HIMEDIA

Technical Data

M377

Lysine Iron Agar

Intended Use

Recommended for the differentiation of enteric organisms especially *Salmonella* Arizonae based on their ability to decarboxylate or deaminate lysine and to form hydrogen sulphide (H_2S).The composition of this medium is in accordance with FDA BAM and APHA.

Composition**

Ingredients	**Gms / Litre**
Peptone	5.000
Yeast extract	3.000
Dextrose (Glucose)	1.000
L-Lysine	10.000
Ferric ammonium citrate	0.500
Sodium thiosulphate	0.040
Bromocresol purple	0.020
Agar	15.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 34.56 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense into tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubes in slanted position to form slants with deep butts.

Principle And Interpretation

Lysine Iron Agar was developed by Edwards and Fife (1) to detect lactose fermenting *Salmonellae*. *Salmonella*e are known to decarboxylate lysine rapidly and produce large amounts of hydrogen sulphide (2,3). This medium is a sensitive medium for the detection of lactose fermenting and lactose non-fermenting *Salmonella* species. Many strains of this group ferment lactose very rapidly thus suppressing H_2S production on Triple Sugar Iron Agar (M021). So there is a possibility that the organisms frequently found in food poisoning outbreaks could be overlooked. Thatcher and Clark described the isolation of *Salmonella* species from foods from selective agar and to inoculate it on Lysine Iron Agar and Triple Sugar Iron (M021) together. Using these two media greater discrimination can be made between coliform organisms e.g. *Escherichia coli* and *Shigella* species (4,5). This medium is recommended by FDA BAM (6) and APHA (7) for biochemical testing of *Salmonella* from food samples.

Peptone and yeast extract provide essential nutrients. Dextrose is a source of fermentable carbohydrate. Ferric ammonium citrate and sodium thiosulphate are indicators of H_2Sformation. Cultures that produce hydrogen sulphide cause blackening of the medium due to ferrous sulphide production. Lysine decarboxylation causes an alkaline reaction (purple colour) to give the amine cadaverine and the organisms which do not decarboxylate lysine, produce acid butt (yellow colour). Organisms that deaminate lysine, form alpha - ketocarboxylic acid, which reacts with iron salt near the surface of the medium under the influence of oxygen to form reddish-brown compound. The medium is stabbed to the base of the butt and streaked on slant.

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to greyish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Purple coloured, clear to slightly opalescent gel forms in tubes as slants

Reaction

Reaction of 3.45% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH

6.50-6.90

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	Butt	Slant	H_2S
Citrobacter freundii ATCC 8090	50-100	luxuriant	acidic reaction, yellowing of the medium	alkaline reaction, purple or no colour change	positive reaction, blackening of medium
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	alkaline reaction, purple or no colour change	alkaline reaction, purple or no colour change	negative reaction
Proteus mirabilis ATCC 25933	50-100	luxuriant	acidic reaction, yellowing of the medium	deep red,lysine deamination	positive reaction, blackening of medium
Salmonella Arizonae ATCC 13314	50-100	luxuriant	alkaline reaction, purple or no colour change	alkaline reaction, purple or no colour change	positive reaction, blackening of medium
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	alkaline reaction, purple or no colour change	alkaline reaction, purple or no colour change	positive reaction, blackening of medium
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	alkaline reaction, purple or no colour change	alkaline reaction, purple or no colour change	positive reaction, blackening of medium
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	acidic reaction, yellowing of the medium	alkaline reaction, purple or no colour change	negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Edward P.R. and Fife M.A., 1961, Appl. Microbiol., 9:47
2. Ewing W.H., Davis B.R. and Edward P.R., 1960, Pub. Hlth. Labs., 18:7
3. Moeller V., 1954, Acta Pathol. Microbiol. Scand., 355:25
4. Finegold S.M. and Martin W.J., 1982, Bailey and Scotts Diagnostic Microbiology, 6th ed., The C.V. Mosby Co., St. Louis.
5. Johnson J.G., Kunz L.J., Barron W. and Ewing W.H., 1966, Appl. Microbiol., 14:21
6. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Peter Feng, Stephen D. Weagant, Michael A. Grant, William Burkhard, FDA BAM Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 04/ 2023