LYSINE IRON AGAR (LIA)

INTENDED USE
Remel Lysine Iron Agar (LIA) is a solid medium recommended for use in qualitative procedures for differentiation of enteric gram-negative bacilli based on deamination or decarboxylation of lysine and production of hydrogen sulfide (H_2S).

SUMMARY AND EXPLANATION
In 1961, Edwards and Fife described LIA for detection of lactose-fermenting *Arizona* strains implicated in outbreaks of food-borne disease.[1] The differentiation of *Arizona* from *Salmonella* was necessary because both produce lysine decarboxylase rapidly and form large amounts of H_2S and not all species cause illness in humans. Prior to the introduction of LIA, Triple Sugar Iron (TSI) Agar and Kligler Iron Agar (KIA) were used for detection of H_2S. However, some H_2S-positive enterics were found to produce acid levels in TSI Agar and KIA high enough to suppress H_2S production.[2] In 1966, Johnson et al. expanded the use of LIA to include identification of all *Enterobacteriaceae*.[3] It was determined that lysine-positive enteric gram-negative bacilli will produce detectable levels of H_2S in LIA, even if not in KIA, because the alkaline pH that results from decarboxylation of lysine enhances precipitation of H_2S. In later years, Ewing recommended the use of LIA in conjunction with TSI for the detection of enteric pathogens in routine examination of stools.[4]

PRINCIPLE
Peptone and yeast extract provide nitrogen, amino acids, and vitamins necessary for bacterial growth. Dextrose is a source of fermentable carbohydrate and brom cresol purple is a pH indicator. Ferric ammonium citrate is an indicator of H_2S production. If H_2S is produced from sodium thiosulfate, it reacts with ferric ammonium citrate to form a black precipitate (ferrous sulfate) in the butt of the tube. Lysine is the substrate that serves for detection of the enzymes, lysine decarboxylase and lysine deaminase. When lysine is decarboxylated, as with *Salmonella* spp., the amine converts to cadaverine which results in a purple butt (alkaline). When lysine is deaminated, as with *Proteus* spp., the amine converts to α-ketocarboxylic acid and the slant turns red.

REAGENTS (CLASSICAL FORMULA)*

L-Lysine	10.0 g	Ferric Ammonium Citrate	0.5 g
Gelatin Peptone	5.0 g	Sodium Thiosulfate	0.04 g
Yeast Extract	3.0 g	Brom Cresol Purple	0.02 g
Dextrose	1.0 g	Agar	13.5 g
		Demineralized Water	1000.0 ml

pH 6.7 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. The performance of this medium is dependent on proper inoculation.
2. Inoculate LIA with a single colony from a pure, 18-24 hour culture of the test isolate growing on plated medium. Streak the slant and stab the butt twice. Triple Sugar Iron (TSI) Agar slants should be inoculated in parallel, unless such results have already been obtained.
3. Incubate tubes with caps loosened at 33-37°C for 18-24 hours in an aerobic atmosphere.
4. Examine tubes for lysine deamination or decarboxylation and H_2S production.

INTERPRETATION OF THE TEST
Lysine Decarboxylation (detected in butt):
Positive Test - Purple slant/purple butt (alkaline), the butt reaction may be masked by H_2S production
Negative Test - Purple slant/yellow butt (acid), fermentation of glucose only

Lysine Deamination (detected on slant):
Positive Test - Red slant
Negative Test - Slant remains purple

H_2S Production:
Positive Test - Black precipitate
Negative Test - No black color development

QUALITY CONTROL
All lot numbers of Lysine Iron Agar (LIA) have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Escherichia coli ATCC® 25922	Aerobic, 18-24 h @ 33-37°C	Purple slant, purple butt, H_2S (-)
Proteus mirabilis ATCC® 12453	Aerobic, 18-24 h @ 33-37°C	Red slant, yellow butt, H_2S (-)
Salmonella enterica serovar Typhimurium ATCC® 14028	Aerobic, 18-24 h @ 33-37°C	Purple slant, purple butt, H_2S (+)
Shigella sonnei ATCC® 9290	Aerobic, 18-24 h @ 33-37°C	Purple slant, yellow butt, H_2S (-)

(Continued on back)

LIMITATIONS

1. H_2S production may not be seen with organisms that do not produce lysine decarboxylase, such as *Proteus* spp., since acid in the butt may suppress H_2S formation.[5]
2. LIA is not a substitute for TSI or Moeller Decarboxylase media.[5]
3. Slant reaction with *Morganella morganii* may be variable after 24 hours incubation and may require longer incubation.[5]
4. Gas production may be irregular or suppressed with organisms other than *Citrobacter* spp.[5]
5. *Salmonella enterica* serovar Paratyphi A does not produce lysine decarboxylase.[5]
6. Before inoculation, a slight precipitate may be present on the slant. This will not affect the performance of the medium.[6]

BIBLIOGRAPHY

1. Edwards, P.R. and M.A. Fife. 1961. Appl. Microbiol. 9:478.
2. Bulmash, J.M. and F. MacDonald. 1964. J. Bacteriol. 88:1813.
3. Johnson, J.G., L.J. Kunz, W. Barron, and W.H. Ewing. 1966. Appl. Microbiol. 14:212-217.
4. Ewing, W.H. 1986. Identification of *Enterobacteriaceae*. 4th ed. Elsevier, New York, NY.
5. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
6. Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken. 1995. Manual of Clinical Microbiology. 6th ed. ASM, Washington, D.C.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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