QUALITY CONTROL PROCEDURES

I INTRODUCTION

Lysine Iron Agar is a differential medium for use in the identification of enteric bacilli.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Inoculate samples with an inoculating needle by stabbing the butt and streaking the slant. Use 10^{-1} dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures of the organisms listed below.
   b. Incubate with loosened caps at 35 ± 2 °C in an aerobic atmosphere.
2. Examine tubes after 18–24 h for growth and reactions.
3. Expected Results

Organisms	ATCC™	Slant	Butt	H_2S
**Salmonella enterica* subsp. *arizonae*	13314	Alkaline (purple)	Alkaline (purple)	+
**Citrobacter freundii*	8454	Alkaline (purple)	Acid (yellow)	+ or –
**Proteus vulgaris*	9484	Red	Acid (yellow)	–

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under “Product Deterioration.”
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Lysine Iron Agar is used for the differentiation of enteric organisms based on their ability to decarboxylate or deaminate lysine and to form hydrogen sulfide.

V SUMMARY AND EXPLANATION

Edwards and Fife devised Lysine Iron Agar for the detection of *Arizona* (now *Salmonella enterica* subsp. *arizonae*) cultures, especially those that ferment lactose rapidly.[1] This development followed closely the promulgation by Ewing and Edwards of a taxonomic scheme for the *Enterobacteriaceae* in which the principal division and groups within this family were defined and differentiation procedures described.[2] The various criteria for identification of cultures were summarized by Edwards and Ewing in their treatise on the *Enterobacteriaceae*.[3] However, the taxonomy of the *Enterobacteriaceae* has changed dramatically in recent years.[4-6]

Johnson et al. utilized Lysine Iron Agar and Kligler Iron Agar for primary differentiation of various groups of bacteria within the family *Enterobacteriaceae* and a combination of Lysine Iron Agar with Triple Sugar Iron Agar for identification of *Salmonella*, *Shigella* and *Arizona* group organisms from feces.[7]

Lysine Iron Agar aids in the differentiation of enteric bacilli on the basis of their ability to decarboxylate lysine, to deaminate lysine and to produce hydrogen sulfide. It is designed for use with other media (e.g., Triple Sugar Iron Agar) in appropriate identification schemes.

VI PRINCIPLES OF THE PROCEDURE

Dextrose serves as a source of fermentable carbohydrate. The pH indicator, bromcresol purple, is changed to a yellow color at or below pH 5.2 and is purple at or above pH 6.8.[8] Ferric ammonium citrate and sodium thiosulfate are indicators of hydrogen sulfide formation. Lysine is the substrate for use in detecting the enzymes, lysine decarboxylase and lysine deaminase.

Cultures of enteric bacilli that produce hydrogen sulfide cause blackening of the medium due to the production of ferrous sulfides. Those that produce lysine decarboxylase produce an alkaline reaction (purple color) or neutral reaction in the butt of the medium. Organisms that deaminate the lysine cause the development of a red slant over an acid butt. Gas may be formed but its formation is often irregular or suppressed.

VII REAGENTS

Lysine Iron Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	5.0 g	Ferric Ammonium Citrate	0.5 g
Yeast Extract	3.0 g	Sodium Thiosulfate	0.04 g
Dextrose	1.0 g	Bromcresol Purple	0.02 g
L-Lysine	10.0 g	Agar	13.5 g

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. Prior to discarding, sterilize prepared tubes, specimen containers and other contaminated materials by autoclaving.

Storage Instructions: On receipt, store tubes in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[9,10] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Lysine Iron Agar Slants

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Using an inoculating needle, stab the butt twice then streak the slant with growth from a pure culture. Incubate tubes with loosened caps for 18–48 h at 35 ± 2 °C in an aerobic atmosphere.

Triple Sugar Iron Agar slants should be inoculated in parallel unless results from this medium have already been obtained to distinguish coliforms from *Shigella*, for example.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Lysine decarboxylation is detected in the butt by an alkaline (purple) reaction. Lysine deamination is detected by a red slant. Hydrogen sulfide production is detected by the formation of a black precipitate. A negative reaction (purple slant and yellow butt) indicates fermentation of dextrose only.[8]

Hydrogen sulfide may not be detected in this medium by organisms which are negative for lysine decarboxylase activity since acid production in the butt may suppress its formation.[8] For this reason H_2S-producing *Proteus* species do not blacken this medium.[8]

Typical reactions by members of the *Enterobacteriaceae*:

Organism	Slant	Butt	H_2S
Arizona group	Alkaline	Alkaline or Neutral	+
Salmonella enterica subsp. *enterica* serotype Paratyphi A	Alkaline	Acid	–
Other *Salmonella*	Alkaline	Alkaline	+
Shigella	Alkaline	Acid	–
Citrobacter	Alkaline	Acid	+ or –
Klebsiella	Alkaline or Neutral	Alkaline or Neutral	–
Escherichia	Alkaline	Alkaline or Neutral*	–
Proteus	Red	Acid	–
Providencia	Red	Acid	–

Key: Alkaline = purple color • Acid = yellow color • Neutral = bluish-gray color

*An alkaline or neutral reaction indicates decarboxylation.

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[5,9,10]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Lysine Iron Agar slants are tested for performance characteristics. Representative samples of the lot are tested with **Trypticase** Soy Agar cultures or **Trypticase** Soy Broth cultures diluted 10-1 of *Salmonella arizonae* (ATCC 13314), *Proteus vulgaris* (ATCC 9484), and *Citrobacter freundii* (ATCC 8454), by stabbing the butt and streaking the slant with an inoculating needle. The tubes are incubated with loosened caps at 35 ± 2 °C and read after 18–24 h for growth and reactions. Growth of all organisms is moderate to heavy. *S. arizonae* decarboxylates lysine indicated by an alkaline reaction (purple color) in both the slant and the butt, and is positive for hydrogen sulfide production indicated by blackening of the medium. *P. vulgaris* deaminates lysine indicated by a red color reaction in the slant while the butt is acid (yellow color), and is negative for hydrogen sulfide production. *C. freundii* is negative for both lysine deamination and lysine decarboxylation resulting in an alkaline reaction in the slant and an acid reaction in the butt (yellow color), which indicates fermentation of dextrose and may or may not produce hydrogen sulfide.

XIII AVAILABILITY

Cat. No.	Description
220952	**BBL™** Lysine Iron Agar Slants, Pkg. of 10 size K tubes
220953	**BBL™** Lysine Iron Agar Slants, Ctn. of 100 size K tubes
297700	**BBL™** Lysine Iron Agar Slants, Ctn. of 100 size D tubes

XIV REFERENCES

1. Edwards, P.R., and M.A. Fife. 1961. Lysine-Iron Agar in the detection of *Arizona* cultures. Appl. Microbiol. *9*:478-480.
2. Ewing, W.H., and P.R. Edwards. 1960. The principal divisions and groups of *Enterobacteriaceae* and their differentiation. Int. Bull. Bacteriol. Nomencl. Taxon. *10*:1-12.
3. Edwards, P.R., and W.H. Ewing. 1962. Identification of *Enterobacteriaceae*, Burgess Publishing Co., Minneapolis.
4. Ewing, W.H. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York.
5. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
6. Farmer, J.J., III. 1999. *Enterobacteriaceae*: introduction and identification, p. 442-458. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. Johnson, J.G., L.J. Kunz, W. Barron, and W.H. Ewing. 1966. Biochemical differentiation of the *Enterobacteriaceae* with the aid of Lysine-Iron-Agar. Appl. Microbiol. *14*:212-217.
8. MacFaddin, J.F. 1985. Media for isolation-cultivation- identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
9. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Yolken (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
10. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.

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