared with that of a non-attenuated (wild) strain of *R. prowazekii*. The test shall be approved by the national control authority.

A suitable test is to dilute the vaccine and wild strain in 10-fold dilutions and to inoculate each dilution into groups of 5 mice. At the end of the observation period the mice are bled and the sera tested for antibody to the rickettsiae. It is expected that the proportion of mice surviving the vaccine dose will be greater than that surviving an equivalent dose of the wild strain.

The difference in virulence and antibody responses between the attenuated and wild strains shall be approved by the national control authority.

4. FILLING AND CONTAINERS

The requirements concerning filling and containers in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (WHO Technical Report Series, No. 323, 1966, p. 16) shall apply to typhus vaccine.
The containers of the final vaccine shall be of neutral glass of high quality. Single- and multiple-dose containers may be used.

As soon as possible after harvesting, vaccines shall be filled into containers and freeze-dried. Containers shall be sealed under vacuum or filled with dry nitrogen.

Failure to achieve adequate drying may result in a product that is liable to rapid deterioration even at 0°C. National control authorities may require an assay for residual moisture by an approved method.

The manufacturer shall provide the national control authority with adequate data to prove the stability of the product under appropriate conditions of storage and shipping.

5. CONTROL TESTS ON FINAL PRODUCT

5.1 Identity test

An identity test shall be performed on at least one container from each filling lot after reconstituting the vaccine according to the indications of the manufacturer for preparing vaccine for human administration.

The test shall identify the individual attenuated strain of rickettsiae used for the production of the vaccine, and shall be approved by the national control authority.

The virulence test of Part A, Section 3.3.7, may serve this purpose.

5.2 Sterility tests

Each filling lot shall be tested for bacterial and mycotic sterility according to the requirements given in Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (WHO Technical Report Series, No. 530, 1973, p. 48).

5.3 Test for viable units of rickettsiae

The rickettsial content of each filling lot shall be determined by titration in both chick embryos and chick embryo cell cultures using tests approved by the national control authority.
The minimum content of egg infectious doses (EID₅₀) and plaque-forming units (PFU) shall be approved by the national control authority.

5.4 Protein nitrogen content

The vaccine shall be tested for nitrogen content, and the upper limit permissible shall be determined 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) (WHO Technical Report Series, No. 323, 1966, p. 17) shall apply, with the addition of the following:

Written records shall be kept of all seed lots and vaccine lots produced by the manufacturing establishment, irrespective of the results of safety and potency tests.

The format of the records shall be of a type approved by the national control authority.

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) (WHO Technical Report Series, No. 323, 1966, p. 17) shall apply with the addition of the following:

In addition to the samples of vaccine lots, samples of all seed lots shall be retained by the manufacturing laboratory and stored under the same conditions as those pertaining to the remainder of the lot until the expiry date of all vaccine lots prepared from these seed lots.

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)
WHO Technical Report Series, No. 323, 1966, p. 18) shall apply, with the addition of the following:

The label on the package shall include the following additional information:

— the fact that the vaccine fulfils the WHO Requirements;
— the words ‘‘living typhus vaccine prepared in chick embryos or appropriate cell cultures’’;
— the volume and kind of diluent to be added to reconstitute the vaccine;
— the volume of the recommended human dose, and the mode of administration (subcutaneous injection);
— a warning that the vaccine should not be given together with other live vaccines or to pregnant women in the first two trimesters of pregnancy;
— the words ‘‘the dose shall be the same for persons of all ages’’;
— instructions for the administration of the vaccine, including the statement in large bold-face type: ‘‘The reconstituted vaccine must be used or discarded within 3 hours of the opening of the container’’;
— a statement that the vaccine is not recommended for children of less than 6 months of age; and
— a statement that reconstituted vaccine should be held at a temperature close to 0°C.

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) (WHO Technical Report Series, No. 323, 1966, p. 18) shall apply.

10. STORAGE AND EXPIRY DATE

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 vaccines shall be kept constantly at a temperature below −20 °C. The manufacturer shall recommend such conditions of
storage and shipping during distribution and will ensure that the vaccine conforms to the requirements of potency until the expiry date as stated on the label.

Distributed vaccine should normally be stored at a temperature lower than 4 °C, though higher temperatures may be permitted for a short interval.

Whatever the temperature of storage and distribution, the vaccine, at its expiry date, should fulfil the requirements for potency as specified in section 5.3.

10.2 Expiry date

The expiry date shall not be more than 2 years after the date of the last satisfactory potency test, provided that the vaccine has been maintained under the conditions of storage mentioned in section 10.1. The expiry date shall not, however, be more than 12 months from the date on which the vaccine was issued by the manufacturers.

The viable count should be checked for all lots being stored by the manufacturer at 12-month intervals.

PART B.
NATIONAL CONTROL REQUIREMENTS

1. GENERAL

The general requirements for control laboratories given in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (WHO Technical Report Series, No. 323, 1966, p. 19) shall apply.

2. RELEASE AND CERTIFICATION

Written procedures for the preparation of typhus vaccine adopted by the manufacturer must be submitted for approval to the national control authority and to the World Health Organization if the vaccine is to be used to satisfy international requirements for
immunization. Proposals for modification must be submitted for approval to the national control authority.

Protocols of the production and testing procedures of each lot of vaccine must be submitted, prior to release, to the national control authority as required.

3. SURVEILLANCE FOR ADVERSE REACTIONS

In the case of new manufacturers, as well as in the case of change of manufacturing process, or change of seed, the national control authority must ascertain that adequate control of the vaccine has been achieved by arranging for studies in man of some of the lots of vaccine.

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Appendix 1

SUMMARY PROTOCOL FOR LOUSE-BORNE
HUMAN TYPHUS VACCINE (LIVE)

Based on Requirements for Biological Substances No. 33
[Requirements for Louse-borne Human Typhus Vaccine (Live)]

Identification of Final Lot

Name and address of manufacturer

Lot number of final product

Date of manufacture of final lot (namely, date of initiation by the manufacturer of the last valid potency test)

Nature of final product

Volume of single human dose

Expiry date

Information on Manufacture

1. Seed lot(s) of rickettsia/e; used

Type(s), species, and strain(s) of Rickettsia

Name of control authority that approved the attenuation of this strain or these strains

2. Single harvests included in pool

Harvest/number

Strain of Rickettsia

Date of virus inoculation

Date of virus harvest

Results of sterility test

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Rickettsial titre
Results of tests on control eggs
Test for avian leukemia viruses

3. Rickettsial pool

Results of sterility test
Date of test
Was a repeat test necessary?
If so, why?
Result of test for M. tuberculosis
Test method
Tests for adventitious agents after addition of antibiotics
Results
Results of test in fertilized chicken eggs
Test for avian leukemia viruses:
Method
Result
Potency test (viable units)
Date
Method
Number of viable units per human dose
Minimum required
Virulence for mice
Method
Result

4. Final lot

Identity test
Method
Result
Sterility test
Result
### Viable units

<table>
<thead>
<tr>
<th>Method</th>
<th>Chick embryo (EID&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>Chick embryo cell culture (PFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum required</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein nitrogen content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper acceptable limit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has the lot been released by the national control authority?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If so, when? (date)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can a certificate, referred to in the Requirements mentioned above, be supplied by the national control laboratory?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Which laboratory would supply such a certificate?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Signature:**

**Name (typed or block letters):**

**Designation/title:**

**Date:**

The protocol must be accompanied by a sample of the label and a copy of the leaflet.