

Specification

Liquid medium used for the detection and enumeration of coliforms by MPN technique.

Formula * in g/L

Peptone.....	20,000
Lactose.....	10,000
Bile salts.....	5,000
Sodium chloride.....	5,000
Neutral red.....	0,075

Final pH 7,4 ±0,2 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 40 g of powder in 1 L of distilled water. Bring to the boil and distribute into suitable containers fitted with Durham tubes. Sterilize in the autoclave at 121°C for 15 minutes.

Description

MacConkey media is a well known popular enrichment media for coliform bacteria.

At the beginning of the last century, MacConkey made the original formulation and included ox bile as an inhibitor of Gram positive bacteria and litmus as the indicator of acid production from lactose sugar. More recently litmus has been substituted by a neutral red indicator making interpretations easier and more precise.

Advancements in knowledge of bacterial physiology has allowed adaptation of this medium to facilitate coliform detection. The most significant modification to the original formulation has been the substitution of ox bile by purified bile salts that improve the selectivity and avoid the inherent turbidity which is due to the fat composition of bile. The efficiency of the inhibition due to bile salts is variable and depends on the relative concentration of cholate and taurocholate.

Technique

MacConkey Broth can be used for the enumeration of coliforms by the MPN technique, selecting positive tubes that show Turbidity, a colour change to red purple and gas production.

Broth is prepared at single (40 g/L) and double strength (80 g/L) and distributed in a series of five tubes fitted with Durham tube. It is recommended that the single strength Broth is distributed in volumes of 10 mL in tubes sized 16 x 160 mm and the double strength 10 mL volumes in tubes on 20 x 200 mm and 50 mL in flask of 100 mL also fitted with Durham tubes.

The inoculation must be done in the following way: Each flask with 50 mL double strength is inoculated with 50 mL of sample. Each tube with 10 mL of double strength Broth is inoculated with 10 mL of sample. Each tube with 10 mL of single strength broth is inoculated with 1 mL of sample.

All the inoculated tubes and flasks are incubated at 30°C ± 1°C for 24-48 hours. The tubes that show Turbidity change to red purple in colour and show gas production (bubble in the Durham tube) are considered positive.

Quality control

Incubation temperature: 30-35°C

Incubation time: 18-48 h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) 10⁴-10⁶ CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018 .

Microorganism

Growth

Remarks

<i>Enterococcus faecalis</i> ATCC® 29212	Total to partial inhibition	48 h
<i>Citrobacter freundii</i> ATCC® 43864	Good	Medium (yellow) Gas (+)
<i>Escherichia coli</i> ATCC® 8739	Good	Medium (yellow) Gas (+). 18 h
<i>Escherichia coli</i> ATCC® 25922	Good	Medium (yellow) Gas (+). 18 h
<i>Salmonella typhimurium</i> ATCC® 14028	Good	Medium (Red) Gas (-)
<i>Pseudomonas aeruginosa</i> ATCC® 27853	Good	Medium (Red) Gas (-)

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- MacCONKEY, A.T. (1905) Lactose-Fermenting Bacteria in Faeces. J. Hyg 5:333.
- WHO (1963) International Standards for Drinking Waters. 7th ed. Churchill Ltd. London.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).