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TECHNICAL PRODUCT INFORMATION

LYSINE IRON AGAR SLANT (LIA)

Catalog No.: T1500 15 x 125 mm Tube w/Screw Cap Catalog No.: T1501 Culture Tube w/Slip on Cap Catalog No.: T1502 13 x 100 mm Tube w/Screw Cap

INTENDED USE:

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

HISTORY/SUMMARY:

Lysine Iron Agar is prepared according to the formulation of Edwards and Fife, who developed the medium to detect *Salmonella arizona*. *S. arizona* ferments lactose rapidly, and the authors found expected H₂S production on Triple Sugar Iron agar was suppressed. Detection of *S. arizona* is important as it has been implicated in food borne infections. By eliminating lactose and incorporating lysine, Edwards and Fife devised a medium capable of differentiating enteric bacilli based on their ability to decarboxylate or deaminate lysine and produce abundant hydrogen sulfide. This medium is recommended for detecting rapid lactose fermenting *S. arizonae*.

PRINCIPLES:

Enzymatic Digest of gelatin provides carbon, nitrogen, and amino acids required for good growth of a wide variety of organisms. Yeast Extract provides vitamins and cofactors required for growth, and additional sources of nitrogen and carbon. Dextrose is an energy source. L-Lysine is the substrate used to detect lysine decarboxylase and lysine deaminase enzymes. Ferric Ammonium Citrate is an indicator of hydrogen sulfide production. Sodium thiosulfate is added as a source of inorganic sulfur. Bromcresol purple, a pH indicator, is yellow at or below pH 5.2 and purple at or above pH 6.8. Agar is the solidifying agent.

FORMULA:

| INGREDIENTS PER LITER OF DEMINERALIZED WATER | AMOUNT |] |
|--|--------|---|
| Enzymatic Digest of Gelatin | 5.0 g | |
| Yeast Extract | 3.0 g | |
| Dextrose | 1.0 g | 1 |
| L-Lysine | 10.0 g | |
| Ferric Ammonium Citrate | 0.50 g | 1 |
| Sodium Thiosulfate | 0.04 g | 1 |
| Bromcresol Purple | 0.02 g | |
| Agar | 13.5 g |] |

Final pH: 6.7 <u>+</u> 0.2 @ 25°C

PRECAUTIONS:

Since living organisms used with this material can be infectious to the user, proper handling and disposal methods should be established by the laboratory director. This product is for In Vitro Diagnostic Use.

STORAGE:

Store media at 2 - 8°C and use prior to the expiration date.

SPECIMEN COLLECTION:

Lysine Iron Agar must not be used as a medium for primary isolation of microorganisms from clinical specimens. Prior to its use inoculation of specimens on adequate media is necessary. Standard procedure for inoculation of clinical specimens must be followed.

PROCEDURE:

- 1. Using a straight inoculating needle, select an isolated colony from the culture plate.
- 2. Remove tube cap, stab needle into the butt of the medium.
- 3. Withdraw inoculating needle to the slant and streak over the slant surface.
- 4. Replace cap loosely on the tube.
- 5. Incubate aerobically overnight (18–24 hours) at $35 \pm 2^{\circ}$ C, observe and record reactions.
- 6. Good growth must occur in the butt and slant, or equivocal reactions will result.

NOTE:

On occasion, equivocal reactions may result and may be due to the selection of mixed organisms. Whenever there is a question, the purity of the culture should be confirmed by repeat testing with a pure isolate of the organism.

RESULTS:

- 1. Lysine decarboxylase reaction:
 - a. Positive Purple (alkaline butt), purple slant
 - b. Negative Yellow (acid butt), purple (alkaline slant)
- 2. Lysine deaminase reaction:
 - a. Positive red slant
 - b. Negative purple slant
 - c. Proteus spp. and Providencia spp. produce a red slant over a yellow (acid) butt
- 3. Hydrogen Sulfide reaction:
 - a. Blackened medium at the apex of the slant
- 4. Some of the typical reactions of Enterobacteriaceae on LIA are described as follows:

| Organisms | ATCC# | Slant | Butt | H2S |
|------------------------|-------|-----------------------------|-----------------------|-----|
| Proteus mirabilis | 12453 | Red (oxidative deamination) | A - Yellow (Acid) | |
| Escherichia coli | 25922 | K - Purple (Alkaline) | K - Purple (Alkaline) | |
| Citrobacter freundii | 8090 | K - Purple (Alkaline) | A - Yellow (Acid) | + |
| Salmonella typhimurium | 14028 | K - Purple (Alkaline) | K - Purple (Alkaline) | + |

LIMITATIONS OF THE PROCEDURE:

- 1. Salmonella paratyphi A, unlike other Salmonella spp., does not produce lysine decarboxylase resulting in an alkaline slant and an acid butt.
- 2. H₂S-producing *Proteus* spp. do not blacken the medium. It is suggested that Lysine Iron Agar be used in conjunction with Triple Sugar Iron Agar or other media to confirm differentiation.
- 3. The reaction of *Morganella morganii* may be variable after 23 hours incubation and may require longer incubation.

PERFORMANCE CHARACTERISTICS:

NEL approval for each lot of LIA is based on results obtained on a number of tests, among them bacteriological performance with challenge organisms.

QUALITY CONTROL:

It is recommended the user confirm the performance characteristics of this medium. Careful selection of organisms must be made to assure maximum testing success. Incubation temperatures and other environmental conditions must be controlled to further warrant effective test results.

REFERENCES:

- 1. Edwards and Fife. J Applied Microbiology, 9:478. 1961
- 2. Acumedia Product Information Sheet Lysine Iron Agar (7211) Rev: 05 March 2009
- 3. Difco & BBL Manual, Manual of Microbiological Culture Media, 2nd Edition, 2009, Page 313