

WHO Technical Report Series, No. 700, 1984

**SUMMARY PROTOCOL OF TYPHOID VACCINE  
(LIVE ATTENUATED, Ty 21a, ORAL)  
PRODUCTION AND TESTING**

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

**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

---

Name of viable organisms per ampoule

---

**Tests on working seed**

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

---

(b) Antigens when grown without galactose 0:9 + / - H:d + / -  
Vi + / -

Antigens when grown with galactose 0:9 + / - H:d + / -  
Vi + / -

(c) Growth on indicator medium

---

Presence of galactose-fermenting colonies

(d) Colony appearance + / - on indicator medium

---

(e) Result of test on Kligler iron agar H<sub>2</sub>S development + / -

Galactose-induced bacteriolysis + / -

---

Mouse virulence

Number of mice injected

---

Percentage survival

---

Galactose metabolism enzymes Ty 21a

Ty2

Epimerases + / - + / -

Galactokinase (EC 2.1.1.6) ...%

(100)

Galactose-1-phosphate-uridylyl-transferase

(EC 2.1.1.10) ...%

(100)

Galactose-permease ...%

(100)

Uptake and distribution of <sup>14</sup>C galactose

% of galactose taken up after 7 hours ...%

<sup>14</sup>C galactose remaining in bacteria + medium ...%

% incorporated in cell wall ...%

Characterization of LPS

Of cells grown in presence of Smooth ...%

Smooth ...%

1 g/litre <sup>14</sup>C galactose Rough Ra ...%

Rough Ra ...%

Rough Rc ...%

Rough Rc ...%

Ratio KDO/galactose/glucose/  
rhamnose Ty 21a

---

Ratio KDO/galactose/glucose/  
rhamnose Ty 2

---

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

---

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 ... live organism per ...


---

 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.