



Medium 7. MacConkey Agar

MM081

Medium 7. MacConkey Agar is used for selective isolation of *Escherichia coli* enteric bacteria in accordance with Indian Pharmacopoeia, 2010.

Composition**

Ingredients	Gms / Litre
Peptones (meat and casein, equal parts)	3.000
Pancreatic digest of gelatin	17.000
Lactose	10.000
Bile salts	1.500
Sodium chloride	5.000
Crystal violet	0.001
Neutral red	0.030
Agar	13.500
pH after sterilization (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50.03 gms of medium in 1000 ml purified/distilled water. Heat to boiling with gentle swirling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. 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 enteric microorganisms from a variety of clinical specimens (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). These media are also accepted by the Standard Methods for the Examination of Milk and Dairy Products (5) and pharmaceutical preparations as per Indian Pharmacopoeia (6). It is recommended for selective isolation and differentiation of lactose fermenting and lactose non-fermenting enteric bacteria.

Original medium contains protein, bile salts, sodium chloride and two dyes. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Essential nutrients, vitamins and nitrogenous factors are provided by a combination of peptones (meat and casein) and pancreatic digest of gelatin. Lactose is the fermentable source of carbohydrate. Sodium chloride maintains the osmotic balance in the medium. Neutral red is the pH indicator.

After enrichment of *Escherichia coli* in MacConkey Broth (MM083), 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.

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 IP. 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).

Cultural Response

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature	Incubation period
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Growth Promoting + Indicative

<i>Escherichia coli</i> ATCC 8739	50 -100	25 -100	>=50 %	pink-red with bile precipitate	30 -35 °C	18 -72 hrs
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Additional Microbiological testing

<i>Escherichia coli</i> ATCC 25922	50 -100	25 -100	>=50 %	pink to red with bile precipitate	30 -35 °C	18 -24 hrs
<i>Escherichia coli</i> NCTC 9002	50 -100	25 -100	>=50 %	pink to red with bile precipitate	30 -35 °C	18 -24 hrs
<i>Enterobacter aerogenes</i> ATCC 13048	50 -100	25 -100	>=50 %	pink to red	30 -35 °C	18 -24 hrs
<i>Enterococcus faecalis</i> ATCC 29212	50 -100	15 -40	30 -40 %	colourless to pale pink	30 -35 °C	18 -24 hrs
<i>Salmonella</i> Typhimurium ATCC 14028	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
<i>Staphylococcus aureus</i> ATCC 6538	>=10 ³	0	0 %		30 -35 °C	>=24 hrs
<i>Staphylococcus aureus</i> ATCC 25923	>=10 ³	0	0 %		30 -35 °C	>=24 hrs
<i>Salmonella</i> Enteritidis ATCC 13076	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
<i>Salmonella</i> Paratyphi A ATCC 9150	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
<i>Salmonella</i> Paratyphi B ATCC 8759	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
<i>Salmonella</i> Typhi ATCC 6539	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
<i>Salmonella</i> Abony NCTC 6017	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
<i>Proteus vulgaris</i> ATCC 13315	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
<i>Shigella flexneri</i> ATCC 12022	50 -100	15 -40	30 -40 %	colourless	30 -35 °C	18 -24 hrs

<i>Staphylococcus epidermidis</i> >=10 ³ ATCC 12228	0	<=0 %	30 -35 °C	>=24 hrs
<i>Corynebacterium</i> <i>diphtheriae type gravis</i>	0	<=0 %	30 -35 °C	>=24 hrs

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

- 1.MacConkey, 1900, The Lancet, ii:20.
- 2.MacConkey, 1905, J. Hyg., 5:333.
- 3.Speck M. (Ed.), 1985, Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., APHA, Washington, D.C.
- 4.Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 1992, Standard Methods for the Examination of Water and Wastewater, 18th ed., APHA, Washington, D.C.
- 5.Marshall R. (Ed.), 1992, Standard Methods For the Examination of Dairy Products, 16th ed., APHA, Washington, D.C.
- 6.Indian Pharmacopoeia, 2010 Ministry of Health and Family Welfare, Govt. of India

Revision : 2 / 2015

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