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

M081

Intended Use:

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 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

Directions

Suspend 51.53 grams in 1000 ml distilled water. 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.

Principle And Interpretation

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative 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 coli-aerogenes 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. 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.

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, foods stuffs 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 container. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Though the medium is recommended for selective isolation, further biochemical and serological testing must be carried out for further confirmation.
2.The surface of the medium should be dry when inoculated.

Performance and Evaluation

Performace 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
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.

Reaction
Reaction of 4.95% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH
6.90-7.30
Cultural Response
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.

Organism	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
Staphylococcus epidermidis ATCC 12228 (00036)*	>=10^3	inhibited	0%	
Escherichia coli ATCC 8739 (00012)*	50 -100	luxuriant	>=50 %	pink-red with bile precipitate

Staphylococcus 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	none - poor	<=10 %	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%	

Key :- * *Corresponding WDCM numbers*
 # Formerly known as Enterobacter aerogenes

Storage and Shelf Life

Store below 30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,10).

Reference

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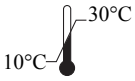
IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
	Do not use if package is damaged
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