PREPARATION OF BACTERIAL VACCINE

A. Killed bacterial vaccine preparation (Example: Cholera vaccine)

i. Selection of an antigen: Each strain is carefully checked for freedom from variation and absence of contaminating organisms.

ii. Inoculation into media and incubation:

- Bacteria grown in media rich in proteins, vitamins and salts
- Selected strain is inoculated onto a solid or liquid medium
- Incubated under optimum conditions for 1-3 days
- At the end, cells are harvested from:

✓ Solid medium- by scraping the organism from solid surface with sterile saline centrifuged to remove pieces of agar washing off

✓Liquid medium- by centrifugation washed free from broth constituents

iii. Inactivation of cell suspension : Cell suspension can be inactivated by:

- Heat: 56ºC for one hour
- Chemicals:

✓ 0.5% formalin for plague and pertussis,

✓ Phenol for cholera,

✓ Thiomersol for pertussis,

✓ 75% alcohol for TAB and TABC

iv. Standardization: The total number of organisms per mL is determined by any one of the following methods:

- Direct: Helber cell or hemocytometer method
- Indirect: Opacity method such as Brown's tube or photoelectric merthod

v. Formulation: By incorporating some other substances including

- Acidity regulators: Sodium or potassium phosphate
- Preservatives: Thiomersol
- Stabilizers: Formaldehyde or phenol

vi. Storage:

- Stored in original packing at optimum temp. (mostly 2-8ºC) and protected from light
- All vaccines are sensitive to some extent to heat and cold as,

✓ Heat may speed up the decline in potency

✓ Freezing may cause increased reactogenicity, loss of potency and hairline cracks in the container, leading to content contamination,

B. Attenuated bacterial vaccine preparation

- Steps involved in preparation of attenuated bacterial vaccine are same as for killed bacterial vaccine.
- The only difference is that there is no sterilization or inactivation stage
- Following strict regulations are laid down for the manufacture of attenuated vaccine:

✓Use of completely self contained laboratory suit in which no living organism except the desired one are allowed

✓Superlative air conditioning

✓Regular X-ray examination of staff to prevent contamination from virulent bacilli

- Example: BCG vaccine