PRODUCT SPECIFICATION SHEET

Motility-Indole-Lysine Medium (MIL Medium) (DM567)

Intended Use

Motility-Indole-Lysine Medium (MIL Medium) (DM567) is recommended for identification of members of *Enterobacteriaceae* on the basis of motility, lysine decarboxylase, lysine deaminase and indole production.

Product Summary and Explanation

Motility-Indole-Lysine Medium (MIL Medium) is designed as per the formulation of Reller and Merrett.[1] It is a single culture medium that provides four differentiating biochemical reactions. It is a highly useful medium in the identification of Enterobacteriaceae. When used in conjunction with Triple Sugar Iron Agar (TSI) and Urea Agar, as many as nine reactions are provided. This combination enables reliable initial identification of *Enterobacteriaceae* from faecal specimens. Extensive testing of 890 enteric cultures by Reller and Mirrett gave essentially the same results with MIL Medium as with the standard motility, indole and lysine decarboxylase (Moeller) test media.[2-5]

Principles of the Procedure

Peptic digest of animal tissue, casein enzymic hydrolysate and yeast extract supply amino acids and other complex nitrogenous substances for growth of wide variety of organisms. Dextrose is an energy source. A small amount of agar is added for demonstrating motility along the stab line of inoculation. Growth of motile organisms extends out from the stab line of inoculation, while non-motile organisms grow only along the stab line. Bromocresol purple serves as the pH indicator. When a dextrose fermenting organism is inoculated, acids are produced that lower the pH, which causes the indicator in the medium to change from the medium color 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, which elevates the pH and causes the medium in the bottom portion of the tube to revert to a purple color. Lysine decarboxylation yields cadaverine. 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. On Lysine deamination a color change is observed in the uper 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, which degrades typtophan present in the casein peptone, yielding indole. It can be detected in the medium by adding Kovac's reagent to the agar surface. Indole combines with the p-dimethylaminobenzaldehyde of Kovacs reagent and produces a red complex.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	10.00
Casein enzymic hydrolysate	10.00
Yeast extract	3.00
L-Lysine hydrochloride	10.00
Dextrose	1.00
Ferric ammonium citrate	0.50
Bromocresol purple	0.02
Agar	2.00
Final pH: 6.6 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

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Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 36.52 grams of the medium in one liter of distilled water.
2. Heat if necessary, to dissolve the medium completely.
3. Dispense into tubes in 5 ml amounts.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Cool the tubes in an upright position.

Quality Control Specifications

Dehydrated Appearance	Cream to greenish yellow homogeneous free flowing powder
Prepared Medium	Reddish purple coloured clear to slightly opalescent gel forms in tubes as butts
Reaction of 3.65% Solution	pH : 6.6 ± 0.2 at 25°C
Gel Strength	Semisolid, comparable with 0.2% Agar gel

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

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Motility	Indole production (on addition of Kovac's reagent)	Lysine Deaminase	Lysine Decarboxylase
1.	*Enterobacter aerogenes ATCC 13048*	50 -100	positive, growth away from stabline	negative reaction	negative	positive reaction, purple colour
2.	*Escherichia coli ATCC 25922*	50 -100	positive, growth away from stabline	positive reaction, red ring at the interface of the medium	negative	positive reaction, purple colour
3.	*Klebsiella pneumonia ATCC 13883*	50-100	negative, growth along the stabline	occasional reaction	negative	positive reaction, purple colour
4.	*Proteus mirabilis ATCC 25933*	50 -100	positive, growth away from stabline	negative reaction	positive reaction, red brown colour reaction at the top	negative reaction
5.	*Proteus vulgaris ATCC 13315*	50 -100	positive, growth away from stabline	positive reaction, red ring at the interface of the medium	positive reaction, red brown colour reaction at the top	negative reaction
6.	*Salmonella enteritidis ATCC 13076*	50-100	positive, growth away from stabline	negative reaction	negative	positive reaction, purple colour
7.	*Shigella flexneri ATCC 12022*	50-100	negative, growth along the stabline	occasional reaction	negative	negative reaction

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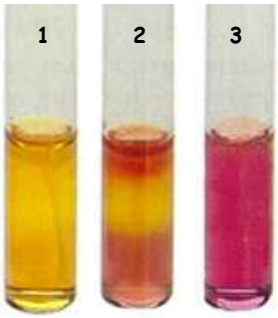
The organisms listed are the minimum that should be used for quality control testing.



Motility-Indole-Lysine Medium (DM567)
1. Control
2. *Escherichia coli ATCC 25922*
3. *Shigella flexneri ATCC 12022*

Test Procedure
1. Using an inoculating needle, stab tubes (containing 5mL medium) with growth from an 18-24 hours pure culture.
2. Incubate the tubes at 35 - 37°C for 18-24 hours.
3. After incubation, examine tubes for evidence of lysine deaminase, motility, lysine decarboxylase reactions and after addition of Kovac's reagent, indole production.

Results
1. Lysine deaminase is indicated by red brown color in the top centimeter of the medium.
2. Motility is indicated by growth extending from the stab line.
3. Lysine decarboxylase is indicated by a purple color throughout the medium. This color may vary in intensity and may be bleached out to a pale light color due to reduction of the indicator.
4. Lysine-negative cultures produce a yellow medium that may be purple or red on the top. Tubes that show a purple reaction with a red color on top should be incubated for a longer period of time.
5. Add 3 or 4 drops of Kovac's Reagent (Cat. No. IR002) to the top of each tube. The appearance of a pink to red color in the reagent is interpreted as a positive indole test.

Storage
Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure
1. Kovacs reagent for indole should not be added until the final lysine deaminase, lysine decarboxylase and motility results have been interpreted.
2. Occasionally, the indole test produces false-negative or falsely weak reactions.
3. Consult appropriate texts for detailed information and recommended procedures.

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Packaging
Product Name : Motility-Indole-Lysine Medium (MIL Medium).
Product Code : DM567
Available Pack sizes : 100gm / 500gm

References

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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., Yolken 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.

Further Information

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