Mi4 : Fecal contamination indicators : thermoduric and total coliform and streptococci in drinking water

Filtration technique

1. Generalities

The intestinal flora includes several germs, indicated following this decreasing predominance :
- rod shaped Gram negative bacteria, anaerobic and called bacteroids
- the genus Bifidobacterium (rod shaped, Gram positive, anaerobe)
- genus Clostridium (cocci, gram positive and sporulating) and lactic bacteria

These three kind of bacteria are difficult to grow in laboratory.
- the specie E.coli
- the genus Enterococcus

These two types of bacteria are easy to grow
And finally,
- enterobacteria (like Citrobacter), genus Staphylococcus, genus Bacillus, yeasts…

FCI are commensally of the intestine ; when they are present in water, there is a risk of contamination due to pathogenic bacteria like Salmonella, Shigella, Vibrio cholerae…
Fecal contamination is due to the discharge of waste water in surface water ; MWW contain 10^6 to $10^{è}$ bacteria / mL.
There are three categories of FCI :
- total coliforms, thermoduric coliforms and E.coli :they are commensally of the intestines and can't survive for a long time in water ; E.coli is the main representant of thermoduric coliforms
- fecal streptococci (Enterococcus faecali) , also commensally of the intestine, but can survive longer in water : they are the indicator of an older contamination
- sulphite reducing Clostridium, which are the less reliable FCI because they can also live like saprophytes in water.

The most reliable FCI in water are thermoduric coliforms.

2. Principle and definitions

Coliforms :
Micro-organism growing at 37°C on lactose-bile salt containing agar (called tergitol 7 TTC agar), acid producing in 24h
and oxidase negative .
. The selective agar medium Tergitol 7 TTC contains molecules that inhibit cocci gram positive growth (like streptococci…)

Thermoduric coliform :
Same proprieties than coliforms, but at 44°C.

There are two steps : presumption and then confirmation
- growing on tergitol 7 TTC
- inoculating each colony from Tergitol 7 on nutritive agar in order<to examine the presence of oxidase :
We will just carry out the first step, i.e. presumption

Streptococci :

Micro-organism growing at 37°C on glucose- azide containing agar (called Slanetz Bartley agar), generating typical TTC reducing colonies, and generating positive reaction in 24h at 37°C on esculine –bile containing agar. The selective agar medium Slanetz Bartley contains molecules that inhibit rod shaped gram negative growth (like coliforms…)

We will just carry out the first step

3. Operation

Requirement for one experimentation :
One flask containing 300 mL of 100 to 300 E.coli / L suspension
One flask containing 300 mL of 100 to 300 Enterococcus faecalis / L suspension

How to prepare a solution containing a precise bacteria concentration ?
- inoculate 10mL of broth with the tested bacterium
- incubate 24 h at 37°C :

the concentration is maximum and ≈ 10^7 /ml = 10^{10}/L

Dilute this suspension 10^8 times : 100 µL in 100 mL, then 1OOµL in 100mL, then 1 in 100mL and you'll have a concentration equal to 100 bacteria /L

3 sterile filtration membranes 55mm, 45µm
One filtration ramp
Two tergitol 7-TTC agar plate 55mm
One slanetz barley agar plate 55 mm
Two 100 mL flask of sterile distillated water
Two incubators : 37 and 44°C

- Prepare aseptically the filtration apparatus and the membrane : **page 1**
and **page 2** : figures 1 to 5 (but no absorbent pad)
- filtrate 100ml of water sample (3 samples)
- place the membrane : (figures 6 to 9 **page 2** but between figure 7 and 8, rinse the funnel with sterile water)
    - on Tergitol 7 TTC agar plate and incubate at 37°C in order to analyse total coliform
    - on Tergitol 7 TTC agar plate and incubate at 44°C in order to analyse thermoduric coliform
    - on Slanetz Bartley agar plate and incubate at 37°C in order to analyse fecal streptococci

Duration of incubation : 24h

Second day : Count positive colonies :
- on Tergitol 7 : yellow colonies surrounded by a yellow halo
- on Slanetz : red pink or brown colonies

4. Report
Each colony is issued from one bacterium growth
We'll consider that each characteristic colony (first step, i.e. presumption step) represents the definitive analysed bacterium (no confirmation test).

Determinate the : (bacteria / 100mL)
- total coliform concentration
- thermoduric concentration
- fecal streptococci concentration, in the three samples.

*The result should be equal to **approximately** 10 colonies / plate, i.e. 10 bacteria / 100mL. (between 1 and 100 bacteria / 100 mL)*