



## Motility-Indole-Lysine Medium (MIL Medium)

M847

Motility-Indole-Lysine Medium is used as an aid for the identification of members of *Enterobacteriaceae* on the basis of motility, lysine decarboxylase, lysine deaminase and indole production.

### Composition\*\*

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Casein enzymic hydrolysate	10.000
Yeast extract	3.000
L-Lysine hydrochloride	10.000
Dextrose	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 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 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 (2-5).

Peptic digest of animal tissue, casein enzymic hydrolysate 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.

## 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

M847: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Motility	Indole production	Lysine Deaminase	Lysine decarboxylase
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	positive, growth away from stabline	negative reaction	negative	positive reaction, purple colour
<i>Escherichia coli</i> ATCC 25922	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
<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	negative, growth along the stabline	occasional reaction	negative	positive reaction, purple colour
<i>Proteus mirabilis</i> ATCC 25933	50-100	positive, growth away from stabline	negative reaction	positive reaction, red-brown colour reaction at the top	negative reaction
<i>Proteus vulgaris</i> 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
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	positive, growth away from stabline	negative reaction	negative	positive reaction, purple colour
<i>Shigella flexneri</i> ATCC 12022	50-100	negative, growth along the stabline	occasional reaction	negative	negative reaction

## Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

## Reference

1. Reller L. B. and Mirrett S., 1975, J. Clin. Microbiol., 2:247.
2. Ewing W. H., 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc., New York, N.Y.
3. Forbes B. A, Sahm A. S. and Weissfeld D. F., 1998, Bailey & Scotts Diagnostic Microbiology, 10th Ed., Mosby, Inc., St. Louis, Mo.
4. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Tenover F. C., Tenover R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

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