



Lysine Iron Cystine Broth Base

M845

Intended Use:

Recommended for rapid presumptive detection of Salmonellae in food, food ingredients and feed materials.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Yeast extract	3.000
L-Lysine hydrochloride	10.000
Mannitol	5.000
Dextrose (Glucose)	1.000
Salicin	1.000
L-Cystine	0.100
Ferric ammonium citrate	0.500
Sodium thiosulphate	0.100
Neutral red	0.025
Final pH (at 25°C)	6.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25.7 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to room temperature and aseptically add one vial of reconstituted Novobiocin Selective Supplement (FD101). Mix well before dispensing in sterile tubes or flasks as desired.

Principle And Interpretation

Lysine Iron Cystine Broth is a modification of the formula of Hoben, Aston and Peterson (1). They described the usefulness of this medium for detecting Salmonellae in food samples in three days, thus reducing the holding time for foods and food ingredients.

Tryptone and L-Cystine provide carbonaceous and nitrogenous compounds. Yeast extract supplies Vitamin B complex. Dextrose, mannitol and salicin are the fermentable carbohydrates. Ferric ammonium citrate and sodium thiosulphate are the indicators of hydrogen sulphide 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. The organisms, which do not decarboxylate lysine, produce acid butt (yellow colour). Organisms that deaminate the lysine form α -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

The medium is stabbed to the base of the butt and streaked on slant. 25 gram of the test sample is added to Lactose Broth (M026) and blended and incubated at $35 \pm 2^\circ\text{C}$ for 24 hours. 1 ml of this enriched culture is added to 10 ml of Tetrathionate Broth (M032) and incubated at $35 \pm 2^\circ\text{C}$ for 24 hours. From this secondary culture, 1 ml is added to 10 ml Lysine Iron Cystine Broth with Novobiocin and incubated at $35 \pm 2^\circ\text{C}$ for 24 hours. To eliminate the possibility of non- H_2S producing Salmonellae, incubate for an additional 16-24 hours. 0.1 ml bromothymol blue solution (0.3%) in 0.1 N NaOH and 50% ethanol is added to each tube. If the colour changes from yellow to dark green or blue, it indicates an alkaline reaction and the presence of *Salmonella* species.

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. Well isolated colonies must be used to avoid erroneous results.

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 pink homogeneous free flowing powder

Colour and Clarity of prepared medium

Red coloured, clear solution which may have slight particles in tubes.

Reaction

Reaction of 2.57% w/v aqueous solution at 25°C. pH : 6.2±0.2

pH

6.00-6.40

Cultural Response

Cultural characteristics observed with added Novobiocin Selective Supplement (FD101), after an incubation at 35-37°C for 24-48 hours. (*- After addition of Bromothymol blue)

Organism	Inoculum (CFU)	Growth	Colour of medium	Colour of medium *	H2S
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	good-luxuriant	red	red-blue	negative reaction
<i>Salmonella</i> Typhi ATCC 19430	50-100	good-luxuriant	yellow	dark green-blue	positive reaction, blackening of medium
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	yellow	dark green-blue	positive, blackening of medium
<i>Shigella flexneri</i> ATCC 12022 (00126*)	50-100	good-luxuriant	red	red-blue	negative reaction, no blackening of medium

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 (2,3).

Reference

1. Hoben, Ashton and Peterson, 1973, Applied Microbiol., 25:123.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.

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