Annex 3

REQUIREMENTS FOR TYPHOID VACCINE
(LIVE ATTENUATED, Ty 21a, ORAL)

(Requirements for Biological Substances No. 34)

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INTRODUCTION

Many health authorities require a vaccine against typhoid fever and international requirements for such a prophylactic are essential.

The formulation of requirements was first considered at the twelfth WHO Expert Committee on Biological Standardization, over 20 years ago. At that time, however, there were few satisfactory laboratory tests available for the control of such vaccines. It was several years before any requirements could be formulated. The many field trials, showing efficacy of some of the vaccines, gave important leads towards meaningful tests to be embodied in requirements and these were formulated and accepted in 1966.

Such requirements concerned only the vaccine made from Salmonella typhi Ty 2, killed by chemicals and given by the parenteral route. Although it was recognized that there was no satisfactory laboratory test for potency, a test in which immunized mice were challenged was included as the best available.

With the recent advancement made in the isolation of an avirulent mutant that can be given orally as a live vaccine, and its proven success in a field trial, the Sub-Committee of the Scientific Working Group on Bacterial Enteric Infections expressed the need for requirements specifically concerned with such a strain. Since there are no other mutants under consideration, and since the tests characterizing the strain are unique, the requirements have been formulated exclusively for the strain known as Salmonella typhi Ty 21a.

Having regard to these considerations, the following international requirements for typhoid vaccine (live attenuated, Ty 21a, oral) have been formulated and fitted into the framework adopted in the Requirements for Biological Substances Nos. 1 to 32 already published by WHO (1). In drafting these requirements, account has been taken of the opinions of consultants as well as information from published and unpublished reports. In addition, opinions and data have been received from a number of experts, whose assistance is gratefully acknowledged below.

GENERAL CONSIDERATIONS

Presently available typhoid vaccines are not wholly satisfactory. Although some killed vaccines given by the parenteral route have been shown, in large controlled field trials sponsored by WHO, to
confer good and long-lasting protection, they tend to cause undesired local and systemic reactions. Furthermore, as has been demonstrated in volunteer challenge studies, the protection is not absolute but can be overwhelmed by large challenge doses. Oral killed vaccines, on the other hand, are well tolerated but their efficacy could never be demonstrated in volunteer challenge studies or controlled field trials. They cannot therefore be recommended.

Several studies with a *Salmonella typhimurium*-mouse typhoid model have clearly demonstrated that vaccination with live attenuated *Salmonella* produces a more effective immunity than does parenteral killed vaccine. The superiority of attenuated live vaccines is particularly well demonstrated when vaccine and challenge are administered by the natural oral route. Based on the results obtained with such an animal model, an attenuated *Salmonella typhi* has been developed and prepared as a candidate for an oral live typhoid vaccine strain. The main characteristic of this *Salmonella typhi* mutant (strain Ty 21a) is the lack of the enzyme uridine-diphosphate-galactose-4-epimerase; therefore, biosynthesis of the cell wall lipopolysaccharides, which are responsible for both immunogenicity and virulence, occurs in this mutant only under conditions that induce bacteriolysis and thereby inactivation of the bacteria.

The safety of the Ty 21a vaccine strain has been proved by volunteer challenge studies in the United States of America, by a large field trial in Egypt, and in a pilot study in Chile. In the study in the United States of America, 5–8 doses of vaccine containing $3 \times 10^9$ viable bacteria per dose were given to 155 adult males without significant side-effects. Three doses of vaccine containing $1 \times 10^9$ viable bacteria were given to 16,000 schoolchildren in Egypt and 3 doses containing $1 \times 5 \times 10^9$ viable bacteria were given to 338 schoolchildren in Chile in the pilot study and to about 60,000 children in a larger trial in Chile, again with no ill effects.

As far as excretion is concerned, the vaccine strain in stools of volunteers given the high doses ($3 \times 10^9$) was low and was never excreted for more than three days. The majority of isolates occurred on the first day after vaccination. None of the 358 stool isolates examined showed any sign of reversion to virulence. The vaccine strain could not be isolated from the stools of the Egyptian and Chilean schoolchildren who received the lower vaccine dosage ($1 \times 8 \times 10^9$).

Furthermore, the protective efficacy was demonstrated in both the
volunteer challenge studies in the United States of America and the field trial in Egypt. In the volunteer studies, the vaccine showed an efficacy of 87% against a challenge dose that provoked typhoid fever in 57% of the control group. In earlier studies using killed vaccine given parenterally, no protection was demonstrated against such a high challenge dose. In addition to protecting against the disease, Ty 21a vaccine also shortened the excretion period of the Salmonella typhi challenge strain in the stools, compared with that of the non-vaccinated subjects. In the Egyptian field trial, in which the population was carefully monitored for three years, the efficacy was 96%. These results indicate that, in the dose schedules and formulation used, the Ty 21a vaccine is stable and safe, and is highly protective for a period of at least three years.

The only difficulty encountered is that, in order to protect the vaccine bacteria from inactivation by gastric acid, the gastric juice has to be neutralized by sodium bicarbonate before the vaccine is given. The Ty 21a vaccine at present available in several countries of Europe, South America, and Asia is administered in three doses given every second day; each dose consists of one capsule containing the vaccine strain and two capsules each containing 0.4 g of sodium bicarbonate. In May 1982 a field trial was launched in Chile in order to investigate if a more practical form of vaccine, consisting of only one or two enteric-coated capsules, each containing $1-5 \times 10^9$ Ty 21a bacteria, would be as effective as the formulation used in Egypt which was not in the form of enteric-coated capsules.

As there is no reliable potency test available to predict the efficacy of typhoid vaccines in man, more research is necessary and clinical trials must continue. Furthermore, since there is no animal model to give guidance on the safety of a strain, emphasis must be placed on the biochemical and immunological characterization of those strains that have been shown by clinical trials to be safe and effective in man.

Since no other mutants are being studied, the present requirements relate to a vaccine intended for oral administration and made only from the mutant Ty 21a.

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 of their national regulations concerning live attenuated typhoid vaccine given orally, it is recommended that a clause be included permitting modifications of manufacturing requirements on condition that it be demonstrated to the satisfaction of the national control authority that such modified requirements ensure a degree of safety and a potency of the vaccine at least equal to those provided by the requirements formulated below. It is desirable that the World Health Organization should then be informed of the action taken.

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

PART A.

MANUFACTURING REQUIREMENTS

1. DEFINITIONS

1.1 International name and proper name

The international name shall be *Vaccinum febris typhoidi* (Ty 21a) *perorale vivum*. The proper name shall be equivalent to the international name in the language of the country of origin.

The use of the international name should be limited to vac-

cines that satisfy the requirements formulated below.

1.2 Descriptive definition

Live oral typhoid vaccine Ty 21a is a freeze-dried preparation contained in gelatin capsules prepared from the *Salmonella typhi* strain Ty 21a. Each capsule shall contain $1-5 \times 10^9$ viable Ty 21a organisms (see Part A, section 3.3.1).

Enteric-coated capsules are being tested in field trials and these requirements apply also to such capsules.
1.3 International reference preparations and international units

There is a need for a freeze-dried international reference preparation of typhoid vaccine Ty 21a for comparison of the characteristics of the strain. The parent seed lot could serve this purpose.

1.4 Terminology

*Parent seed lot:* A quantity of living *Salmonella typhi* Ty 21a organisms derived from a single colony, processed together and of uniform composition. A parent seed lot shall be maintained in the freeze-dried form.

*Working seed lot:* A quantity of living *Salmonella typhi* Ty 21a organisms produced from the parent seed lot which is maintained in aliquots in the freeze-dried form.

*Single harvest:* One or more sediments obtained by centrifugation of cultures within 48 hours and harvested at the same time from an ampoule of the working seed lot.

*Final bulk:* A pool of single harvests freeze-dried in the same freeze-drying run and thereafter homogenized to form a uniform powder. The final bulk is contained in a single vessel.

*Final lot:* A collection of filled capsules that are homogeneous with respect to the risk of contamination during filling of one or more final bulks. A final lot must, therefore, have been filled in one working session.

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)* (2) shall apply, with the addition of the following:

Production areas shall be decontaminated before they are used for the manufacture of typhoid Ty 21a vaccine.

Formaldehyde and glutaraldehyde have been found useful for this purpose.

The production of Ty 21a vaccine shall be conducted by a staff of healthy persons, who shall be examined medically at regular intervals. Steps shall be taken to ensure that all such persons in the
production area have been immunized against typhoid and do not excrete *S. typhi*.

The medical authority of the production area shall determine the frequency of the medical examinations.

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 Bacterial strain

The attenuated *S. typhi* mutant strain Ty 21a has been shown to be safe and effective in man and has the characteristics described in Part A, section 3.1.4.

##### 3.1.2 Production of parent seed lot

A single colony of organisms shall be grown using a suitable galactose-free medium and incubated at a temperature to produce luxuriant growth. As the culture reaches the stationary phase of growth the organisms shall be harvested, centrifuged, suspended in a stabilizer, dispensed into ampoules, and freeze-dried. Each ampoule shall contain at least $10^9$ viable organisms and satisfy the tests in Part A, section 3.1.4.

It has been found that brain heart infusion broth is suitable for this purpose.

The parent seed shall be tested according to the requirements in Part A, section 3.1.4. It shall be stored at $5^\circ\text{C} \pm 3^\circ\text{C}$.

##### 3.1.3 Production of working seed lot

A working seed shall be produced from the growth of organisms obtained from an ampoule of the parent seed. The working seed shall be treated in a similar manner to the parent seed and freeze-dried. Each ampoule shall contain at least $10^9$ viable organisms.

The working seed shall be tested according to the requirements in Part A, section 3.1.4. It shall be stored at $5^\circ\text{C} \pm 3^\circ\text{C}$.
3.1.4 Tests on parent and working seed lots

The parent and working seeds shall satisfy the following tests:

3.1.4.1 Identity and purity

(a) The growth from the seeds shall confirm that the Ty 21a organisms are Gram-negative rods and that they are motile.

(b) Organisms grown on media for three passages with or without galactose agglutinate with H : d antiserum but not with Vi antiserum, whereas only those organisms grown on medium containing galactose (1 g/litre) will agglutinate with 0 : 9 antiserum.

(c) Samples, when plated on galactose containing indicator medium (for example Endo agar to which galactose has been added) and incubated at 37°C for 7 days, develop a coloured confluent growth which, due to lysis, gradually becomes transparent. Galactose-fermenting colonies shall not appear at any time during the 7 days’ incubation.

The observation for 7 days is a sensitive test for reversion.

Other indicator media such as bromthymol blue agar are also useful for this test.

(d) Samples plated on galactose containing indicator medium and incubated for 48 hours at 37°C shall have concave colonies with a pale border, due to active growth, and darker centres, due to death and lysis; such is a typical appearance of Ty 21a on galactose containing agar medium.

(e) Growth on Kligler iron agar slants shows no blackening, indicating that the organisms do not produce hydrogen sulfide.

The use of lead acetate paper is also suitable for the detection of H2S.

3.1.4.2 Galactose-induced bacteriolysis

Shake-cultures growing in the exponential phase in heart infusion broth at 37°C shall begin to lyse within 1 hour of the addition of galactose at a final concentration of 100 g/litre. When a similar concentration of galactose is added at the time of inoculation the growth is limited before lysis takes place.

3.1.4.3 Mouse virulence

The organisms grown for 6 hours at 37°C in brain heart infusion broth shall be inoculated into 5 mice (18–20 g) by the intraperitoneal
route. A suspension containing at least $5 \times 10^7$ bacteria shall not kill any mice within a 7 day observation period.

The NIH-GI strain of mice are suitable for this test.

3.1.4.4 \textit{Determination of enzymes involved in galactose metabolism}

Tests for the enzymes involved in galactose metabolism shall show that the Ty 21a organisms have lower enzyme activities than those of Ty 2.

When compared with the enzyme activity of Ty 2, taken as 100%, the activities of Ty 21a are epimerase 0%, galactokinase (EC 2.7.1.6) 5–20%, galactose-1-phosphate-uridylyltransferase (EC 2.7.7.10) 25–50%, and galactose-permease 40–50%.

The tests suitable for this test are mentioned in references (3–5).

3.1.4.5 \textit{Uptake and intracellular distribution of $^{14}$C galactose}

The kinetics of the uptake and of the distribution of radioactive $^{14}$C galactose in the cells shall be shown to be typical for Ty 21a and shall differentiate it from other mutants free from epimerase.

After 7 hours' growth at 30°C, at least 90% of the galactose in the medium (1 g/litre) shall be shown to have been taken up by the organisms by the measurement of $^{14}$C in the cells. It shall be shown also that approximately 75% of the galactose is in the cell wall.

It should be noted that the galactose uptake is not parallel to the growth curve and should be measured in 2 independent cultures at 1 hour intervals over a period of 7 hours.

3.1.4.6 \textit{Characterization of lipopolysaccharide (LPS)}

Lipopolysaccharide extracted from the cell walls of the organisms grown in brain heart infusion broth containing 1 g/litre $^{14}$C galactose for 7 hours at 30°C, shall be hydrolysed in 1% acetic acid and the polysaccharides examined.

It shall be shown by gel chromatographic methods, using Sephadex G50, that the organisms contain LPS of both smooth type and rough type, in a similar ratio to that of the virulent strain \textit{S. typhi} Ty 2.

The solvent system used for chromatography on Sephadex G50 of the polysaccharide portion of the LPS is pyridine acetate buffer at pH 5.4.
Some countries may permit the use of SDS-polyacrylamide gel electrophoresis for this test.

The rough LPS of S. typhi Ty 21a are of both Ra (complete core but no O-side-chain) and Rc (no O-side-chain and incomplete core without galactose) types, while Ty 2 rough LPS are of the Ra type only.

Paper chromatography of $^{14}$C-labelled LPS hydrolysed in 0.5 mol/l sulfuric acid shows that the $^{14}$C-label is present only on the galactose spot. (This is a sensitive test to show that the mutation in the epimerase gene is not leaky.)

Furthermore, it shall be shown that the distribution of the sugars keto-deoxy-octonate (KDO), galactose, glucose, and rhamnose of the LPS are similar in their ratios for both the Ty 21a and the Ty 2 strains.

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) (2) shall apply.

In addition, the production process from the growth of the working seed to the harvesting, centrifugation, and resuspension must be carried out in a closed system. The virulent Ty 2 strain shall be excluded from the production area.

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 when ingested.

3.2.2 Temperature and time of incubation

The cultures shall be incubated at such a temperature and for such a time that they will be harvested in the early stationary phase.

The number of passages to provide the inoculum for the final fermenter from the working seed shall be kept to a minimum and in any event it shall not exceed 4 passages.

At every passage a sample of the bacterial suspension should be Gram stained and plated on blood agar to demonstrate the purity of the culture. In addition, plating on an indicator medium is used to confirm identity.
3.3 Control of single fermenters

Before centrifugation of the contents of the fermenters, samples shall be taken from each fermenter and tested to demonstrate that the bacteria have identity with the Ty 21a strain of the working seed, as shown by the tests of Part A, section 3.1.4.1 (c) and (d).

3.3.1 Viable count

The viable count of the contents of each fermenter shall be made on brain heart infusion agar and incubated at 37°C for 36 hours.

3.3.2 Pooling of contents of fermenters to form a single harvest

The contents of several fermenters may be centrifuged, resuspended in a stabilizer, and pooled to form a single harvest.

In some countries the contents of each fermenter are kept separate until after freeze-drying.

The stabilizer used shall be approved by the national control authority and the single harvests shall be maintained frozen until freeze-dried.

After freeze-drying, several single harvests may be blended to form the bulk powder, which is mixed thoroughly.

3.4 Control of final bulk

The final bulk is subjected to the following tests:

3.4.1 Test for identity

A sample of the powder shall be reconstituted and tested for identity by the test shown in Part A, section 3.1.4.1 (d).

3.4.2 Test for viable organisms

The viable count in a weighed quantity shall be measured by the test formulated in Part A, section 3.3.1.

The viable count shall be compared with that of the single harvests stored before freeze-drying and a minimum of 10% of the organisms shall have survived the freeze-drying process.
3.4.3 Test for residual moisture

The final bulk shall be tested for residual moisture which shall be shown to be not greater than a level approved by the national control authority.

Two methods have been shown to be useful. When the Karl Fischer method is used with Aquavit-Tacussel equipment the moisture content should be less than 3%.

4. FILLING AND CONTAINERS

The requirements concerning filling and containers given in Part A, section 4 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2) shall apply to vaccine filled in the dried form.

Care should be taken to ensure that the material of which the container is made does not adversely affect the bacterial content of the vaccine under the recommended conditions of storage.

4.1 Filling into capsules

The final bulk shall be homogenized with a calculated quantity of dry filling material such that at least $2.5 \times 10^9$ live organisms shall be filled into each gelatin capsule.

The range of $2.5 \times 10^9$ is to ensure that the capsules contain at least $1 \times 10^9$ as the human dose at the time the subject takes the capsule.

5. CONTROL TESTS ON FINAL PRODUCT

Samples shall be taken from each filling lot for the tests in the following sections.

5.1 Identity test

The contents of 3 capsules shall be tested for identity by the test described in Part A, section 3.1.4.1 (d).
5.2 Viable count

The viable count of the contents of 5 capsules shall be determined by the test described in Part A, section 3.3.1; it shall be between 2 and $5 \times 10^9$ viable organisms per capsule. This test of viable count shall be regarded as the test for potency.

A suitable method is to empty the contents of at least 5 capsules into a flat-bottom sterile Kolle-type flask with glass beads and then to suspend in 20 ml or 50 ml of physiological saline. The suspending is accomplished by swirling for approximately 15 minutes on a rotary shaker. Serial dilutions are prepared in physiological saline and—according to the presumed live count of capsules—the appropriate dilutions are plated on at least 5 brain heart infusion agar plates per dilution, with each plate receiving 0.1 ml. After aerobic incubation at 35–37 °C for 30–36 hours or longer, the typical colonies are counted and the average live cell content of each capsule is calculated.

5.3 Test for freedom from gross contamination

The contents of at least 3 capsules shall be tested on selective media for freedom from pathogens such as *Salmonellae* (other than Ty 21a), *Shigella*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus*. It shall be shown that none is present.

In addition, the number of non-pathogenic organisms that may be present shall not exceed $2 \times 10^5$ bacteria and 20 fungi per capsule.

5.4 Innocuity test

Each final lot shall be tested for abnormal toxicity by the inoculation of 0.01 human dose intraperitoneally into each of 5 mice and by giving a human dose orally to each of 3 guinea-pigs. The mice shall be observed for 7 days and the guinea-pigs for 14 days. None of the animals shall show signs of infection due to the vaccine.

5.5 Test for stability

The vaccine in its final form shall be tested for stability by a method approved by the national control laboratory. The viable
count of the vaccine shall not fall below \(2 \times 10^9\) live organisms per gelatin capsule throughout its permitted storage period.

5.6 Inspection of final containers

Samples of each final lot shall be inspected visually, and those that show abnormalities shall be discarded.

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) (2) 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

As it is not possible to label the gelatin capsules, the package or the leaflet of the capsules shall be labelled according to 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); (2).

It should include an indication that inactivation of the vaccine by gastric acid should be prevented by sodium bicarbonate.

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) (2) shall apply.

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 the present requirements until the expiry date as stated on the label.

The capsules should be stored in a dry place and in the dark at 5°C ± 3°C.

10.2 Expiry date

The expiry date for the capsules shall be not more than 18 months from the date of manufacture.

If data showing greater stability are available a later expiry date may be permitted.

PART B.

NATIONAL CONTROL REQUIREMENTS

1. GENERAL

The general requirements for control laboratories contained in Part B of the revised Requirements for Biological Substance No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2) shall apply.
2. RELEASE AND CERTIFICATION

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

The national control authority shall ensure that the presentation of the vaccine is such that living Ty 21a bacteria are able to cross the acidic gastric secretions and to infect the intestines.

A statement signed by the appropriate official of the national control laboratory shall be provided at the request of the manufacturing establishment and shall certify whether the lot of vaccine in question meets all national requirements as well as Part A of the present requirements. The certificate shall also state the date of the last satisfactory test for viable count, 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.

AUTHORS

The Requirements for Typhoid Vaccine (Live Attenuated, Ty 21a, Oral) were prepared by:
Professor R. Germanier, Director, Division of Bacteriology, Swiss Serum and Vaccine Institute, Berne, Switzerland (Consultant)
Dr I. Joó, “HUMAN” Institute for Serobiological Production and Research, Budapest, Hungary (Consultant)
Dr F.T. Perkins, Chief, Biologicals, WHO, Geneva, Switzerland
Dr J.D. van Ramhorst, Biologicals, WHO, Geneva, Switzerland

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Dr J. Cameron, Director, Biological Products, Institute Armand- Frappier, Laval-des-Rapides, Quebec, Canada
Dr V.F. Davey, Group Director, Science, Commonwealth Serum Laboratories, Parkville, Victoria, Australia
Mr I. Davidson, Head, Biological Products and Standards Department, Central Veterinary Laboratory, Weybridge, Surrey, England
Dr I. Di Tommaso, Responsible Head of Establishment, “Selavo” Tuscan Institute for Serotherapy and Vaccine Production, Siena, Italy
Dr J.C. Feeley, Assistant Director for Laboratory Science, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA, USA

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REFERENCES

Appendix

SUMMARY PROTOCOL OF TYPHOID VACCINE
(LIVE ATTENUATED, Ty 21a, ORAL)

PRODUCTION AND TESTING

Based on Requirements for Biological Substances No. 34,
Requirements for Typhoid Vaccine (Live Attenuated, Ty 21a,

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 viable count)

Nature of final product (gelatin capsules/
enteric-coated capsules)

Information on Manufacture

As the information on the seed lot will be identical for many batches, the producer is advised to make copies of this part of the protocol for future batches.

Working seed lot of *S. typhi* Ty 21a used

Date of manufacture of parent seed

Date of manufacture of working seed

Number of viable organisms per ampoule

Tests on working seed

(a) Appearance of growth (Gram stain
and motility)

(b) Antigens when grown without
galactose

Antigens when grown with galactose

(c) Growth on indicator medium

Presence of galactose-fermenting colonies

65
(d) Colony appearance on indicator medium

(e) Result of test on Kliger iron agar

Galactose-induced bacteriolysis

Mouse virulence

<table>
<thead>
<tr>
<th>Number of mice injected</th>
<th>Percentage survival</th>
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Galactose metabolism enzymes

<table>
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<tr>
<th>Ty 21a</th>
<th>Ty 2</th>
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<tr>
<td>Epimerases</td>
<td>+/−</td>
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<tr>
<td>Galactokinase (EC 2.1.1.6)</td>
<td>...% (100)</td>
</tr>
<tr>
<td>Galactose-1-phosphate-uridylyltransferase (EC 2.1.1.10)</td>
<td>...% (100)</td>
</tr>
<tr>
<td>Galactose-permease</td>
<td>...% (100)</td>
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Uptake and distribution of 14C galactose

<table>
<thead>
<tr>
<th>Ty 21a</th>
<th>Ty 2</th>
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<tr>
<td>% of galactose taken up after 7 hours</td>
<td>...%</td>
</tr>
<tr>
<td>14C galactose remaining in bacteria + medium</td>
<td>...%</td>
</tr>
<tr>
<td>% incorporated in cell wall</td>
<td>...%</td>
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Characterization of LPS

<table>
<thead>
<tr>
<th>Ty 21a</th>
<th>Ty 2</th>
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<tr>
<td>Of cells grown in presence of 1 g/litre 14C galactose</td>
<td>Smooth ...%</td>
</tr>
<tr>
<td>Rough Ra ...%</td>
<td>Rough Ra ...%</td>
</tr>
<tr>
<td>Rough Re ...%</td>
<td>Rough Re ...%</td>
</tr>
<tr>
<td>Ratio KDO/galactose/glucose/ rhamnose</td>
<td></td>
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<tr>
<td>Ratio KDO/galactose/glucose/ rhamnose Ty 2</td>
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This section of the protocol shall be completed for each final lot.

Production

Culture medium used

Temperature and time of incubation

Number of passages to final fermenter

Number of single harvests (fermenters) used in final bulk

66
Viable count in each separate fermenter

Date of blending to final bulk

**Tests on final bulk**

1. **Identity**
   - Growth on indicator medium
   - Presence of galactose-fermenting colonies
   - Colony appearance on indicator medium

2. **Viable organisms**
   - Viable count
   - Percentage of survival after freeze-drying

3. **Residual moisture**

**Filling into capsules**

- Date of filling
- Filling material

**Tests on final product**

1. **Identity**
   - Colony appearance on indicator medium

2. **Viable count**
   - Number of viable organisms per capsule

3. **Gross contamination**
   - Media used

   Pathogens found
   - Number of non-pathogenic bacteria per capsule
   - Number of fungi per capsule
4. Innocuity
   Number of mice injected
   Quantity injected
   Number of guinea-pigs on test
   Quantity given orally
   Result of tests

5. Result of mouse protection test, if done

6. Visual inspection result

Expiry date
   Has the lot been released by the national control authority?
   If so, date:
   Can a certificate, referred to in the
   Requirements for Typhoid Vaccine (Live Attenuated, Ty 21a, Oral), be supplied by the national control laboratory?
   Which laboratory would supply such a certificate?

   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.