Tryptone Lactose Iron Agar

M321

Tryptone Lactose Iron Agar is used for identification of anaerobes on the basis of motility, hydrogen sulphide production and lactose fermentation.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Lactose	10.000
Ferrous sulphate	0.200
Sodium sulphite	0.400
Sodium thiosulphate	0.080
Phenol red	0.020
Agar	3.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 34.2 grams in 1000 ml distilled water. Heat boiling to dissolve the medium completely. Dispense in test tubes. Sterilize by autoclaving at * 118°C for 15 minutes. Cool the tubes in an upright position. * -corresponds to 12lbs pressure.

Principle And Interpretation

Tryptone Agar was developed by Vera (1) for the accurate differentiation and identification of aerobes and anaerobes by means of motility and fermentation reactions.Tryptone Lactose Iron Agar medium is recommended to study motility of organism and simultaneous sulphite reduction in acidic environment. Due to presence of phenol red in the medium, on fermentation of lactose the medium turns yellow due to production of acid and gas (2). The ability of an organism to produce H2S is a consistent characteristics and an H2S producer usually produce gas (CO2 + H2) in carbohydrate media (2) which is visualized as air bubbles in the medium.

Casein enzymic hydrolysate provides essential growth nutrients to support the growth of organisms. Phenol red is the pH indicator. Even small amount of agar renders it suitable for study of motility. Small amounts of acid produced do not readily get dispersed throughout the medium and hence positive reaction can be more quickly determined in this medium than in liquid medium. Lactose is the fermentable carbohydrate.

H2S production takes place in the presence of R1-SH group provided by cystine present in casein enzymic hydrolysate or through reduction of an inorganic sulphur source such as thiosulphate. H2S is a colourless gas, which upon contact with ferrous salt produces ferrous sulphide, a black precipitate indicated by a visible black reaction (3-6). Sodium sulphite at a concentration less than 0.05% is not inhibitory to *Clostridium sporogenes* (7).

Quality Control

Appearance
Light yellow to light pink homogeneous free flowing powder

Gelling
Semisolid, comparable with 0.35% Agar gel.

Colour and Clarity of prepared medium
Red coloured clear to slightly opalescent gel forms in tubes as butts.

Reaction
Reaction of 3.4% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response

M321: Cultural characteristics observed when incubated anerobically, after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Acid	H2S	Motility
Clostridium perfringens ATCC 13124	50-100	luxuriant	positive reaction, yellow colour	positive, blackening of medium	positive, growth away from stabline causing turbidity
Clostridium sporogenes ATCC 11437	50-100	luxuriant	positive reaction, yellow colour	negative, no blackening of medium	positive, growth away from stabline causing turbidity

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.Vera, 1944, J. Bacteriol., 47:455.
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4.Fieser L. F. ad Fieser M., 1956, Organic Chemistry, 3rd Ed., New York Reinhold Publishing Corporation. pg 155.
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6.Padron A. P. and Dockstader W. B., 1972, Appl. Microbiol., 23:1107.
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