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HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

LYSINE IRON AGAR (LIA)

Cat. no. L25	LIA, 16x125mm Tube, 8ml Slant	20 or 100 tubes/box
Cat. no. R22	LIA, 13x100mm Tube, 4.5ml Slant	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics Lysine Iron Agar (LIA) is recommended for use in differentiating certain members of the Enterobacteriaceae, especially *Salmonella* species, by demonstrating hydrogen sulfide production and the decarboxylation or deamination of lysine.

SUMMARY

Edwards and Fife designed LIA in 1961 to presumptively identify *Salmonella* species, including lactose fermenting *Salmonella arizonae*, which has been implicated in food-borne outbreaks of gastroenteritis.[3] This is accomplished by the elimination of lactose from the medium, as some strains of *S. arizonae* actively ferment lactose, which may suppress the production of hydrogen sulfide. As a result these colonies may be overlooked on mediums containing lactose, since the characteristic black or darkened colonies are not seen. By eliminating lactose, these organisms will produce an abundance of hydrogen sulfide in the medium and may be detected. The incorporation of lysine is another important addition, as most species of *Salmonella* produce the lysine decarboxylase enzyme. *Proteus* and *Providencia* species also may be presumptively identified on this medium, as they produce a red slant and an alkaline butt.

The indicator in LIA is bromcresol purple. An alkaline reaction is seen by the presence of a purple color, and an acidic reaction is indicated by the appearance of a yellow color. Sodium thiosulfate is incorporated into this medium as the source of hydrogen sulfide, and ferric ammonium citrate as the indicator, which turns the butt black in the presence of free hydrogen sulfide gas. Dextrose is the carbohydrate source, and is added in a concentration of 0.1 %. Enteric organisms that are capable of fermenting dextrose will produce acid (a yellow butt and a purple slant), and sometimes gas, seen as cracks and bubbles in the medium. Lysine is added to show the decarboxylation reaction, which causes an alkaline situation to occur, seen as a purple butt. The yellow color is seen only if lysine decarboxylation does not occur, as this reaction overcomes any acidic (yellow) conditions. If lysine is deaminated in the presence of oxygen (the reaction seen in the presence of *Proteus* and *Providencia* species), a red color change is seen on the slant.

LIA is contained in a tube, and slanted to form a deep butt and short slant. Inoculation is performed with a straight needle by stabbing to the base of the butt, and streaking the slant when the needle is removed. The cap is replaced loosely to facilitate an aerobic atmosphere.

FORMULA

Ingredients per liter of deionized water:*

L-Lysine Hydrochloride	10.0gm
Peptone	5.0gm

Yeast Extract	3.0gm
Dextrose	1.0gm
Ferric Ammonium Citrate	0.5gm
Sodium Thiosulfate	0.04gm
Bromcresol Purple	0.02gm
Agar	15.0gm

Final pH 6.7 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8 degrees C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: This product is not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organism. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism.

Method of Use:

1. Let the LIA medium warm to room temperature before use.

2. Using an inoculation needle, obtain a pure culture of the organism to be tested. Select well-isolated colonies.

3. Inoculate by stabbing to the bottom of the tube. Streak the surface of the slant while withdrawing the needle.

4. Incubate tubes aerobically with loose caps at 35-37ºC. for 18-48 hours. Examine reaction of medium.

INTERPRETATION OF RESULTS

Lysine decarboxylation is indicated by an alkaline (purple) medium. Deamination of lysine is detected by a red slant. *Proteus* and *Providencia* spp. deaminate lysine which results in a distinctive red slant over an acid (yellow) butt. Dextrose fermentation is indicated by a purple slant and a yellow butt. H_2S production results in a blackening of the medium, especially in the butt. Gas production is demonstrated by the presence of bubbles or cracks in the medium. Consult listed references for the identification of colony morphology and further biochemical tests required for identification.[1,2,4,6]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

It is important to stab the butt of the medium. Failure to stab the butt invalidates this test. The integrity of the agar must be maintained when stabbing. Caps must be loosened during this test or erroneous results will occur.

An organism that produces hydrogen sulfide may mask acid production in the butt of the medium. However, hydrogen sulfide production requires an acid environment, thus the butt portion should be considered acid.

LIA is not as sensitive in detecting hydrogen sulfide in comparison to other iron containing mediums, such as Sulfide Indole Motility (SIM) Medium (Cat. no. Q30). Thus organisms that have weak hydrogen sulfide production may show only trace hydrogen sulfide activity, or none at all. Some species may require up to seven days incubation time to show this reaction.

Certain species or strains may give delayed reactions or completely fail to ferment the carbohydrate in the stated manner. However, in most cases if the organism fails to ferment dextrose within 48 hours, and growth is definitely present, the organism is most likely not in the Enterobacteriaceae family.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Salmonella typhimurium *** ATCC® 14028	C	18-48hr	35°C	Aerobic	Growth; purple slant and butt, blackening with H_2S positive

Proteus mirabilis *** ATCC® 12453	C	18-48hr	35°C	Aerobic	Growth; red slant, yellow butt, H_2S negative
Shigella flexneri *** ATCC® 12022	C	18-48hr	35°C	Aerobic	Growth; purple slant, yellow butt, H_2S negative

* Refer to the document "Inoculation Procedures for Media QC" for more information.

*** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

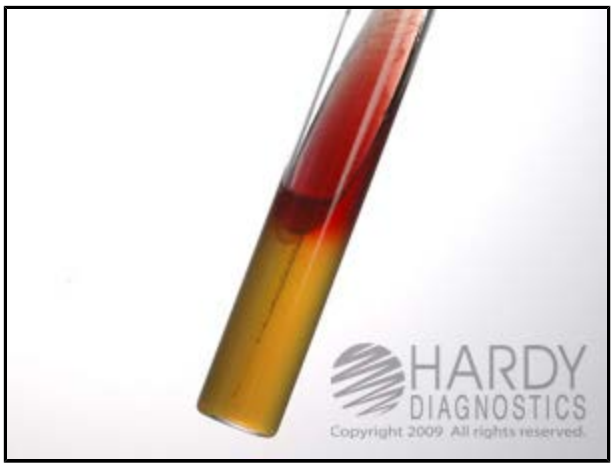
USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

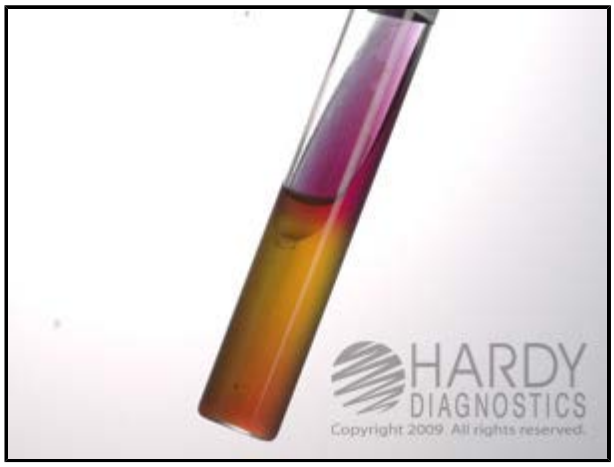
PHYSICAL APPEARANCE

Lysine Iron Agar (LIA) should appear slightly opalescent, and reddish-purple in color.

Salmonella typhimurium (ATCC® 14028) growing on Lysine Iron Agar (Cat. no. L25). Incubated aerobically for 24 hours at 35ºC.



Proteus mirabilis (ATCC® 12453) growing on Lysine Iron Agar (Cat. no. L25). Incubated aerobically for 24 hours at 35ºC.



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Shigella flexneri (ATCC 12022) growing on Lysine Iron Agar (Cat. no. L25). Incubated aerobically for 24 hours at 35ºC.

Uninoculated tube of Lysine Iron Agar (Cat. no. L25).

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

3. Edwards, P.R. and M.A. Fife. 1961. *Appl. Microbiol.*; 9:478.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

6. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]