Peptone Iron Agar

M440

Peptone Iron Agar is used for detection of hydrogen sulfide production by microorganisms.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	15.000
Proteose peptone	5.000
Ferric ammonium citrate	0.500
Sodium glycerophosphate	1.000
Sodium thiosulphate	0.080
Agar	15.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.58 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in test tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubed medium to cool in an upright position or in a slanting position to form slants.

Principle And Interpretation

The ability of certain bacterial species to liberate sulfur from sulfur-containing amino acids or other compounds in the form of hydrogen sulphide is an important characteristic for their identification. Hydrogen sulphide production can be detected by incorporating a sulfur source and an H2S indicator system in the medium (1). Peptone Iron Agar which is modification of Levin`s original formula (2, 3) is used to detect H2S production by organisms. This medium utilizes sodium thiosulphate, an inorganic compound as a supplemental source of sulfur and ferric ammonium citrate as the H2S indicator in the medium. Peptone Iron Agar scores over Lead Acetate Agar, a medium to detect H2S, in giving clear and early results (4). This is because ferric ammonium citrate is a better indicator of hydrogen sulphide, as compared to lead acetate.

Peptic digest of animal tissue and proteose peptone provide carbonaceous and nitrogenous compounds, including trace elements. Sodium thiosulphate and ferric ammonium citrate form the H2S detecting system. Sulphide is released from thiosulphate due to the action of bacterial enzymes. This sulphide then couples with a hydrogen ion to form H2S, which then reacts with the ferric ions from ferric ammonium citrate to produce insoluble heavy metal sulphides that appear as a black precipitate (1). Sodium glycerophosphate buffers the medium.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light amber coloured clear to slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 3.66% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH
6.50-6.90

Cultural Response
M440: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	H2S production

Cultural Response

Escherichia coli ATCC 25922	50-100	negative reaction, no blackening of medium
Enterobacter aerogenes ATCC 13048	50-100	negative reaction, no blackening of medium
Salmonella Typhi ATCC 6539	50-100	positive reaction, blackening of medium
Salmonella Enteritidis ATCC 13076	50-100	positive reaction, 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. Koneman E. W, Allen S. D., Janda W. M., Schreckenberger P. C., Winn W.C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed, J. B. Lippincott Company, Philadelphia.
2. Levine M., Vaughn R., Epstein S. S. and Anderson D., 1932, Proc. Soc. Exp. Biol. Med. 29:1022.
3. Levine M., Epstein S. S. and Vaughn R., 1934, Am. J. Public Health 24 :505.
4. Tittsler R. P. and Sandholzer L. A., 1937, Am. J. Public Health 27:1240.

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