MacConkey Agar

MU081

MacConkey Agar is recommended for selective isolation of *Escherichia coli* from pharmaceutical products and is in accordance harmonized methodology of USP.

Composition**

Ingredients	Gms / Litre
Peptones (meat and casein)	3.000
Pancreatic digest of gelatin	17.000
Lactose monohydrate	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 49.53 grams of dehydrated medium in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes i.e. validated cycle. 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

Mac Conkey Agar is the earliest selective and differential medium for cultivation of coliform organisms (1,2). Subsequently Mac Conkey 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). United States pharmacopoeia (6) has recommended this medium for the subculture and identification of *Escherichia coli* . It is in accordance with the harmonized method of EP/BP/JP (7,8,9)

Pancreatic digest of gelatin and peptones (meat and casein) 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 (MU083), 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 USP. 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
Growth Promoting + Indicative						
Escherichia coli ATCC 8739	50 -100	25 -100	>=50 %	pink-red with bile precipitate	30 -35 °C	18 -72 hrs
Additional Microbiological testing						
Escherichia coli ATCC 25922	50 -100	25 -100	>=50 %	pink to red with bile precipitate	30 -35 °C	18 -24 hrs
Escherichia coli NCTC 9002	50 -100	25 -100	>=50 %	pink to red with bile precipitate	30 -35 °C	18 -24 hrs
Enterobacter aerogenes ATCC 13048	50 -100	25 -100	>=50 %	pink to red	30 -35 °C	18 -24 hrs
Enterococcus faecalis ATCC 29212	50 -100	15 -40	30 -40 %	colourless to pale pink	30 -35 °C	18 -24 hrs
Salmonella Typhimurium ATCC 14028	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
Staphylococcus aureus ATCC 6538	>=10^3	0	0 %		30 -35 °C	>=24 hrs
Staphylococcus aureus ATCC 25923	>=10^3	0	0 %		30 -35 °C	>=24 hrs
Salmonella Enteritidis ATCC 13076	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
Salmonella Paratyphi A ATCC 9150	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
Salmonella Paratyphi B ATCC 8759	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
Salmonella Typhi ATCC 6539	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
Salmonella Abony NCTC 6017	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
Proteus vulgaris ATCC 13315	50 -100	25 -100	>=50 %	colourless	30 -35 °C	18 -24 hrs
Shigella flexneri ATCC 12022	50 -100	15 -40	30 -40 %	colourless	30 -35 °C	18 -24 hrs
Staphylococcus epidermidis ATCC 12228	>=10^3	0	0 %		30 -35 °C	>=24 hrs
Corynebacterium diphtheriae type gravis	>=10^3	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.Downes F P and Ito K(Eds.), 2001, Compendium of Methods For The Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C
4.Eaton A. D., Clesceri L. S. and Greenberg A W.,(Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.5. ,,
5.Wehr H M and Frank J H., 2004, Standard Methods for the Examination of Dairy Products, 17th ed., APHA Inc., Washington, D.C.
6.The United States Pharmacopoeia, 2011, The United States Pharmacopeial Convention. Rockville, MD.
7.British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
8.European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
9.Japanese Pharmacopoeia, 2008.

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