MIL Medium

Intended Use

MIL Medium is used for differentiating *Enterobacteriaceae* based on motility, lysine decarboxylation, lysine deamination and indole production.

Summary and Explanation

MIL (Motility-Indole-Lysine) Medium, prepared according to the formula of Reller and Mirrett,[1] is a single culture medium that provides four differentiating biochemical reactions. 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*.[2,3] Extensive testing of 890 enteric cultures by Reller and Mirrett[1] gave essentially the same results with MIL Medium as with the standard motility, indole and lysine decarboxylase (Moeller) test media.

Principles of the Procedure

Peptones provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Yeast extract provides vitamins and cofactors required for growth. Lysine hydrochloride is present as a substrate to detect lysine decarboxylase or lysine deaminase activity. Dextrose is an energy source. Ferric ammonium citrate is an H_2S indicator. Bromcresol purple is a pH indicator. Agar is the solidifying agent.

Formula

Difco™ MIL Medium

Approximate Formula* Per Liter

Peptone	10.0	g
Pancreatic Digest of Casein	10.0	g
Yeast Extract	3.0	g
L-Lysine HCl	10.0	g
Dextrose	1.0	g
Ferric Ammonium Citrate	0.5	g
Bromcresol Purple	0.02	g
Agar	2.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 36.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ MIL Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.65% solution, soluble in purified water upon boiling. Solution is reddish-purple, clear.
Prepared Appearance:	Reddish purple, clear, semisolid.
Reaction of 3.65% Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

Difco™ MIL Medium

Prepare the medium per label directions. Stab inoculate using fresh cultures and incubate at 35 ± 2°C for 18-24 hours. After reading the lysine decarboxylase, motility and lysine deaminase reactions, add Indole Reagent Kovacs to determine the indole reaction.

ORGANISM	ATCC™	LYSINE DECARBOXYLASE	MOTILITY	LYSINE DEAMINASE	INDOLE PRODUCTION
Escherichia coli	25922	+	+	–	+
Providencia alcalifaciens	9886	–	+	+	–
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	+	+	–	–
Shigella flexneri	12022	–	–	–	–

Uninoculated Tube | *Escherichia coli* ATCC™ 25922 | *Shigella flexneri* ATCC™ 12022

All with Indole Reagent

Procedure

1. Using an inoculating needle, stab tubes (13 × 100 mm screw-capped tubes containing 5 mL) with growth from an 18-24 hour pure culture.
2. Incubate the tubes at 35 ± 2°C for 18-24 hours.
3. After incubation, examine tubes for evidence of lysine deaminase, motility, lysine decarboxylase reactions and, after addition of Indole Reagent Kovacs, indole production.

Expected Results

Lysine deaminase is indicated by a red or red-brown color in the top centimeter of the medium.

Motility is indicated by a clouding of the medium or by growth extending from the inoculation line.

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. 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.

After examining the medium for lysine deaminase, motility and lysine decarboxylase reactions, add 3 or 4 drops of Indole Reagent Kovacs (Cat. No. 261185) to the top of each tube. The appearance of a pink to red color in the reagent is interpreted as a positive indole test.

Positive and negative reactions are based on 90% or more occurrences. When an aberrant reaction occurs, subcultures should be plated on differential media to ensure the purity of the culture.

Limitations of the Procedure

1. Do not add Indole Reagent Kovacs 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.[4]

References

1. Reller and Mirrett. 1975. J. Clin. Microbiol. 2:247.
2. Murray, Baron, Jorgensen, Landry and Pfaller. (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Forbes, Sahm and Weissfeld. 2007. Bailey and Scott's diagnostic microbiology, 12th ed. Mosby Inc., St. Louis, Mo.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ MIL Medium

Cat. No. 218041 Dehydrated – 500 g

Difco™/BBL™ Indole Reagent

Cat. No. 261185 Droppers, 0.5 mL – Ctn. of 50