Detection and Confirmation of *Salmonella species* in foods.

Environmental sources:

water, soil, insects, factory surfaces, kitchen surfaces, animal feces, raw meats, raw poultry, and raw sea foods, to name only a few.

PURPOSE:

To determine the presence of *Salmonella* in processed foods and soils/water used for the growth of foods intended for human consumption.

Culture Media:

1-Per-enrichment :

- Lactose broth
- Trypticase Soy Broth **(TSB) .**
- Trypticase Soy Broth Containing Potassium Sulfite at a final concentration of 0.5%.
- Reconstituted Non-Fat Dry Milk
- 1% aqueous Brilliant Green Dye Solution.

2- Selective Enrichment :

- Selenite Cystine Broth **(SCB)** .
- Tetrathionate Broth**(TB).**

3- Selective media plating

- Xylose Lysine Deoxycholate **(XLD)** Agar
- Hektoen Enteric Agar **(HEA)**
- Bismuth Sulphite Agar **(BSA)**

Procedure:

Preparation of sample and pre-enrichment

Aseptically open the sample container and weigh 25g sample into a sterile empty wide mouth container with screw cap or suitable closure.

Add 225ml of sterile **lactose broth** to the sample. Buffered peptone water, **Trypticase soy broth**, and **nutrient broth** can also be used for pre- enrichment. Make a uniform suspension by blending if necessary.

Cap container and let stand at room temperature for 60 min.

Instead of lactose broth the recommended pre-enrichment broth for the following food samples is as follows :

-Non fat dry milk and dry whole milk - Sterile distilled water. Add 0.45 ml of 1% aqueous briliant green dye before incubation.

-Dried active yeast - Trypticase soy broth

-Onion-garlic powder - Trypticase soy broth containing potassium sulfite at a final concentration of 0.5%

-Milk Chocolate - Reconstituted non fat dry milk.

Shake and adjust pH (if necessary) to 6.8±0.2 with sterile 1N NaOH or 1N HCl. Incubate at 35 C for 24±2 hours .

Selective enrichment

Gently shake incubated sample mixture and transfer 1 ml to 10 ml of **selenite cystine broth** and an additional 1 ml to **tetrathionate broth**.

Incubate 24±2 hours at 35 C.

Selective media plating

Vortex - mix and streak 3 mm loopful of incubated selenite cystine broth on selective media plates of XLD, HEA and BSA. Repeat with 3mm loopful of incubated tetrathionate broth.

Incubate plates at 35 C for 24±2 hours and 48±2 hours.

Observe plates for typical Salmonella colonies

On XLD (after 24h) - Pink colonies with or without black centres.

On HEA (after 24h) - Blue green to blue colonies with or without black centers.

On BSA (after 24 to 48h) - Brown, grey or black colonies sometimes with metallic sheen. Surrounding medium is usually brown at first, turning black with increasing incubation time.

Calculation:

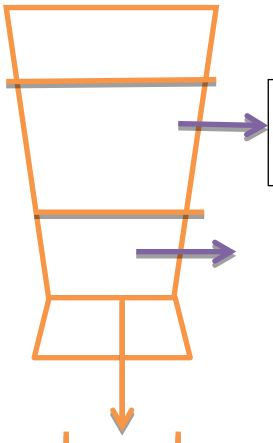
NA

Expression of Result:

Salmonella = Present/Absent per 25 g

Detection of salmonella

1-Food homogenate



225mi buffered peptone water

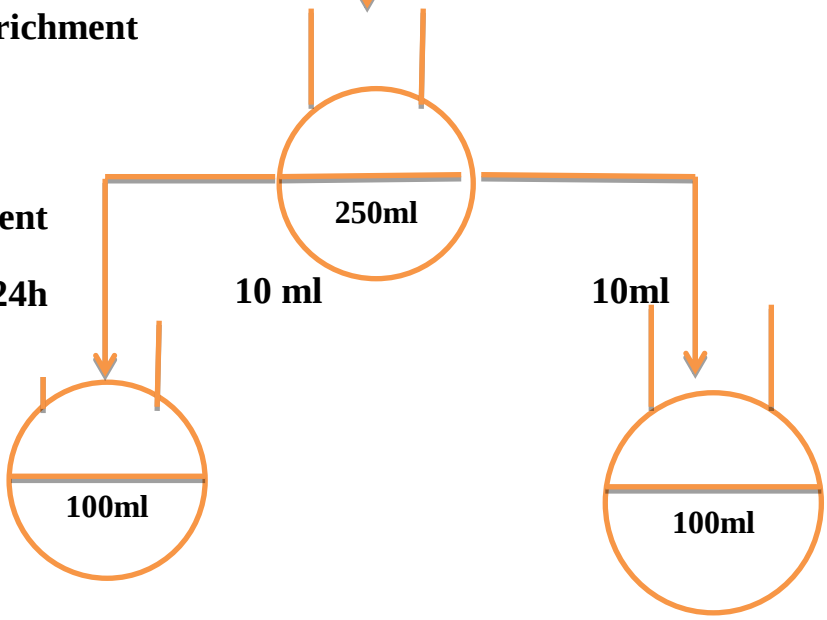
25g food

Blend at 15000-20000

2-Pre-enrichment

3-Enrichment

Incubate at 35C for 24h

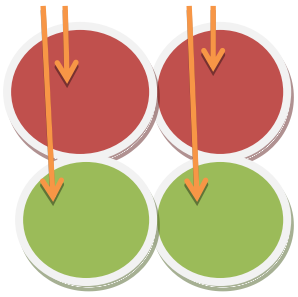


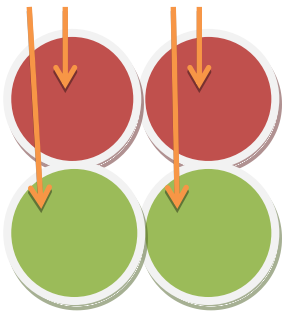
Tetrathionate Broth

Selenite Cystine Broth

4-plating

Incubate at 35C for 24h



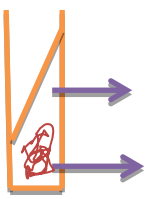


XLD agar

Bismuth sulphite agar(BSA)

5-secreening

On TSI



Slant red

Butt; yellow. Black.spots,

6-confirmation

a-Biochemical

b-serological