Technical Information

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

Product Code: PM 1081

Application: -Recommended for the selective isolation and differentiation of coliform organisms and other enteric pathogens from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Gelatin peptone	17.000
Tryptone	1.500
Peptone	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 slightly selective and differential plating media mainly used for the detection and isolation of gramnegative organisms from clinical (10), dairy (14), food (5,11), water (1), pharmaceutical (3,12) and industrial sources (15). It is also recommended for the selection and recovery of the *Enterobacteriaceae* and related enteric gram-negative bacilli. USP recommends this medium for use in the performance of Microbial Limit Tests (12).

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 M081, which corresponds with, that recommended by APHA can be used for the direct plating of water samples for coliform bacilli, for the examination of food samples for food poisoning organisms (11) and for the isolation of Salmonella and *Shigella* species in cheese (14). Other than that this medium is also used for count of coli-aerogenes bacteria in cattle and sheep faeces (9), the count of coli-aerogenes and non-lactose fermenters in poultry carcasses (9), bacterial counts on irradiated canned minced chicken (13) and the recognition of coliaerogenes bacteria during investigations on the genus Aeromonas (4).

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (7,8). 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. Lactosefermenting 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.

Peptone, Tryptone and gelatin peptone are sources of nitrogen, carbon, long chain amino acids and other nutrients. Lactose is a fermentable carbohydrate,Sodium chloride maintains the osmotic equilibrium. Bile salts and crystal violet are selective agents that inhibit growth of gram-positive organisms. Neutral red is the pH indicator dye.

Type of specimen

Clinical - faeces, urine and other pathological material, foodstuffs and dairy samples, water samples, pharmaceutical samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,10).For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,11,14.). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(1) For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines.(3,12) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use only. 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 ta 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 w/ 0.15% Bile salts, CV and NaCl in 90 mm disposable plates.
pH
6.90-7.30
Quantity of medium
25 ml of medium in 90 mm disposable plates.
Colour of medium
Red with purplish tinge coloured medium
Sterility Test
Passes release criteria
Cultural Response
Cultural response was observed after incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Oragnism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Corynebacterium diphtheriae type gravis	>=10^3	inhibited	0%	
Shigella flexneri ATCC 12022 (00126*)	50 -100	fair to good	30 -40%	colourless
Salmonella paratyphi A ATCC 9150	50 -100	luxuriant	>=50 %	colourless
Salmonella abony NCTC 6017 (00029*)	50 -100	luxuriant	>=50 %	colourless
Proteus vulgaris ATCC 13315	50 -100	luxuriant	>=50 %	colourless
Salmonella typhi ATCC 6539	50 -100	luxuriant	>=50 %	colourless
Staphyloccus aureus Subsp.*aureus* ATCC 6538 (00032*)	>=10^3	inhibited	0%	_
Salmonella paratyphi B ATCC 8759	50 -100	luxuriant	>=50 %	colourless
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	>=50 %	pink to red with bile precipitate

Escherichia coli NCTC 9002	50 -100	luxuriant	>=50 %	pink to red with bile precipitate
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	luxuriant	>=50 %	pink to red
Salmonella typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	>=50 %	colourless
Enterococcus faecalis ATCC 29212 (00087*)	50 -100	non-poor	>=50 %	colourless to pale pink
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	>=50 %	colourless
Staphylococcus aureus subsp.*aureus* ATCC 25923 (00034*)	>=10^3	inhibited	0%	_

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (2, 4).

Further Reading

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5. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.C.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition
7. MacConkey A., 1905, J. Hyg., 5:333.
8. MacConkey A., 1900, The Lancet, ii:20.
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10. .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.
11. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
12. The United States Pharmacopoeia, 2018, The United States Pharmacopeial Convention, Rockville, M.D.
13. Thornley Margaret J., 1957, J. Appl. Bacteriol., 20(2), 273-285.
14. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
15. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC , Washington, D.C

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and developmentwork carried at **CDH** is true and accurate
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