

Investigation of pathogenic bacteria in water
Isolation and identification of Salmonella and Shigella:

Salmonella

Bacilli, gram negative stain ,non forming spores , motile by peripheral flagella , mostly non fermented sugar lactose forming hydrogen sulfide (H₂S) , differ from other Enterobacteriaceae by non fermented ,non capsulated. when growth on Macconkey agar or S-S agar so be pale colonies .

Shigella

Bacilli , gram negative stain , non forming spores, non motile, late fermented sugar lactose and non hydrogen sulfide production (H₂S) .

Differ Salmonella about Shigella by :

1. Forming gas and acid when grow in sugar solution
2. Non Motile .
3. Non Forming (H₂S) .
4. Most of them do not have ability to produce indole .

Salmonella causes typhoid and salmonellosis food poison when eating food and drinks contaminated while Shigella causes dysentery disease with diarrhea and Rose Bengal

Procedure

1. Taken different sample of water .
2. Add sample water to enrichment broth (size equal to the size of the enrichment broth) .

Enrichment broth

Selenite broth is used as a selective enrichment for Salmonella spp. And reduced growth of fecal coliforms. Incubate at 24 h and when appear turbidity or orange color, streaking it on the selective media agar.

GN broth : is used a selective enrichment for Shigella inhibition growth E.coli and fecal streptococci.

Note growth on GN broth media (turbid form) and on Selenite broth media on the form of turbidity and orange color.

Divided the dish S.S agar into two parts and streaking on the first part by loop from Selenite broth and on second part from GN broth.

3. Incubate petri dish at 37°C for 24 h.
4. Note colonies.

Pale colonies +FeS -----Salmonella

Pale colonies without FeS-----Shigella

5. Prepare smear and staining by gram stain to know from bacteria under microscope

Differential selective media because it contains bile salt material inhibitory gram negative bacteria and contain lactose sugar to differentiate between fermented and non fermented to lactose because it contains evidence neutral red, differentiates this media between Salmonella and Shigella because the Salmonella produce H₂S which react with iron giving FeS similar a black precipitate in the center of the colony, while Shigella has pale colonies without black center because it non produce to H₂S.

Isolation and diagnosis Vibrio cholera :

Bacilli, gram negative stain, short curved (vibrio form), motile by flagellum, non forming spores and capsule and gas, fermented sugar except lactose, live in salt and fresh water, cause cholera disease where the disease caused profuse diarrhea lead to the loss of body fluids and may lead to death prefer to grow in the alkaline media pH = 8.5 – 9.5 and halophiles.

Procedure

1. Taken different water sample
2. Added water sample to enrichment media (alkaline peptone water pH = 8.5) in addition the same media size, this media contain salt therefore this bacteria live in seas water because halophiles.
3. Incubate media at 37°C for 18 h but not more than this temp because if increased this period lead to the pH become acidic, this affect the growth of bacteria and promote bacteria acidophilus.
4. Taken 2 ml from this culture media and added to second alkaline peptone water and incubate at 37°C for 6 h.
5. Streaking from enrichment media on selective agar media.

Culture on selective media :

1. After note growth on enrichment media taken from surface culture media (note growth in the form of a white crust on surface) streaking on selective agar media by method ABCD for the purpose separation the colonies.
 2. Incubate the petri dish at 37°C for 18 h
- Note the growth colonies to Vibrio where a small, circular, diameter 1-3 Mm soft smooth, convex, yellow color.
4. Selective media use in isolated Vibrio cholera is (T.C.B.S.) (thiosulfate-citrate-bile-sucrose) pH = 8.5.