

## MacConkey Agar Plate (Triple Pack)

MPH081T

### 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	Gms / Litre
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

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

### Directions

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

### 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 pack. 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. It is recommended to store the plates at 24-30°C to avoid minimum condensation.
4. Though the medium is recommended for selective isolation, further biochemical and serological testing must be carried out for further confirmation.
5. 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

Sterile MacConkey Agar in 90 mm disposable plates (Triple pack)

### Colour of medium

Red with purplish tinge coloured medium

### Quantity of medium

27 ml of medium in 90 mm disposable plates.

### pH

6.90-7.30

### Sterility Check

Passes release criteria

### 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
<b>Growth Promoting + Indicative</b>						
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	luxuriant	25 -100	≥50 %	pink-red with bile precipitate	18 -72 hrs
<b>Additional Microbiological testing</b>						
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	luxuriant	25 -100	≥50 %	pink to red with bile precipitate	18 -24 hrs
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50 -100	luxuriant	25 -100	≥50 %	pink to red	18 -24 hrs

<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	50 -100	none - poor	0 - 10	<=10 %	colourless to pale pink	18 -24 hrs
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
<i>Staphylococcus aureus</i> <i>subsp. aureus</i> ATCC 6538 (00032*)	>=10 <sup>3</sup>	inhibited	0	0 %		>=24 hrs
<i>Staphylococcus aureus</i> <i>subsp. aureus</i> ATCC 25923 (00034*)	>=10 <sup>3</sup>	inhibited	0	0 %		>=24 hrs
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
<i>Salmonella</i> Paratyphi A ATCC 9150	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
<i>Salmonella</i> Paratyphi B ATCC 8759	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
<i>Salmonella</i> Typhi ATCC 6539	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
<i>Proteus vulgaris</i> ATCC 13315	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
<i>Shigella flexneri</i> ATCC 12022 (00126*)	50 -100	fair to good	15 -40	30 -40 %	colourless	18 -24 hrs
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	>=10 <sup>3</sup>	inhibited	0	0 %		>=24 hrs
<i>Corynebacterium</i> <i>diphtheriae type gravis</i>	>=10 <sup>3</sup>	inhibited	0	0 %		>=24 hrs

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

## Storage and Shelf Life

On receipt store between 20-30°C. Use before expiry date on the label. 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

1. MacConkey, 1900, The Lancet, ii:20.
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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.
6. The United States Pharmacopoeia-National Formulary (USP-NF), 2022.
7. The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
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10. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition
11. 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.

Revision : 03/2023

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