



MacConkey Agar Medium

M081I

Recommended for the selective isolation and differentiation of coliform organisms and other enteric pathogens from cosmetic products. The composition and performance criteria are in accordance with ISO 21150:2015

Composition**

ISO 21150:2015- MacConkey Agar Medium

Ingredients	g / L
Pancreatic digest of gelatin	17.000
Pancreatic digest of casein	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	13.500
Final pH (at 25°C)	7.1±0.2

M081I- MacConkey Agar Medium

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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	13.500
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**Formula adjusted, standardized to suit performance parameters
 \$ Equivalent to Pancreatic digest of casein

Equivalent to Pancreatic digest of gelatin
 ## Equivalent to Peptic digest animal tissue

Directions

Suspend 50.03 grams in 1000 ml purified/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. Mix well 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 (1), dairy (2), food (3,4), water (5), pharmaceutical (6,7) and industrial sources (8). 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 (7).

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 (4) and for the isolation of *Salmonella* and *Shigella* species in cheese (2). 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 (10) and the recognition of coli-aerogenes bacteria during investigations on the genus *Aeromonas* (11,12).

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (13,14). MacConkey Agar Medium is also recommended by ISO Committee (15). 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

Cosmetic samples

Specimen Collection and Handling

For cosmetics samples follow appropriate techniques for handling specimens as per established guidelines (15).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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 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.
3. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
4. 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.

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

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 5.00% 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
<i>Corynebacterium diphtheriae</i> type gravis	>=10 ⁴	inhibited	0%	
<i>Shigella flexneri</i> ATCC 12022 (00126*)	50 -100	fair to good	30 -40 %	colourless
<i>Salmonella</i> Paratyphi A ATCC 9150	50 -100	luxuriant	>=50 %	colourless
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	luxuriant	>=50 %	colourless
## <i>Proteus hauseri</i> ATCC 13315	50 -100	luxuriant	>=50 %	colourless
<i>Salmonella</i> Typhi ATCC 6539	50 -100	luxuriant	>=50 %	colourless
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	>=10 ⁴	inhibited	0%	
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	luxuriant	>=50 %	pink-red with bile precipitate

<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	>=10 ⁴	inhibited	0%	
<i>Salmonella</i> Paratyphi B ATCC 8759	50 -100	luxuriant	>=50 %	colourless
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	luxuriant	>=50 %	pink to red with bile precipitate
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50 -100	luxuriant	>=50 %	pink to red
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	>=50 %	colourless
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	50 -100	none - poor	<=10 %	colourless to pale pink
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	>=50 %	colourless
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	>=10 ⁴	inhibited	0%	

Key :- * Corresponding WDCM numbers, # Formerly known as *Enterobacter aerogenes*,
Formerly known as *Proteus vulgaris*.

Storage and Shelf Life

Store between 10-30°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. 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 (1,16).

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