Laboratory (5) Water Microbiology Dr Duaa Suhail

Isolation of *Clostridium perfringens* from Water

Characteristics of *Clostridium perfringens*:

1. Gram-positive bacteria.
2. Anaerobic or microaerophilic bacteria.
3. Rod-shaped, rounded end bacilli.
4. The arrangement in single or double or short chains.
5. Non-motile, spore-forming (endospore) and capsule-forming bacteria when they are present in tissues.
6. The bacterium is present in nature inhabiting decaying vegetation, marine sediment and soil. Low numbers of this bacterium are found in stool samples of human and animal (vertebrates and insects), since their intestinal tract is considered as a natural bacterial habitat. However, in case of food poisoning or gas gangrene they are present in large numbers. Furthermore, they can be the cause of an intestinal syndrome called "Clostridial enterotoxicosis" brought on by abnormally high levels of bacteria.
7. It is a good indicator of fecal pollution even in the absence of *E. coli* and fecal streptococci. Its presence in the water with the absence of these bacteria is considered as a sign of old fecal contamination.
8. Its ability to form spores allow the bacterium to stay for a long time in the water and resist unsuitable conditions such as chemical disinfectants (chlorine used for water disinfection), antiseptics and physical factors (heating and dryness).

9. It has the ability to ferment sugars like sucrose, maltose, and glucose producing acid and gas showing a significant feature called “stormy fermentation” which can be used for the diagnosis of bacterial species.
10. It has the ability to analyze proteins and amino acids containing sulfur.
11. When the bacterium grows on culture media containing reductase factors such as carbohydrate sources (glucose or maltose) and protein sources (amino acids). It utilizes the elements from medium nutrients like C, H, O, N, S. P, K, Mg, Fe, Ca and Mn which are required for energy and cellular biosynthesis. It can utilize these elements by producing special enzymes.
12. Clostridium can reduce sulphite to sulphide at 37° C within 24 hrs.

Detection methods of *Clostridium*:

1. The most probable number method (MPN).

2. Litmus milk method.

3- Wilson and Blair sulfite medium.

The Most Probable Number Method (MPN):

Method Principle:

Differential Reinforced Clostridium Medium (DRCM) is used in this method. This medium contains sodium disulfite and ferric citrate. The method is based on the ability of *C. perfringens* to reduce ferric sulphite into ferrous sulphide which can be detected as black precipitate in the medium.

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Presumptive Test:

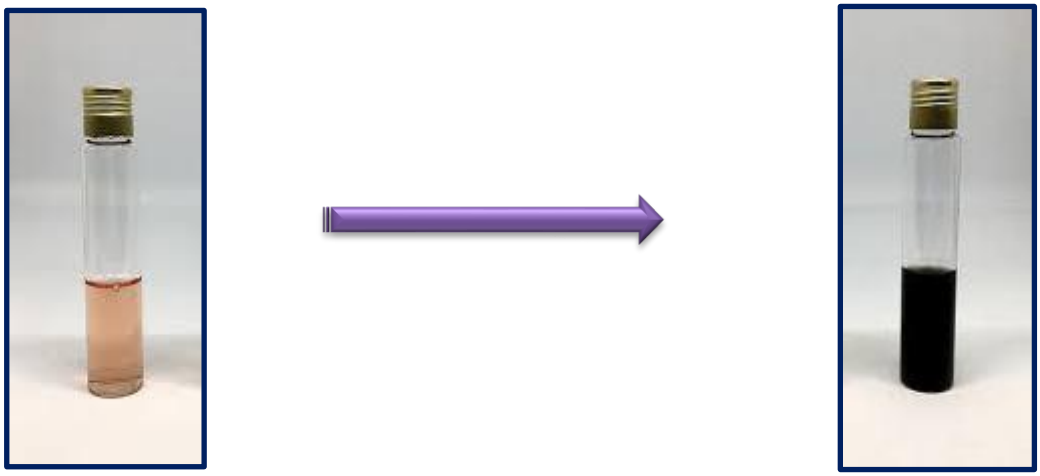
1. Vegetative forms of bacteria must be firstly removed from water sample by pasteurization at 70-80º C for 10 mins. The pasteurization temperature is much lower than the boiling temperature, so the bacterial spores are able to resist it. Pasteurization is conducted to eliminate non-spore forming bacteria. Follow the same steps involved in counting coliform bacteria.

2. Double concentration of medium is used for large volumes of water and single concentration for small volumes.

3. To provide anaerobic conditions for bacterial growth, fill the tube with medium. In case the tubes are closed strongly, pressure will form. Therefore, to avoid tubes burst, close the tubes with plugs but do not close or fill up completely to allow the gases formed during growth out.

4. Incubate the inoculated tubes at 37º C for 48 hrs. After the incubation period, check the black precipitate in the medium as a result of bacterial growth and reduction of sulfite into FeS, which indicates the positive test result (the presence of *C. perfringens*).

5. Refer to the McCradys tables for MPN/100 ml of water.



Confirmed Test:

1. Inoculate tubes containing Litmus milk medium from the tubes which showed positive presumptive test by using a sterile loop**.**

2. Incubate at 37º C for 48 hrs under anaerobic conditions (Add Paraffin Wax).

3. Record the results and observe the stormy fermentation phenomenon occurs by the appearance of a broken clot or cracks in the curd.

4. A Gram staining could be also carried out for the grown bacterium to check the bacterial cells.

Results Reading:

If the bacterium ferment lactose, an acid and gas will be formed leading to stormy fermentation phenomenon that appears center-aligned in thrombus rise to the top as a result of gas formation. The medium turns into a transparent liquid and the thrombus formed because of curdling casein (milk protein).

- Litmus milk: it is a multipurpose medium distinguish between different species of bacteria. It contains lactose (milk sugar), casein (milk protein) and litmus (pH indicator). The bacterium can use either sugar or protein, both of them, neither of them.

- Species of bacteria which ferment lactose will lead to acid reaction causing a color change of medium into pink, whereas species that utilize casein protein will result in alkaline reaction and change the medium color into blue. If the casein is completely hydrolyzed, the medium turns into a clear brown. Moreover, some species produce a renin enzyme (curd formed) and gas in the curd.

Pink = Acid reaction (lactose fermentation with acid production)

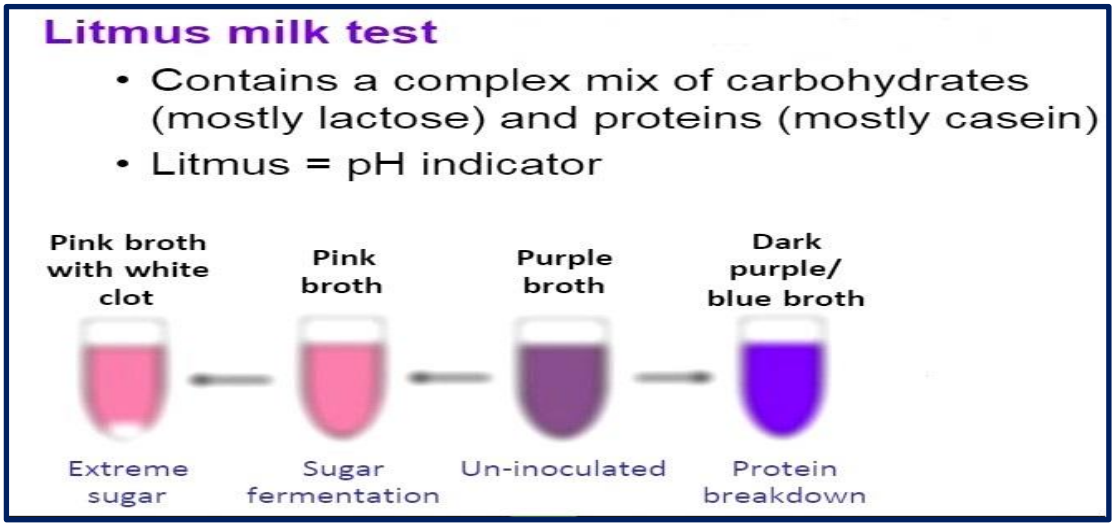
Purple = No lactose fermentation

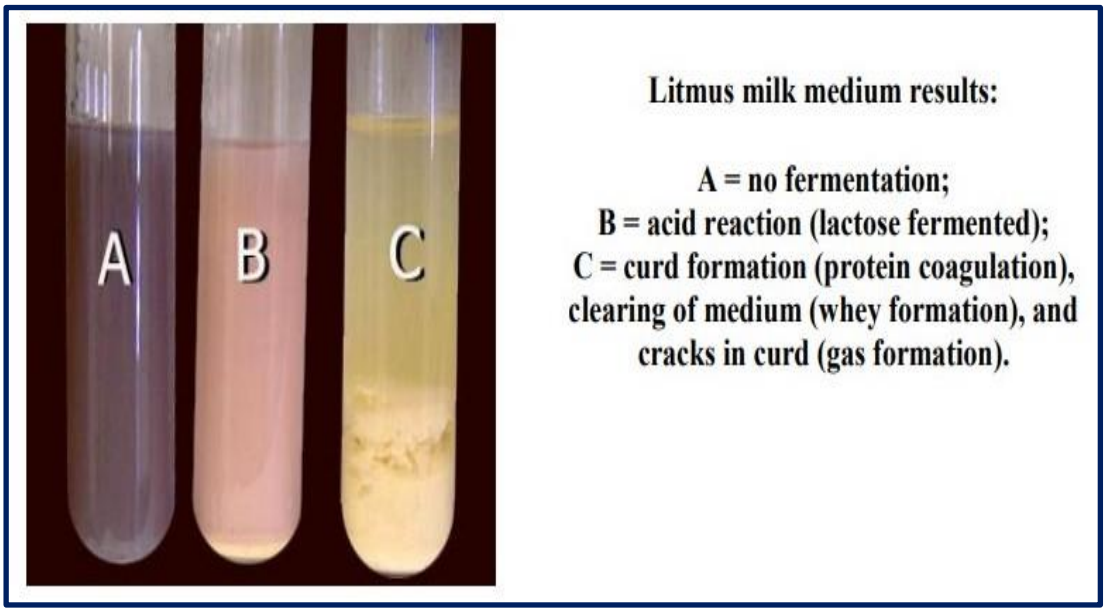
Blue = Alkaline reaction (degradation of amino acid in milk)

Clots or curds = Milk protein coagulation

Clearing of medium = Whey formation (digestion of milk protein)

Cracks in curds = Stormy fermentation / Too much gas formation (CO2 and/or H2).





Diagnostics: Litmus Milk Reaction showing

Saccharolytic and proteolytic properties of Cl. *perfringnes*

1- Acidic Reaction

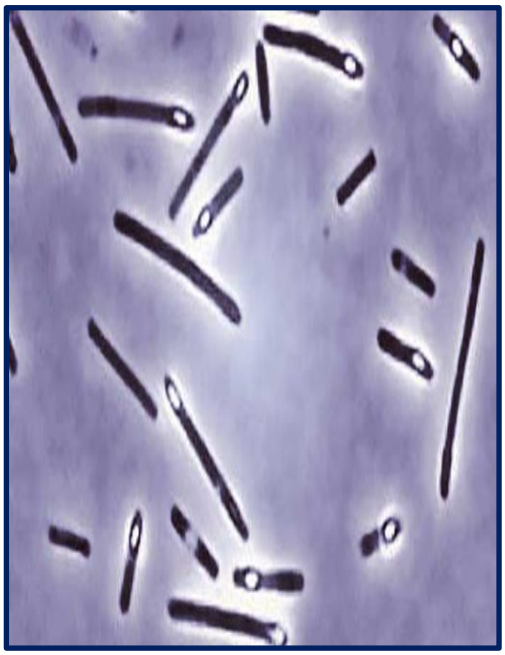
Lactose in Milk → Fermented to Acid → Litmus Indicator Pink Color

2- Basic Reaction

Casein in Milk → Digestion → Alkaline amines → Litmus Indicator Blue Color

2- Stormy fermentation

Too much acid and gas



Wilson and Blair sulfite (Bismuth Sulphite) medium procedure:

1. After the pasteurization of water sample (70-80º C) for 10 mins, mix 20 ml of water sample with 20 ml of Wilson and Blair medium.

2. Pour into two plates and incubate anaerobically at 37º C for 48 hrs using a candle jar.

3. Black colonies indicate the presence of *C. perfringens*.