Lysine Iron Cystine Broth Base

M845

Lysine Iron Cystine Broth Base is used for rapid presumptive detection of Salmonellae in foods, food ingredients and feed materials.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	5.000
Yeast extract	3.000
L-Lysine hydrochloride	10.000
Mannitol	5.000
Dextrose	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 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.

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.

Casein enzymic hydrolysate 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 #-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.

25 gram of the test sample is added to Lactose Broth (M026) and blended and incubated at 35 ± 2°C for 24 hours. 1 ml of this enriched culture is added to 10 ml of Tetrathionate Broth (M032) and incubated at 35 ± 2°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 ± 2°C for 24 hours. To eliminate the possibility of non-H2S 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.

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
M845: 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
Escherichia coli ATCC 25922	50-100	good-luxuriant	red	red-blue	negative reaction
Salmonella Typhi ATCC 19430	50-100	good-luxuriant	yellow	dark green-blue	positive reaction, blackening of medium
Salmonella Enteritidis ATCC 13076	50-100	good-luxuriant	yellow	dark green-blue	positive, blackening of medium
Shigella flexneri ATCC 12022	50-100	good-luxuriant	red	red-blue	negative reaction, no blackening of medium

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1. Hoben, Ashton and Peterson, 1973, Applied Microbiol., 25:123.

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