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 (2-5).

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.