MacConkey Broth

MH083

Intended use

Recommended for the selective enrichment of *E.coli* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP.

Composition**

Ingredients	Gms / Litre
Gelatin peptone#	20.000
Lactose monohydrate	10.000
Dehydrated bile##	5.000
Bromo cresol purple	0.010
pH after sterilization (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of gelatin

Equivalent to Dehydrated Ox-bile

Directions

Suspend 34.51 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/ distilled water. Heat if necessaryto dissolve the medium completely. Dispense into test tubes with inverted Durham tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle.

Principle And Interpretation

MacConkey Broth is a modification of MacConkey Medium (1). Childs and Allen (2) demonstrated the inhibitory effect of neutral red and therefore substituted it by the less inhibitory bromocresol purple dye. BCP is more sensitive in recording pH variation in the medium. This medium is prepared in accordance with the harmonized method of USP/BP/JP (3,4,5)

Gelatin peptone provides essential growth nutrients. Lactose is the fermentable carbohydrate. Dehydrated bile inhibits gram-positive organisms. Bromocresol purple is the pH indicator in the medium, which turns yellow under acidic condition. Lactose fermenting organisms turn the medium yellow due to the acidity produced on lactose fermentation. The colour change of the dye is observed when the pH of the medium falls below 6.8. Lactose non-fermenting organisms like *Salmonella* and *Shigella* do not alter the appearance of the medium.

Transfer homogenate in Soyabean Casein Digest Medium (MH011) containing 1 gm or 1 ml of the preparation to be examined to 100 ml MacConkey Broth Incubation is carried at 43°-45°C for 24-48 hours. For further isolation, subculture on MacConkey Agar (MH081). Growth of red generally non-mucoid colonies, sometimes surrounded by a reddish precipitation zone, indicates pressure of coliforms.

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (3-6).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

3.Though the medium is recommended for selective isolation, further biochemical and serological testing must be carried out for further confirmation.
4.For further isolation, subculture on MacConkey Agar (MH081) is required.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow with green tinge homogeneous free flowing powder

Colour and Clarity of prepared medium
Purple coloured clear to slightly opalescent solution in tubes

pH
7.10-7.50

Cultural Response
Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP. For organisms not specified in pharmacopoeia, cultural response was observed after an incubation at 30-35°C for 18-48 hours.

Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 42-44°C for <= 24 hours).

Inhibitory properties
No growth of the test microorganism occurs for the specified temperature for not less than longest period of time specified inoculating >=100 cfu(at 42-44°C for >= 48 hours).

Cultural Response
Cultural characteristics observed after an incubation at 30-35°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Acid	Gas	Incubation temperature	Incubation period
Growth promoting						
Escherichia coli ATCC 8739 (00012)*	50 -100	luxuriant	positive reaction, yellow colour	positive reaction	42 -44 °C	<=24 hrs
Inhibitory						
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	>=10^3	inhibited			42 -44 °C	>=48 hrs
Additional Microbiological testing						
Escherichia coli ATCC 25922 (00013)*	50 -100	luxuriant	positive reaction, yellow colour	positive reaction	30 -35 °C	18 -24 hrs
Escherichia coli NCTC 9002	50 -100	luxuriant	positive reaction, yellow colour	positive reaction	30 -35 °C	18 -24 hrs
# Klebsiella aerogenes ATCC 13048 (00175)*	50 -100	luxuriant	positive reaction, yellow colour	positive reaction	30 -35 °C	18 -24 hrs
Salmonella Choleraesuis ATCC 12011	50 -100	fair-good	negative reaction	negative reaction	30 -35 °C	18 -24 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	>=10^3	inhibited			30 -35 °C	>=48 hrs

Key :- (#) Formerly known as *Enterobacter aerogenes* (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10- 30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1.MacConkey A. T., 1900, The Lancet, ii: 20.
2.Childs E. and Allen, 1953, J. Hyg: Camb. 51:468-477.
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4.British Pharmacopoeia, 2022, The Stationery office British Pharmacopoeia
5.Japanese Pharmacopoeia, 2016.
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7.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition
8.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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