



PRODUCT SPECIFICATION SHEET

MacConkey Agar (DM143B)

Intended Use

MacConkey Agar (DM143B) is recommended for selective isolation and differentiation of coliforms from pharmaceutical products using the microbial limit testing in compliance with the harmonized methodology of USP/EP/BP/JP.

Product Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.⁽¹⁾ The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved the growth of *Shigella* and *Salmonella* strains. These modifications included the addition of 0.5% sodium chloride, decreased agar content, and altered bile salts and neutral red concentrations. The formula improvements gave improved differential reactions between these enteric pathogens and the coliform group. 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⁾ This medium is also accepted by the Standard Methods for the Examination of Milk and Dairy Products.⁽⁵⁾ British pharmacopoeia⁽⁶⁾ has recommended this medium for the subculture and identification of *Escherichia coli*. It is also cited as Agar Medium H. It is also recommended by and in accordance with the harmonized method of USP/BP/EP/JP.^(7, 6, 8, 9)

Principles of the Procedure

MacConkey Agar contains pancreatic digest of gelatin and peptones (meat and casein) which provides the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms. Lactose monohydrate is the fermentable source of carbohydrate. Bile salts and crystal violet attributes to the selective action of this medium, which is inhibitory to most species of gram-positive bacteria. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Neutral red is the indicator dye. Lactose fermentation produces acid, which causes absorption of neutral red and a subsequent colour change of the dye giving pink to red colour colonies, when the pH of medium falls. Sodium chloride in the medium helps to maintain osmotic balance of the cells.

Formula / Liter

Ingredients	Gms / Liter
Peptones (meat and casein)	3.00
Pancreatic digest of gelatin	17.00
Lactose monohydrate	10.00
Bile salts	1.50
Sodium chloride	5.00
Crystal violet	0.001
Neutral red	0.030
Agar	13.50
Final pH: 7.1 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 49.53 grams of the medium in one liter of distilled water.





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2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle. AVOID OVERHEATING.
4. Cool to 45-50°C.
5. Mix well before pouring into sterile Petri plates.
6. The surface of the medium should be dry when inoculated.

Quality Control Specifications

Dehydrated Appearance	Light yellow to pink homogeneous free flowing powder
Prepared Medium	Red with purplish tinge coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 4.95% Solution	pH : 7.1 ± 0.2 at 25°C
Gel Strength	Firm comparable with 1.35% Agar gel.

Expected 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 Casein Soybean 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 <=18-72 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).

Sr. No.	Organisms	Results to be achieved						
		Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation Temperature	Incubation period
Growth Promoting + Indicative Properties								
1.	<i>Escherichia coli</i> ATCC 8739	50 - 100	good-luxuriant	25 -100	>=50 %	pink-red with bile precipitate	30-35 °C	18-72 hrs
Additional Microbiological Testing								
2.	<i>Escherichia coli</i> ATCC 25922	50 - 100	good-luxuriant	25 -100	>=50 %	pink-red with bile precipitate	30-35 °C	18-24 hrs
3.	<i>Escherichia coli</i> NCTC 9002	50 - 100	good-luxuriant	25 -100	>=50 %	pink-red with bile precipitate	30-35 °C	18-24 hrs
4.	<i>Enterobacter aerogenes</i> ATCC 13048	50 - 100	good-luxuriant	25 -100	>=50 %	pink to red	30-35 °C	18-24 hrs
5.	<i>Enterococcus faecalis</i> ATCC 29212	50 -100	fair-good	15 -40	30 -40 %	colourless to pale pink	30-35 °C	18-24 hrs





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6.	<i>Salmonella Typhimurium</i> ATCC 14028	50 - 100	good-luxuriant	25 -100	≥50 %	colourless	30-35 °C	18-24 hrs
7.	<i>Staphylococcus aureus</i> ATCC 6538	≥10 ³	inhibited	0	0%	-	30 -35 °C	≥24 hrs
8.	<i>Staphylococcus aureus</i> ATCC 25923	≥10 ³	inhibited	0	0%	-	30 -35 °C	≥24 hrs
9.	<i>Salmonella Enteritidis</i> ATCC 13076	50 - 100	good-luxuriant	25 -100	≥50 %	colourless	30-35 °C	18-24 hrs
10.	<i>Salmonella Paratyphi A</i> ATCC 9150	50 - 100	good-luxuriant	25 -100	≥50 %	colourless	30-35 °C	18-24 hrs
11.	<i>Salmonella Paratyphi B</i> ATCC 8759	50 - 100	good-luxuriant	25 -100	≥50 %	colourless	30-35 °C	18-24 hrs

Sr. No.	Organisms	Results to be achieved						
		Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation Temperature	Incubation period
12.	<i>Salmonella Typhi</i> ATCC 6539	50 - 100	good-luxuriant	25 -100	≥50 %	colourless	30-35 °C	18-24 hrs
13.	<i>Salmonella Abony</i> NCTC6017	50 - 100	good-luxuriant	25 -100	≥50 %	colourless	30-35 °C	18-24 hrs
14.	<i>Proteus vulgaris</i> ATCC 13315	50 - 100	good-luxuriant	25 -100	≥50 %	colourless	30-35 °C	18-24 hrs
15.	<i>Shigella flexneri</i> ATCC 12022	50 - 100	fair-good	15 -40	30 -40 %	colourless	30-35 °C	18-24 hrs
16.	<i>Staphylococcus epidermidis</i> ATCC 12228	≥10 ³	inhibited	0	0%	-	30 -35 °C	≥24 hrs
17.	<i>Corynebacterium diphtheriae type gravis</i>	≥10 ³	inhibited	0	0%	-	30 -35 °C	≥24 hrs

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Refer to appropriate references for standard test procedures.

Results

1. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose.
2. Lactose fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile.
3. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless and transparent and typically do not alter appearance of the medium.
4. *Yersinia enterocolitica* may appear as small, non-lactose fermenting colonies after incubation at room temperature.





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Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Although, MacConkey media are selective primarily for gram-negative enteric bacilli, for complete identification, biochemical and, if indicated, serological testing using pure cultures are recommended. Consult appropriate texts for detailed information and recommended procedures.
2. Incubation of MacConkey Agar plates under increased CO₂ has been reported to reduce the growth and recovery of a number of strains of gram-negative bacilli.

Packaging

Product Name : MacConkey Agar

Product Code : DM143B

Available Pack sizes : 100gm/ 500gm

References

1. MacConkey, 1905, J. Hyg., 5:333.
2. MacConkey, 1900, The Lancet, ii:20.
3. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
4. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.
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. British Pharmacopoeia 2011, The Stationery office British Pharmacopoeia
7. The United States Pharmacopoeia 2011, The United States Pharmacopoeial Convention. Rockville, MD.
8. European Pharmacopoeia 2011, European Dept. for the quality of Medicines
9. Japanese Pharmacopoeia, 2008.

Further Information

For further information please contact your local MICROMASTER Representative.



MICROMASTER LABORATORIES PRIVATE LIMITED

Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall', Thane (W) - 400607. M.S. INDIA.
Ph: +91-22-25895505, 4760, 4681. Cell: 9320126789.

Email: micromaster@micromasterlab.com

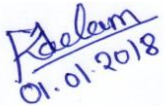
sales@micromasterlab.com

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Prepared By	Checked By	Approved By
		
Microbiologist	Head Quality Control	Head Quality Assurance

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