IFU

Instructions for Use

MODIFIED LYSINE IRON AGAR (MLIA)

Cat. no. G86	Modified Lysine Iron Agar (MLIA), 15x100ml Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Modified Lysine Iron Agar (MLIA) is recommended for the selective and differential isolation of *Salmonella* spp. from food.

This product is not intended to be used for the diagnosis of human disease.

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.[2] However, some *Salmonella* spp. could be overlooked on LIA due to their atypical appearance and the overgrowth of non-salmonella *Enterobacteriaceae* . LIA was modified in 1979 by Rappold and Bolderdijk to provide better detection of hydrogen sulfide-positive and -negative *Salmonella* spp. through the addition of novobicin, bile salts, lactose, and sucrose.[7]

Hardy Diagnostics MLIA is based upon Rappold and Bolderdijk's modification. The indicator in MLIA 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 the source of hydrogen sulfide, and ferric ammonium citrate is used as an indicator, which forms a black precipitate in the presence of free hydrogen sulfide gas generated by colonies of certain species. Dextrose is the primary carbohydrate source for *Salmonella* spp., but lactose and sucrose are added to allow differentiation. Enteric organisms that are capable of fermenting dextrose, lactose, or sucrose will produce acid, resulting in yellow media. Lysine is used to show the decarboxylation reaction, which causes an alkaline reaction and the medium color to remain purple. The yellow color is seen only if lysine decarboxylation does not occur, as this alkaline reaction overcomes any acidic (yellow) conditions. If lysine is de-animated in the presence of oxygen, a red color change is exhibited in the colony.

FORMULA

Ingredients per liter of deionized water:*

Lysine Iron Agar	34.0gm
Bile Salts No. 3	1.5gm
Lactose	10.0gm
Sucrose	10.0gm
Sodium thiosulfate	6.76gm
Ferric Ammonium Citrate	0.3gm

Novobiocin	0.015gm

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 2-8ºC. 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 blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory 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: Consult listed references for information on sample collection.[1-4,8]

Samples should be submitted directly to the laboratory without delay and protected from excessive heat and cold.

1. Process the specimen as appropriate.

2. Inoculate a *Salmonella* enrichment broth (Hanja Tetrathionate Broth, Cat. no. K289, Tetrathionate Broth, Cat. no. K65, or Lactose Broth, Cat. no. K137) and incubate for 18-24 hours at 35ºC.

3. Allow the plates to warm to room temperature, and the agar surface to dry before inoculating.

4. Streak for isolation with a sterile loop or swab.

5. Incubate plates aerobically at 35ºC. for 18-24 hours.

6. Examine colonial morphology, characteristics, and color reactions.

INTERPRETATION OF RESULTS

After 18-24 hours incubation, *Salmonella* colonies appear as purple colonies with a black-center or black periphery. *Escherichia coli* , *Citrobacter* , *Enterobacter* and *Proteus* colonies that are not inhibited will be yellow with no blackening. *Citrobacter freundii* colonies can appear as purple colonies with black-centers and be mistaken for hydrogen sulfide-positive strains of *Salmonella* .

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.

Citrobacter freundii colonies can appear as purple colonies with black-centers and be mistaken for hydrogen sulfide-positive strains of *Salmonella* .

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, swabs, applicator sticks, other culture media such as Tetrathionate Broth (Cat. no. K65) and Lactose Broth (Cat. no. K137), 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 enteritidis ATCC® 13076	A	18-24 hrs	35°C	Aerobic	Growth; Clear colonies with black centers, H_2S positive, medium remains purple
Escherichia coli ATCC® 25922	A	18-24 hrs	35°C	Aerobic	Partial to complete inhibition; If present, yellow colonies without black centers, H_2S negative
Proteus mirabilis ATCC® 12453	A	18-24 hrs	35°C	Aerobic	Partial to complete inhibition

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

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

Modified Lysine Iron Agar should appear slightly opalescent, and purple in color.

REFERENCES

1. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods,* APHA, Washington, D.C.

2. Tille, P., et al. 2007. *Bailey and Scott's Diagnostic Microbiology,* 12th ed. C.V. Mosby Company, St. Louis, MO.

3. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology,* J.B. Lippincott Company, Philadelphia, PA.

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

5. The Official Compendia of Standards. 2008. *USP27-NF22* . United States Pharmacopeial Convention, Rockville, MD.

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

7. Rappold, H., and Bolderdijk, R.F. 1979. Modified lysine iron agar for isolation of *Salmonella* from food. *Appl. Environ. Microbiol.* ; 38(1):162-3.

8. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

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

HDQA 2207D [D]