Lysine Iron Agar

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.

Summary and Explanation

Edwards and Fife devised Lysine Iron Agar for the detection of *Salmonella enterica* subsp. *arizonae* (previously *Arizona 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 principle 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.

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.

Formulae

Difco™ Lysine Iron Agar

Approximate Formula* Per Liter		
Peptone	5.0	g
Yeast Extract	3.0	g
Dextrose	1.0	g
L-Lysine HCl	10.0	g
Ferric Ammonium Citrate	0.5	g
Sodium Thiosulfate	0.04	g
Bromcresol Purple	0.02	g
Agar	15.0	g

BBL™ Lysine Iron Agar

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

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

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Lysine Iron Agar

Dehydrated Appearance:	Beige to greenish beige, free flowing, homogeneous.
Solution:	3.45% solution, soluble in purified water upon boiling. Solution is reddish purple, slightly opalescent.
Prepared Appearance:	Purple, slightly opalescent.
Reaction of 3.45% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Lysine Iron Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	RECOVERY	REACTION SLANT/BUTT	H_2S
Proteus mirabilis	25933	Good	Red/acid	–
Salmonella enterica subsp. *arizonae*	13314	Good	Alkaline/alkaline	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	Good	Alkaline/alkaline	+
Shigella flexneri	12022	Good	Alkaline/acid	–

Alkaline = red purple, no change in color
Acid = yellow
Red = lysine deaminase
+ H_2S = black precipitate
– H_2S = no black precipitate

Identity Specifications

BBL™ Lysine Iron Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.3% solution, soluble in purified water upon boiling. Solution is medium to dark, rose purple, clear to slightly hazy.
Prepared Appearance:	Medium to dark, rose purple, clear to slightly hazy.
Reaction of 3.3% Solution at 25°C:	pH 6.7 ± 0.2

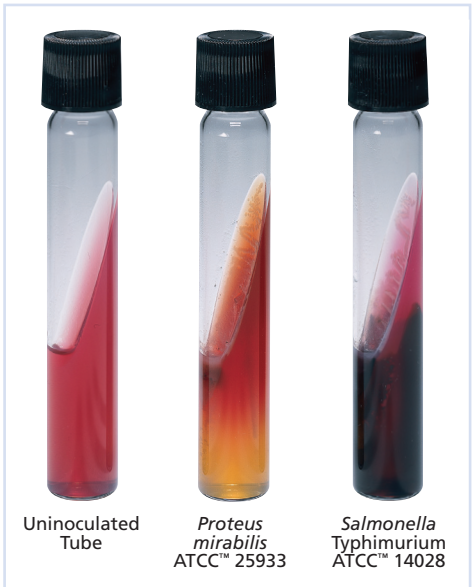
Cultural Response

BBL™ Lysine Iron Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC™	RECOVERY	REACTION SLANT/BUTT	H_2S
Citrobacter freundii	8454	Good	K/A, w/ or w/o gas	+
Escherichia coli	25922	Good	K/Weak K to K, w/ or w/o gas	–
Proteus vulgaris	9484	Good	R/A, w/ or w/o gas	–
Providencia rustigianii	13159	Good	R/A, w/ or w/o gas	–
Salmonella enterica subsp. *arizonae*	13314	Good	K /K, w/ or w/o gas	+
Salmonella enterica subsp. *enterica* serotype Paratyphi A	9150	Good	K/A, w/ or w/o gas	–
Salmonella enterica subsp. *enterica* serotype Typhi	19430	Good	K/K, w/ or w/o gas	+

A = Acid (yellow)
K = Alkaline (red purple, no change in color)
R = Red (lysine deaminase)
+ H_2S = black precipitate
– H_2S = no black precipitate



Uninoculated Tube | *Proteus mirabilis* ATCC™ 25933 | *Salmonella* Typhimurium ATCC™ 14028

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Lysine Iron Agar – 34.5 g;
   BBL™ Lysine Iron Agar – 33 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 12 minutes.
4. Cool tubes in a slanted position to form slants with deep butts.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

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 hours 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.

Expected 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 that 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]

References

1. Edwards and Fife. 1961. Appl. Microbiol. 9:478.
2. Ewing and Edwards. 1960. Int. Bull. Bacteriol. Nomencl. Taxon. *10*:1.
3. Edwards and Ewing. 1962. Identification of *Enterobacteriaceae*. Burgess Publishing Co., Minneapolis, Minn.
4. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
7. Johnson, Kunz, Barron and Ewing. 1966. Appl. Microbiol. *14*:212.
8. MacFaddin. 1985. Media for isola on-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore Md.

Availability

Difco™ Lysine Iron Agar

AOAC | BAM | CCAM | CMPH2 | COMPF | MCM9 | SMD | SMWW | USDA

Cat. No.	284920	Dehydrated – 500 g

BBL™ Lysine Iron Agar

AOAC | BAM | CCAM | CMPH2 | COMPF | MCM9 | SMD | SMWW | USDA

Cat. No.	211363	Dehydrated – 500 g
	220952	Prepared Slants – Pkg. of 10*
	220953	Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*