MacConkey Agar, Modified

M051

Intended use

MacConkey Agar, Modified is recommended for isolation of *Klebsiella* species from water samples.

Composition**

Ingredients	Gms / Litre
Peptone	17.000
Proteose peptone	3.000
Bile salts	1.500
Inositol	10.000
Sodium chloride	5.000
Crystal violet	0.001
Neutral red	0.030
Agar	13.500
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50.03 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Cool to 45-50°C and aseptically add two vials of Klebsiella Selective Supplement(FD225). Mix well and pour into sterile Petri plates.

Principle And Interpretation

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (1, 2). 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. *Klebsiella* species are often associated with coliforms in water supply distribution systems and are present as a major component in industrial wastes of paper mill, textile and other industries. Thom (1970) (3) developed a medium based on MacConkey Agar in which lactose is replaced by inositol with the addition of 100µg of carbenicillin per ml. Bagley and Seidler (1978) (4) devised a similar medium with only 50µg of carbenicillin per ml. In the modified MacConkey agar medium (M051), inositol is incorporated in place of lactose while added carbenicillin makes the medium selective for *Klebsiella* species. Further, this method reduces the necessity for biochemical testing of pure strains; however, preliminary verification of differentiated colonies is recommended.

Peptones are sources of nitrogen and other nutrients. Inositol is a fermentable carbohydrate, bile salts and crystal violet are selective agents that inhibit growth of gram-positive organisms.

Type of specimen

Water samples.

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(3) 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 this medium is recommended for the selective isolation of *Klebsiella* species, further biochemical and serological tests should be carried out for confirmation.

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 1.35% Agar gel.

Colour and Clarity of prepared medium

Purplish red coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.0% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

M051: Cultural characteristics, after addition of 2vials of Klebsiella Selective Supplement(FD225), observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
# Klebsiella aerogenes ATCC 13048 (00175*)	>=10³	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	>=10³	inhibited	0%	
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	>=50%	pink
Salmonella Typhi ATCC 6539	>=10³	inhibited	0%	
Serratia marcescens ATCC 8100	>=10³	inhibited	0%	

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

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. 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 (4,5).

Reference

1. Bagley S. T. , Seidler R. J. , Tablbot H. W. and Morrow J. C., 1978, Appl. Environ. Microbiol., 36:178-185

2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

3.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

4. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. MacConkey, 1900, The Lancet, ii:20.
6. MacConkey, 1905, J. Hyg., 5:333.
7. Thom B. T., 1970, Lancet 2:1033

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