M-FC Agar Base, Modified

M1124

Intended Use:

Recommended for detection and enumeration of *Klebsiella* in water samples by using membrane filtration technique.

Composition**

Ingredients	g / L
Tryptose	10.000
Proteose peptone	5.000
Yeast extract	3.000
Sodium chloride	5.000
Inositol	10.000
Bile salts mixture	1.500
Aniline blue	0.100
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 49.6 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE.** Add 10 ml of 1% Rosolic Acid (FD058).Cool to 45-50°C and add 50 mg Carbenicillin. Mix well and pour into sterile Petri plates.

Principle And Interpretation

M-FC Agar Base, Modified is used for the enumeration of *Klebsiella* using membrane filter technique. *Klebsiella* are widely distributed in nature, occurring in soil, water, grains, vegetation etc. Wood pulp, paper mills, textile finishing plants and sugarcane processing operations contain large numbers of *Klebsiella* in their effluents and are often in the predominant coliform in such effluents. M-FC Agar, Modified is formulated as per APHA (1) for enumeration of *Klebsiella*. M-FC Agar is modified by replacing lactose by inositol and adding Carbenicillin.

Proteose peptone, tryptose and yeast extract in the medium provide necessary nutrients for the growth of faecal coliforms. Inositol is the fermentable carbohydrate and the carbon source in the medium. Bile salts mixture inhibits the growth of contaminating gram-positive microorganisms. Aniline blue is a triphenyl methane dye, which suppresses the growth of many gram-positive microorganisms. Also, along with rosolic acid it forms the indicator system in the medium. Carbenicillin inhibits accompanying coliforms and other bacteria and helps in selective isolation of *Klebsiella* species. Sample volume is selected to yield 20 to 60 *Klebsiella* colonies per membrane. This membrane filter is aseptically placed on agar surface. Occasional false positive results may be occurred due to *Enterobacter* species. *Klebsiella* colonies appear deep blue to blue grey due to aniline blue present in the medium. *Klebsiella* colonies will form blue or bluish gray coloured. Presumptive colonies should be further confirmed by performing the biochemical tests.

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 (1). 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. Occasional false positive results may be occurred due to *Enterobacter* species.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

3.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 greyish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

After Addition of 1% Rosolic Acid : Red coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.96% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed with added 1% Rosolic Acid (FD058) and 50 mg/l Carbenicillin after an incubation at 35 - 37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Colour of colony (on membrane filter)
#*Klebsiella aerogenes* ATCC 13048 (00175*)	>=10^4	inhibited	-
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good-luxuriant	deep blue to blue grey

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 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder 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 (2,3).

Reference

1. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.

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