LYSINE IRON AGAR (LIA)

PRODUCT:

Tube Media:

Lysine Iron Agar, item no. T6860, T6863, T3350, T3340

PURPOSE:

Lysine Iron Agar (LIA) is a differential medium used as an aid in the identification of certain members of the family *Enterobacteriaceae*, in particular, *Salmonella* species, by demonstrating decarboxylation or deamination of lysine and H_2S production.

PRINCIPLE:

Edwards and Fife[1] designed LIA for the purpose of making a preliminary identification of *Salmonella* species. Levine and coworkers added ferric citrate as an indicator for the production of H_2S.[4,5]

Dextrose is incorporated into the medium in a 0.1% concentration. The organism is stabbed into the butt portion of the medium using a long wire or pipette, approximately 3-5 mm from the bottom. As the inoculating instrument is removed, the slant is streaked and the tubes are incubated at 35°C for 18-24 hours with loose caps. Organisms that ferment dextrose produce acid, often accompanied by gas production demonstrated as bubbles or cracks in the medium. Organisms which decarboxylate lysine produce alkaline by-products that revert the medium to the alkaline range, demonstrated by a purple color throughout the medium. Organisms that do not decarboxylate lysine will produce an acid reaction in the butt, resulting in a yellow color. The slant portion may remain alkaline due to oxidative decarboxylation of proteins, proteoses, and amino acids in the medium.

Proteus species deaminase lysine in the presence of oxygen and will produce a red color change on the slant portion.

H_2S-producing organisms react with sodium thiosulfate to produce gas. The gas then reacts with the iron salts, ferrous sulfate and ferric ammonium citrate, to produce an insoluble black precipitate in the medium.

FORMULA:

Approximate, per liter deionized filtered water.

Pancreatic Digest	5.00 g
Yeast Extract	3.00
L-Lysine	10.00
Ferric Ammonium Citrate	0.50
Dextrose	1.00
Agar	13.50
Sodium Thiosulfate	40.00 mg
Bromcresol Purple	20.00

Final pH 6.7 ± 0.2 at 25°C

PRECAUTIONS:*

For in vitro diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Limitations: An H_2S-producing organism may mask the acid production in the butt. H_2S production requires an acid environment; therefore, the butt portion should be considered acid.

LIA is less sensitive in detecting H_2S, in comparison to other iron-containing media such as Sulfide-Indole-Motility (SIM) Medium. Organisms that have weak H_2S production may show only a trace of H_2S activity or will be negative.

It is important to stab the butt of the agar; failure to do so will make the test results invalid. Be careful to maintain the integrity of the agar when stabbing.

Organisms that give consistent biochemical patterns for enteric pathogens must be further tested using other biochemical and/or serological means.

Failure to incubate with caps loose may give erroneous results.

PROCEDURE:*

Specimen Collection: Not applicable since this medium is not for primary isolation. This medium is used in characterizing pure cultures. Isolated organisms, established isolation techniques, and tests for purity are necessary before inoculating this medium. Direct inoculation of specimens will produce erroneous results. Information on specimen collection may be found in standard reference texts.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. Inoculate from a single colony from a pure 18- to 24-hour culture, or from turbid growth from a 4- to 8-hour tryptone broth. Using a straight needle stab the butt to approximately 1/4 inch from the bottom of the tube then streak the slant, being careful not to disturb the integrity of the medium. If a pipette is used, caution must be taken not to introduce air into the butt. Incubate aerobically at 35°C with the caps loose for 18-24 hours, and then observe reactions.

Interpretation: If lysine is decarboxylated, the medium will be alkaline (purple) throughout. *Proteus* species and *Providencia* species show a distinctive red slant over acid butt, indicating the deamination of lysine. An acid butt indicates the fermentation of glucose; lysine decarboxylation did not take place. A blackening of the medium, particularly in the butt, indicates the production of H_2S.

Materials Required but Not Provided: Standard microbiological supplies and equipment such as loops, needles, pipettes, incubator, incinerator, and inoculation system are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC #):	Expected Results:
Proteus mirabilis (12453)	Red slant, H_2S, acid butt
Salmonella cholerasuis ssp. *cholerasuis* (14028)	Alkaline slant, H_2S, alkaline butt
Shigella flexneri (12022)	Alkaline slant, no H_2S, acid butt
	Key: See “Interpretation”

User Quality Control: Check for signs of contamination and deterioration.

BIBLIOGRAPHY:

1. Edwards, P. R., and M. A. Fife, *Appl. Microbiol.*, 9:478, 1961.
2. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology*, 3rd ed., J. B. Lippincott, Philadelphia, 1988.
3. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D. C., 1985.
4. Levine, M., et al., *Am. J. Publ. Health*, 24:505, 1934.
5. Levine, M., et al., *Proc. Soc. Exp. Biol. Med*., 2:1022, 1932.

*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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