Double Modified Lysine Iron Agar Base

M1909

Intended Use:

Recommended for selective and differential cultivation of *Salmonella* species

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Yeast extract	3.000
Dextrose (Glucose)	1.000
L-Lysine	10.000
Ferric ammonium citrate	0.800
Sodium thiosulphate	6.800
Bile salt	1.500
Lactose	10.000
Sucrose	10.000
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 63.12 grams in 1000 ml purifies / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of NO 15 Selective Supplement (FD101). Mix well and dispense into sterile Petri plates.

Principle And Interpretation

Salmonella is the main agent of foodborne diseases in several parts of the world, belonging to the family *Enterobacteriaceae*. Most serovars, however, have a wide spectrum of hosts and typically cause gastroenteritis. Double

Modified Lysine Iron Agar is used to for isolation and identification of *Salmonella* from food (1). *Salmonella* are known to decarboxylate lysine rapidly and produce large amounts of hydrogen sulphide (2, 3). 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 (4) 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* and *Shigella* (5,6). Peptone and yeast extract provides nitrogen, carbon compounds, long chain amino acids, vitamins and other essential nutrients. Dextrose (Glucose) is a source of fermentable carbohydrate. Ferric ammonium citrate and sodium thiosulphate are indicators of H_2S formation. 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 a -ketocarboxylic acid, which reacts with iron salt near the surface of the medium under the influence of oxygen to form reddish-brown compound.

Type of specimen

Food samples.

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (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. The medium is selective for *Salmonella* may not support the growth of other microorganisms.
2. Due to nutritional variations, some strains may show poor growth.
3. Final confirmation of suspected colonies must be carried out by serological and biochemical tests
4. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
5. Further biochemical and serological test are necessary for confirmation.

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 Petri plates.

Reaction

Reaction of 6.31% 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	Colour of colony
Citrobacter freundii ATCC 8090	50-100	luxuriant	yellow
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	yellow
Proteus mirabilis ATCC 25933	50-100	luxuriant	red with black center
Salmonella Arizonae *ATCC 13314*	50-100	luxuriant	purple with black center
Salmonella Enteritidis *ATCC 13076* (00030*)	50-100	luxuriant	purple with black center
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	purple with black center
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	colourless

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Microbiology Laboratory guidebook,MLG/FSIS/USDA (2011),Washington,Food Safety and Inspection Service.
2. Ewing W.H., Davis B.R. and Edward P.R., 1960, Pub. Hlth. Labs., 18:77.
3. Moeller V., 1954, Acta Pathol. Microbiol. Scand., 355:259.
4. Thatcher F.S. and Clark D.S., 1968, University of Toronto Press, p. 100.
5. Finegold S.M. and Martin W.J., 1982, Bailey and Scotts Diagnostic Microbiology, 6th ed., The C.V. Mosby Co., St. Louis.
6. Johnson J.G., Kunz L.J., Barron W. and Ewing W.H., 1966, Appl. Microbiol., 14:212.
7. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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 : 02/2024