TM 225 - MOTILITY-INDOLE-LYSINE MEDIUM (MIL MEDIUM)

INTENDED USE

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

PRODUCT SUMMARY AND EXPLANATION

MIL Medium is prepared as per the formulation of Reller and Merrett. 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) and Urea Agar so as to enable presumptive identification of members of *Enterobacteriaceae* from faecal specimens.

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). 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.

COMPOSITION

Ingredients	Gms / Ltr
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

PRINCIPLE

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.

INSTRUCTION FOR USE

- Dissolve 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 psi pressure (121°C) for 15 minutes.
- Cool the tubes to 45-50°C in an upright position.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to greenish yellow homogeneous free flowing powder.

Appearance of prepared medium : Reddish purple coloured clear to slightly opalescent gel forms in tubes as butts.

pH (at 25°C) : 6.6±0.2

INTERPRETATION

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Motility	Indole production	Lysine Deaminase	Lysine decarboxylase	Incubation Temperature	Incubation Period
Enterobacter aerogenes	13048	50-100	Positive, growth away from stabline	Negative reaction	Negative	Positive reaction, purple colour	35-37°C	18-24 Hours
Escherichia coli	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	35-37°C	18-24 Hours
Klebsiella pneumoniae	13883	50-100	Negative, growth along the stabline	Occasional reaction	Negative	Positive reaction, purple colour	35-37°C	18-24 Hours
Proteus mirabilis	25933	50-100	Positive, growth away from stabline	Negative reaction	Positive reaction, red- brown colour reaction at the top	Negative reaction	35-37°C	18-24 Hours
Proteus vulgaris	13315	50-100	Positive, growth away from stabline	Positive, 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	35-37°C	18-24 Hours
Salmonella Enteritidis	13076	50-100	Positive, growth away from stabline	Negative reaction	Negative	Positive reaction, purple colour	35-37°C	18-24 Hours
Shigella flexneri	12022	50-100	Negative, growth along the stabline	Occasional reaction	Negative	Negative reaction	35-37°C	18-24 Hours

PACKAGING:
In pack size of 100 gm and 500 gm bottles.

STORAGE
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES
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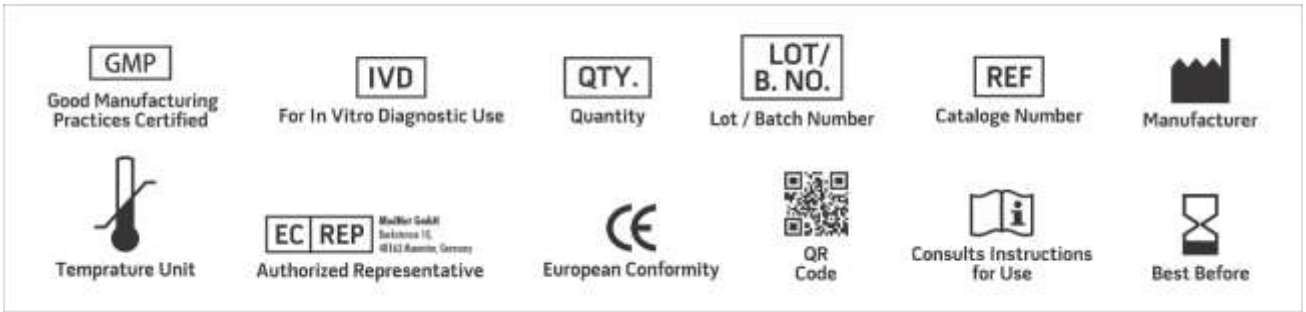

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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