Technical Information

MacConkey Agar w/ 0.15% Bile salts, CV and NaCl

Product Code: DM 1081

Application: MacConkey Agar w/ 0.15% Bile salts, CV and NaCl is recommended for the selective isolation and differentiation of coliform organisms and other enteric pathogens.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of gelatin	17.000
Casein enzymic hydrolysate	1.500
Peptic digest of animal tissue	1.500
Lactose	10.000
Bile salts	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.001
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

MacConkey agars are both selective and differential plating media used mainly for the detection and isolation of gram-negative organisms from clinical, dairy, food, water, pharmaceutical and industrial sources [1-7&14]. It is also recommended for the identification and recovery of the *Enterobacteriaceae* and related enteric gram-negative bacilli. USP recommends this medium for in the performance of Microbial Limit Tests [6].

These agar media are selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric plating media. The medium DM1081, which fulfill the recommendation of by APHA can be used for the direct plating of water samples for coliform bacilli, of food samples for food poisoning organisms [3] and for the isolation of *Salmonella* and *Shigella* species in cheese [2]. Even this medium is also used for count of coli-aerogenes bacteria in cattle and sheep faeces [8] and non-lactose fermenters in poultry carcasses [9], bacterial counts on irradiated canned minced chicken [10] and the recognition of coliaerogenes bacteria during investigations on the genus *Aeromonas* [11].

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens [13, 12]. The 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. 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 colonies 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, transparent and typically do not alter appearance of the medium.

Peptones are sources of nitrogen and other nutrients. Lactose is a fermentable carbohydrate; bile salts and crystal violet are selective agents that inhibit growth of gram-positive organisms. Neutral red is the pH indicator dye.

Methodology

Suspend 51.53 grams of powder media in 1000 ml distilled water. Shake well & heat to boiling with gentle swirling to dissolve the agar completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid overheating. Cool to 45 - 50°C and pour into sterile Petri plates. The surface of the medium should be dry when inoculated.

Quality Control

Physical Appearance
Light yellow to pink homogeneous free flowing powder

Gelling
Firm comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium
Red with purplish tinge coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 5.15% w/v aqueous solution at 25°C .pH:-7. 1±0.2
pH range 6.90-7.30

Cultural Response/Characteristics
DM 1081: Cultural response was observed after an incubation at 30-35°C for 18-72 hours. The recovery rate is considered as bacteria growth on Soyabean Casein Digest Agar

Organism	Inoculum (CFU)	Growth	Observed value (CFU)	Recovery	Colour of colony	Incubation temperature
Escherichia coli ATCC 8739	50-100	luxuriant	25-100	>=50%	pink-red with bile precipitate	18-72 hrs
Escherichia coli ATCC25922	50-100	luxuriant	25-100	>=50%	Pink to red with bile precipitate	18-72 hrs
Escherichia coli NCTC 9002	50-100	luxuriant	25-100	>=50%	Pink to red with bile precipitate	18-72 hrs
Enterobacter aerogenes ATCC 13048	50-100	Luxuriant	25-100	>=50%	Pink to red	18-72 hrs
Enterococcus faecalis ATCC 29212	50-100	Fair to good	15-40	>=50%	Colourless to pale pink	18-72 hrs
Salmonella Typhimurium ATCC 14028	50-100	Luxuriant	25-100	>=50%	Colourless	18-72 hrs
Staphylococcus aureus ATCC 6538	$>10^3$	Inhibited	0	0%		>=24 hrs
Staphylococcus aureus ATCC 25923	$>10^3$	Inhibited	0	0%		>=24 hrs
Salmonella Enteritidis ATCC 13076	50-100	Luxuriant	25-100	>=50%	Colourless	18-72 hrs
Salmonella Paratyphi A ATCC 9150	50-100	Luxuriant	25-100	>=50%	Colourless	18-72 hrs
Salmonella Paratyphi B ATCC 8759	50-100	Luxuriant	25-100	>=50%	Colourless	18-72 hrs
Salmonella Typhi ATCC 6539	50-100	Luxuriant	25-100	>=50%	Colourless	18-72 hrs
Salmonella A bony NCTC 6017	50-100	Luxuriant	25-100	>=50%	Colourless	18-72 hrs
Proteus vulgaris ATCC 13315	50-100	Luxuriant	25-100	>=50%	Colourless	18-72 hrs
Shigella flexneri ATCC12022	50-100	Fair to good	15-40	>=50%	Colourless	18-72 hrs
Staphylococcus epidermidis ATCC 12228	$>10^3$	Inhibited	0	0%		>=24 hrs

Corynebacterium diphtheriae type gravis	$>10^3$	Inhibited	0	0%	>=24 hrs

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Murray P. R, Baron E, J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

2. 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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4. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.C.

5.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.

6. The United States Pharmacopoeia, 2009, The United States Pharmacopeial Convention, Rockville, M.D.

7.Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C

8. Medrek T. F and Barnes Ella M., 1962, J. Appl. Bacteriol., 25(2),159-168

9. Barnes Ella M. and Shrimpton D. H., 1957, J. Appl. Bacteriol., 20(2), 273-285.

10. Thornley Margaret J., 1957, J. Appl. Bacteriol., 20(2), 273-285.

11. Eddy B. P., 1960, J. Appl. Bacteriol., 23(2).216-249.

12. MacConkey A., 1905, J. Hyg., 5:333.

13. MacConkey A., 1900, The Lancet, ii: 20.

14. British Pharmacopoeia, 2009, The Stationery office British Pharmacopoeia.

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