MacConkey Agar

MH081

Intended Use

Recommended for selective isolation and differentiation of *E.coli* and other enteric bacteria from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP.

Composition**

Ingredients	g / L
Gelatin peptone #	17.000
HMC peptone ##	3.000
Lactose monohydrate	10.000
Sodium chloride	5.000
Bile salts	1.500
Neutral red	0.030
Crystal violet	0.001
Agar	13.500
pH after sterilization (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Pancreatic digest of gelatin ## Equivalent to Peptones (meat and casein)

Directions

Suspend 49.53 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Boil for 1 minute with constant stirring. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Avoid overheating. Cool to 45-50°C. Mix well before pouring into sterile Petri plates. The surface of the medium should be dry when inoculated.

Principle And Interpretation

MacConkey Agar is the earliest selective and differential medium for cultivation of coliform organisms (1,2). Subsequently MacConkey Agar and Broth have been recommended for use in microbiological examination of foodstuffs (3) and for direct plating / inoculation of water samples for coliform counts (4). This medium is also accepted by the Standard Methods for the Examination of Milk and Dairy Products (5). It is recommended in pharmaceutical preparations and is in accordance with the harmonized method of USP/EP/BP/JP (6-9).

Gelatin peptone and HMC peptone provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms. Lactose monohydrate is the fermentable source of carbohydrate. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Sodium chloride maintains the osmotic balance in the medium.

After enrichment of *Escherichia coli* in MacConkey Broth (MH083), it is then subcultured on MacConkey Agar. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless and transparent and typically do not alter appearance of the medium. *Yersinia enterocolitica* may appear as small, non-lactose fermenting colonies after incubation at room temperature.

Type of specimen

Pharmaceutical samples, Food and dairy samples; Water samples.

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (6-9).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,5). For water samples, follow appropriate techniques for sample collection and processing as per guidelines (4).

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 clinical 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. The surface of the medium should be dry when inoculated.

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

Light yellow to pink homogeneous free flowing powder

Gelling

Firm comparable with 1.35% Agar gel.

Colour and Clarity of prepared medium

Red with purplish tinge coloured clear to slightly opalescent gel forms in Petri plates.

pH

6.90-7.30

Cultural Response

Growth Promotion is carried out in accordance with the harmonized method of ICH (USP/EP/BP/JP). Cultural response was observed after an incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Growth promoting properties

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 30-35°C for <=18 hours).

Indicative properties

Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100 cfu (at 30-35°C for 18-72 hours).

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation period
Growth Promoting + Indicative						
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	25 -100	>=50 %	pink-red with bile precipitate	18 -72 hrs
Additional Microbiological testing						
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	25 -100	>=50 %	pink to red with bile precipitate	18 -24 hrs
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	luxuriant	25 -100	>=50 %	pink to red	18 -24 hrs
Enterococcus faecalis ATCC 29212 (00087*)	50 -100	fair-good	0 - 10	<=10 %	colourless to pale pink	18 -24 hrs
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs

Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10³	inhibited	0	0 %		>=24 hrs
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10³	inhibited	0	0 %		>=24 hrs
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Salmonella Paratyphi A ATCC 9150	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Salmonella Paratyphi B ATCC 8759	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Salmonella Typhi ATCC 6539	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Salmonella Abony NCTC 6017 (00029*)	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
## *Proteus hauseri* ATCC 13315	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Shigella flexneri ATCC 12022 (00126*)	50 -100	fair to good	15 -40	30 -40 %	colourless	18 -24 hrs
Staphylococcus epidermidis ATCC 12228 (00036*)	>=10³	inhibited	0	0 %		>=24 hrs
Corynebacterium diphtheriae type *gravis*	>=10³	inhibited	0	0 %		>=24 hrs

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

Storage and Shelf Life

Store between 10- 30°C in a tightly closed container and the prepared medium at 20 - 30°C. For better performance ot is advised to store the plates at 2-8°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 (10,11).

Reference

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4. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
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7. European Pharmacopoeia, 2022 European Dept. for the quality of Medicines.
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10. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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