Laboratory (6) Water Microbiology Dr Duaa Suhail

Isolation and Enumeration of Fecal Streptococci

Fecal streptococci are present in human and animal intestine, therefore, their existence in water is considered as an indication of recent fecal contamination. Furthermore, these bacteria are present in soil, grass and some insects.

Characteristics of Fecal Streptococci:

1. Gram-positive bacteria
2. Cocci, arranged in pairs or in short cjains.
3. Include several species such as:

Streptococcus faecalis var. *liquefaciens*
Streptococcus feacalis var. *zymogenes*
Streptococcus faecium
Streptococcus faecium var. *durans*
} Human Waste

Streptococcus equines
Streptococcus bovis
} Animal Waste

The presence of fecal streptococci (Fs) with fecal coliforms (Fc) confirms the source of fecal contamination, whether it is from human or animal. Therefore, the ratio Fc/Fs is used as an indicator for determination of feces source. If the number of Fc in human feces is more than the number of Fs in animal feces (e.g., Fc/Fs = 4/1), this means that the source of contamination is the human. While if the number of Fs is more than the Fc number (e.g., Fc/Fs = 0.6/1), this means that the contamination source is the animal. This test is important for chlorinated water since these bacteria (Fs) are resistant to chlorine than coliforms.

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Count Techniques of Fecal Streptococci

1. Most probable number (MPN) method
2. Membrane filtration method
3. Plate count method

Most probable number (MPN) method

Presumptive test:

1. Different water samples such as river, sea, sewage are collected, 100 ml for each sample.
2. Three sets of five test tubes containing 10 mL of sterile Azide-dextrose broth are prepared for each sample resulting in a total of 15 tubes for each water sample.
3. Label each tube according to the amount of water to be dispensed to it: 10 ml, 1.0 ml and 0.1 ml, respectively.
4. Mix the water sample by shaking several times to ensure homogeneity
5. Ten ml, 1 ml and 0.1 ml samples are added sequentially in 5 test tubes containing 10 ml of double concentration Azide-dextrose broth (2X), 5 tubes containing 10 ml of single concentration Azide-dextrose broth (1X) and 5 test tubes containing 10 ml of (1X) Azide-dextrose broth.
6. Incubate the tubes at 37º C for 24-72 hrs.
7. Check the tubes for positive result (positive presumptive test) and compare the number of tubes giving a positive reaction to a standard chart and record the number of bacteria present in it.

Note: Positive result is indicated by the appearance of turbidity in medium and change in color from red (pH indicator = phenol red) or form

purple (pH indicator = bromocresol purple) to yellow as a result of sugar fermentation and acid production.

If the result of presumptive test is positive, proceed to the confirmed and completed test. While, if it is negative, there is no need to perform other tests and presumptive test would be enough to determine the presence of fecal streptococci.

Note: Azide- dextrose broth is a selective medium as it inhibits the growth of Gram-negative bacteria and allows fecal streptococci growth. It contains the following substances:

1- Sodium azide (NaN_3) which inhibits the growth of Gram-negative bacteria.
2- NaCl 7.5% that inhibits the growth of non-enterococci.

Confirmed test:

1. Transfer a loopful from positive tubes in presumptive test to other test tubes containing Ethyl violate broth and incubate at 37ºC for 24 hr.

2. After the incubation period, a purple ring is observed at the bottom of test tubes with the appearance of high turbidity which indicates the positive result of this test. A bacterial smear is prepared from positive tubes and stained with Gram-staining to visualize the bacterial cells of fecal streptococci