

Isolated& count fecal Streptococci

Streptococci exist in intestines of human and animals such as :

1. *Streptococcus faecalis*: live in human intestines.
2. *S. bovis* live in cows intestines.
3. *S. equines*: live in horses intestines.

The presence of these bacteria with *E. coli* and *Fecal Coliform* use to acknowledge the source of contamination whether from humans or animals, where found there is a ratio between Fecal Coliform / Fecal Streptococci resulting this ratio more than one if fecal contamination comes from human because the number of Coliform more than the number of Streptococci in human gut while less than one the fecal contamination comes from animals.

MPN method:

Presumptive test:

- 1- Using the same method used in inoculation with *E. coli* but user media is Azide dextrose broth.
- 2- Add (0.5ml) from water sample to three test tubes contain (4.5 ml) from Azide media this first dilution. Then transport (0.5 ml) from this three test tubes to other three test tubes non inoculation contain (4.5 ml) from media and mix this dilution, produces here 9 tubes and three replications and each repeater has three test tubes to one dilution (10^{-1} , 10^{-2} , 10^{-3}) . do not more dilution sample because the bacteria do not grow easily .
- 3- Incubated tubes at 37c for 24-72 h.
- 4- Note grow and turn red color media to yellow and the result record user reagent is phenol red.
- 5- Record MPN value.
- 6- Compare the resulting number with fecal coliform number, and apply the percentage.

Complete test:

Grow bacteria on PSE media (Pfizer selective agar) where colonies of fecal Streptococci show black- brown surrounded by a halo brown . selective media contain Sodium azide material , a substance tolerant Streptococci and not tolerant other bacteria.

Isolated clostridium:

Large bacillus, gram positive stain, with round end exist in a single form or pairs, forming capsule and spores, non motile, anaerobic because forming spores they consider indicates of old fecal contamination cause food poisoning, Gas gangrene wherein lysis RBC complet hemolysis (B- hemolysis).

The presence *C. perfringens* and absence *E. coli* indicates presence of old fecal contamination but presence *E. coli* with it indicates presence of a new fecal contamination because *E. coli* still exist.

Presumptive test:

Not work dilution of the sample because of the few numbers in water.

- 1- Fill glass tube with water sample then pasteurize for 10 min at 80 c.
- 2- Inoculate tubes contain DRCM media (differential Reinforced Closteridial Media) and to provision anaerobic conditions fill glass tubes and leaves little space near the nozzle of the tube, while if use tubes with a screw cap do not close tightly stoppers and not completely to allow the gas formed during the growth of bacteria out so as not to example tube.
- 3- Added sodium sulfide solution and iron citrate (one ml of each one) to media before inoculate because the device enzymatic to bacteria works best with presence reductive factors and forming black precipitate is an iron sulfide (FeS) (positive results).
- 4- Incubate tubes at 37c for 24 h and note black precipitate.

Confirmed test :

Are being sure the presence of bacteria *C. perfringens* through the stormy fermentation phenomenon where the fermentation of sugar in litmus milk media leads to the acidity of the media and then the bacteria are working of deposition milk protein and appears clot because of the large amount of gas H₂ & CO₂ that produce by bacteria clot rise to the surface of the media and media turn to a transparent liquid.

- 1- Prepare tubes contain (litmus milk) with a screw stoppers and placed in a water bath for 5 min to expel oxygen.
- 2- Transport 1ml from positive result to litmus milk tubes and fill to end close the tubes quickly to prevent exposed to air and O₂ solubility in the media.
- 3- Incubate tubes in anaerobic jar for 48 h at 37 c and note stormy fermentation.
- 4- Prepare slides to examination of bacteria and staining by Gram stain and note colonies bacillus, gram positive stain forming terminal spores.