**TYPHOID VACCINE (LIVE, ORAL, STRAIN Ty 21a)**

Vaccinum febris typhoidis vivum perorale (stirpe Ty 21a)

**DEFINITION**

Typhoid vaccine (live, oral, strain Ty 21a) is a freeze-dried preparation of live Salmonella typhi strain Ty 21a grown in a suitable medium. When presented in capsules, the vaccine complies with the monograph on Capsules (0016).

**PRODUCTION**

**CHOICE OF VACCINE STRAIN**

The main characteristic of the strain is the defect of the enzyme uridine diphosphate-galactose-4-epimerase. The activities of galactoperoxidase, galactokinase and galactose-1-phosphate uridyl-transferase are reduced by 50 per cent to 90 per cent. Whatever the growth conditions, the strain does not contain Vi antigen. The strain agglutinates to anti-O:9 antiserum only if grown in medium containing galactose. It contains the flagellar H:6 antigen and does not produce hydrogen sulphide on Kligler iron agar. The strain is nonvirulent for mice. Cells of strain Ty 21a lyse if grown in the presence of 1 per cent of galactose.

**BACTERIAL SEED lots**

The vaccine is prepared using a seed-lot system. The working seed lots represent not more than one subculture from the master seed lot. The final vaccine represents not more than four subcultures from the original vaccine on which were made the laboratory and clinical tests showing the strain to be suitable.

Only a master seed lot that complies with the following requirements may be used in the preparation of working seed lots.

**Galactose metabolism.** In a spectrophotometric assay, no activity of the enzyme uridine diphosphate-galactose-4-epimerase is found in the cytoplasm of strain Ty 21a compared to strain Ty 2.

**Biogenesis of lipopolysaccharide.** Lipopolysaccharides are extracted by the hot-phenol method and examined by size-exclusion chromatography. Strain Ty 21a grown in medium free of galactose shows only the rough (R) type of lipopolysaccharide.

**Serological characteristics.** Strain Ty 21a grown in a synthetic medium without galactose does not agglutinate to specific anti-O:9 antiserum. Whatever the growth conditions, strain Ty 21a does not agglutinate to Vi antiserum. Strain Ty 21a agglutinates to H:6 flagellar antiserum.

**Biochemical markers.** Strain Ty 21a does not produce hydrogen sulphide on Kligler iron agar. This property serves to distinguish Ty 21a from other galactose-epimerase-negative S. typhi strains.

**Cell growth.** Strain Ty 21a cells lyse when grown in the presence of 1 per cent of galactose.

**BACTERIAL PROPAGATION AND HARVEST**

The bacteria from the working seed lot are multiplied in a preculture, subcultured once and are then grown in a suitable medium containing 0.001 per cent of galactose at 30 °C for 13 h to 15 h. The bacteria are harvested. The harvest must be free from contaminating micro-organisms.

Only a single harvest that complies with the following requirements may be used for the preparation of the freeze-dried harvest.

**pH.** The pH of the culture is 6.9 to 7.6.

**Optical density.** The optical density of the culture, measured at 546 nm, is 6.5 to 11.0. Before carrying out the measurement, dilute the culture so that a reading in the range 0.1 to 0.5 is obtained and correct the reading to take account of the dilution.

**Identification.** Culture bacteria on an agar medium containing 1 per cent of galactose and bromothymol blue. Light blue, concave colonies, transparent due to lysis of cells, are formed. No yellow colonies (galactose-fermenting) are found.

**FREEZE-DRIED HARVEST**

The harvest is mixed with a suitable stabiliser and freeze-dried by a process that ensures the survival of at least 10 per cent of the bacteria and to a water content shown to be favourable to the stability of the vaccine. No antimicrobial preservative is added to the vaccine.

Only a freeze-dried harvest that complies with the following tests may be used for the preparation of the final bulk.

**Identification.** Culture bacteria are examined on an agar medium containing 1 per cent of galactose and bromothymol blue. Light blue, concave colonies, transparent due to lysis of cells, are formed. No yellow colonies (galactose-fermenting) are found.

**Number of live bacteria.** Not fewer than 1 × 10¹¹ live S. typhi strain Ty 21a per gram.

**Water (2.5.12):** 1.5 per cent to 4.0 per cent, determined by the semi-micro determination of water.

**FINAL BULK VACCINE**

The final bulk vaccine is prepared by aseptically mixing one or more freeze-dried harvests with a suitable sterile excipient.

Only a final bulk that complies with the following requirement may be used in the preparation of the final lot.

**Number of live bacteria.** Not fewer than 40 × 10⁸ live S. typhi strain Ty 21a per gram.

**FINAL LOT**

The final bulk vaccine is distributed under aseptic conditions into capsules with a gastro-resistant shell or into suitable containers.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Number of live bacteria may be released for use, except that in the determination of the number of live bacteria each dosage unit must contain not fewer than 4 × 10⁸ live bacteria.

**IDENTIFICATION**

Culture bacteria from the vaccine to be examined on an agar medium containing 1 per cent of galactose and bromothymol blue. Light blue, concave colonies, transparent due to lysis of cells, are formed. No yellow colonies (galactose-fermenting) are found.

**TESTS**

**Contaminating micro-organisms (2.6.12, 2.6.13).** Carry out the test using suitable selective media. Determine the total viable count using the plate-count method. The number of contaminating micro-organisms per dosage unit is not greater than 10² bacteria and 20 fungi. No pathogenic bacterium, particularly Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and no salmonella other than strain Ty 21a are found.
**Water (2.5.12):** 1.5 per cent to 4.0 per cent, determined on the contents of the capsule or of the container by the semi-micro determination of water.

**NUMBER OF LIVE BACTERIA**

Carry out the test using not fewer than five dosage units. Homogenise the contents of the dosage units in a 9 g/l solution of sodium chloride R at 4 °C using a mixer in a cold room with sufficient glass beads to emerge from the liquid. Immediately after homogenisation prepare a suitable dilution of the suspension using cooled diluent and inoculate brain heart infusion agar; incubate at 36 ± 1 °C for 20 h to 36 h. The vaccine contains not fewer than \( 2 \times 10^9 \) live *S. typhi* Ty21a bacteria per dosage unit.

**LABELLING**

The label states:
- the minimum number of live bacteria per dosage unit,
- that the vaccine is for oral use only.

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**VARICELLA VACCINE (LIVE)**

Vaccinum varicellae vivum

**DEFINITION**

Varicella vaccine (live) is a freeze-dried preparation of a suitable attenuated strain of *Herpesvirus varicellae*. The vaccine is reconstituted immediately before use, as stated on the label, to give a clear liquid that may be coloured owing to the presence of a pH indicator.

**PRODUCTION**

The production of vaccine is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to yield consistently live varicella vaccines of adequate immunogenicity and safety in man. The virus in the final vaccine shall not have been passaged in cell cultures beyond the 38th passage from the original isolated virus. The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (2.6.9).

**SUBSTRATE FOR VIRUS PROPAGATION**

The virus is propagated in human diploid cells (5.2.3).

**VIRUS SEED LOT**

The strain of varicella virus shall be identified as being suitable by historical records which shall include information on the origin of the strain and its subsequent manipulation. The virus shall at no time have been passaged in continuous cell lines. Seed lots are prepared in the same kind of cells as those used for the production of the final vaccine. To avoid the unnecessary use of monkeys in the test for neurovirulence, virus seed lots are prepared in large quantities and stored at temperatures below \(-20 °C\), if freeze-dried, or below \(-60 °C\), if not freeze-dried. Only a virus seed lot that complies with the following requirements may be used for virus propagation.

**Identification.** The master and working seed lots are identified as varicella virus by serum neutralisation in cell culture, using specific antibodies.

**Virus concentration.** The virus concentration of the master and working seed lots is determined as prescribed under Assay to monitor consistency of production.

**Extraneous agents (2.6.16).** The working seed lot complies with the requirements for seed lots for live virus vaccines; a sample of 50 ml is taken for the test in cell cultures.

**Neurovirulence (2.6.18).** The working seed lot complies with the test for neurovirulence of live virus vaccines.

**VIRUS PROPAGATION AND HARVEST**

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled. Approved animal (but not human) serum may be used in the media. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and approved antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. 5 per cent, but not less than 50 ml, of the cell cultures employed for vaccine production is set aside as uninfected cell cultures (control cells). The infected cells constituting a single harvest are washed, released from the support surface and pooled. The cell suspension is disrupted by sonication.

Only a virus harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

**Identification.** The virus harvest contains virus that is identified as varicella virus by serum neutralisation in cell culture, using specific antibodies.

**Virus concentration.** The concentration of infective virus in virus harvests is determined as prescribed under Assay to monitor consistency of production and to determine the dilution to be used for the final bulk vaccine.

**Extraneous agents (2.6.16).** Use 50 ml for the test in cell cultures.

**Control cells.** The control cells of the production cell culture from which the single harvest is derived comply with a test for identity and with the requirements for extraneous agents (2.6.16).

**FINAL BULK VACCINE**

Viruses that comply with the above tests are pooled and clarified to remove cells. A suitable stabiliser may be added and the pooled harvests diluted as appropriate.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

**Bacterial and fungal contamination.** Carry out the test for sterility (2.6.1) using 10 ml for each medium.

**FINAL LOT**

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers and freeze-dried to a moisture content shown to be favourable to the stability of the vaccine. The containers are then closed so as to prevent contamination and the introduction of moisture.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

**IDENTIFICATION**

When the vaccine reconstituted as stated on the label is mixed with specific *Herpesvirus varicellae* antibodies, it is no longer able to infect susceptible cell cultures.

**TESTS**

**Bacterial and fungal contamination.** The reconstituted vaccine complies with the test for sterility (2.6.1).