Motility-Indole-Lysine Medium (MIL Medium)

M847

Intended Use:

Recommended for identification of members of *Enterobacteriaceae* on the basis of motility, lysine decarboxylase, lysine deaminase and indole production.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Tryptone	10.000
Yeast extract	3.000
L-Lysine hydrochloride	10.000
Dextrose (Glucose)	1.000
Ferric ammonium citrate	0.500
Bromocresol purple	0.020
Agar	2.000
Final pH (at 25°C)	6.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.52 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense into tubes in 5 ml amounts. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubes to 45-50°C in an upright position.

Principle And Interpretation

MIL Medium is prepared as per the formulation of Reller and Merrett (1). It is a highly useful medium in the identification of *Enterobacteriaceae* as it provides four differential reactions in a single culture tube. It is recommended to be used along with Triple Sugar Iron Agar (TSI) (M021) and Urea Agar (M112) so as to enable presumptive identification of members of *Enterobacteriaceae* from faecal specimens (2,3,4,5).

Peptone, Tryptone and yeast extract supply amino acids and other complex nitrogenous substances. Dextrose is a source of energy. A small amount of agar is added for demonstration of motility along the stab line of inoculation. Growth of motile organisms extends out from the line of inoculation, while non-motile organisms grow only along the stab line. Bromocresol purple serves as the pH indicator.

When inoculated with an organism that ferments dextrose, acids are produced that lower the pH, causing the indicator in the medium to change from purple to yellow. The acidic pH also stimulates decarboxylase enzyme activity. Organisms that possess a specific decarboxylase degrade the amino acid provided in the medium, yielding a corresponding amine. Lysine decarboxylation yields cadaverine. The production of these amines elevates the pH and causes the medium in the bottom portion of the tube to revert to a purple color. The medium in the upper portion of the tube remains acidic because of the higher oxygen tension. If the organism being tested does not produce the required decarboxylase, the medium remains yellow (acidic) throughout or yellow with a purple or red reaction near the top. Lysine deamination produces a colour change in the upper portion of the medium. Oxidative deamination of lysine yields a compound that reacts with ferric ammonium citrate, producing a burgundy red or red-brown color in the top centimeter of the medium (the bottom portion of the medium remains acidic) (3).

This reaction can only be detected if lysine decarboxylase is not produced, which is the case with *Proteus*, *Morganella* and *Providencia* species. Indole is produced in this medium by organisms that possess the enzyme tryptophanase. Tryptophanase degrades typtophan present in the casein peptone, yielding indole. It can be detected in the medium by adding Kovacs reagent to the agar surface. Indole combines with the p-dimethylaminobenzaldehyde of Kovacs reagent and produces a red complex. Cultures are stab-inoculated and incubated at 37°C for 18-24 hours. Motility, lysine deamination and lysine decarboxylation reactions are read before testing indole reaction, since addition of Kovacs reagent causes the colour of the medium to change to yellow. Therefore positive lysine decarboxylase reaction could be misinterpreted as negative.

Type of specimen

Isolated Microorganism from clinical and non-clinical samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use only. For professional 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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:

1. Motility, lysine deamination and lysine decarboxylation reactions are read before testing indole reaction, since addition of Kovacs reagent causes the colour of the medium to change to yellow. Therefore positive lysine decarboxylase reaction could be misinterpreted as negative.

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

Cream to greenish yellow homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.2% Agar gel.

Colour and Clarity of prepared medium

Reddish purple coloured clear to slightly opalescent gel forms in tubes as butts

Reaction

Reaction of 3.65% w/v aqueous solution at 25°C. pH : 6.6±0.2

pH

6.40-6.80

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Motility	Indole production	Lysine Deaminase	Lysine decarboxylase
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	positive, growth away from stabline	negative reaction	negative	positive reaction, purple colour
Escherichia coli ATCC 25922 (00013*)	50-100	positive, growth away from stabline	positive, red ring at the interface of the medium on addition of Kovac's reagent	negative	positive reaction, purple colour
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	negative, growth along the stabline	occasional reaction	negative	positive reaction, purple colour negative reaction
Proteus mirabilis ATCC 25933	50-100	positive, growth away from stabline	negative reaction	positive reaction, red-brown colour reaction at the top	
Shigella flexneri ATCC 12022 (00126*)	50-100	negative, growth along the stabline	occasional reaction	negative	negative reaction
Proteus vulgaris ATCC 13315	50-100	positive, growth away from stabline	positive reaction, red ring at the interface of the medium on addition of Kovac's reagent	positive reaction, red-brown colour reaction at the top	negative reaction
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	positive, growth away from stabline	negative reaction	negative	positive reaction, purple colour

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.
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 (6,7).

Reference

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4.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
5.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.
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7.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.

Revision : 03/2022

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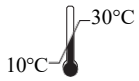
IVD *In vitro* diagnostic medical device



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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www.cepartner4u.eu

CE **CE Marking**

Do not use if package is damaged

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