Triple Sugar Iron Agar Medium

MU021

Triple Sugar Iron Agar Medium is used for the identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production and is in accordance to United States Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Tryptone	10.000
Lactose	10.000
Sucrose	10.000
Dextrose	1.000
Ferrous ammonium sulphate	0.200
Sodium chloride	5.000
Sodium thiosulphate	0.200
Phenol red	0.025
Agar	13.000
pH after sterilization (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 59.42 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Allow the medium to set in form of a slope with a butt about 1 inch long.

Principle And Interpretation

Triple Sugar Iron Agar Medium was originally proposed by Sulkin and Willett (1) and modified by Hajna (2) for identifying *Enterobacteriaceae* . This medium is in accordance with United States Pharmacopoeia (3) and is recommended in pharmaceutical testing for identification of Gram-negative bacilli.

Tryptone and peptone provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and dextrose are the fermentable carbohydrates. Sodium thiosulphate helps in reactivation of sulphur containing compounds and prevents the desiccation of these compounds during storage.It also forms the substrate for enzyme thiosulphate reductase, which breaks it to form H2S. Sodium thiosulphate and ferric or ferrous ions make H2S indicator system. Sodium thiosulphates are also inactivators of halogens and can minimize its toxicity in the testing sample, if any during microbial limit tests. Phenol red is the pH indicator.

Organisms that ferment dextrose produce a variety of acids, varying the colour of the medium from red to yellow. More amounts of acids are liberated in butt region (fermentation) than in the slant (respiration). Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amounts of acid present in the butt. Thus the appearance of an alkaline (red) slant and an acid (yellow) butt after incubation indicates that the organism is a dextrose fermenter but is unable to ferment lactose and/or sucrose. Bacteria that ferment lactose or sucrose (or both), in addition to dextrose, produce large amounts of acid enables no reversion of pH in that region and thus bacteria exhibit an acid slant and acid butt. Gas production (CO2) is detected by the presence of cracks or bubbles in the medium, when the accumulated gas escapes. Thiosulphate is reduced to hydrogen sulphide by several species of bacteria and H2S combines with ferric ions of ferric salts to produce the insoluble black precipitate of ferrous sulphide. Reduction of thiosulphate proceeds only in an acid environment and blackening usually occurs in the butt of the tube.

Triple Sugar Iron Agar should be used in parallel with Urea Agar / Broth (M112/M111) to distinguish between *Salmonella* and *Proteus* species. The reactions can be summarized as follows:

Alkaline slant / acid butt - only dextrose fermented

Acid slant / acid butt - dextrose and sucrose fermented or dextrose and lactose fermented or all the three sugars, dextrose , lactose and sucrose fermented.

Bubbles or cracks present - gas production

Black precipitate present - H2S gas production

Some members of the *Enterobacteriaceae* and H2S producing Salmonella may not be H2S positive on TSI Agar. Some bacteria may show H2S production on Kligler Iron Agar but not on TSI Agar. This can happen because utilization of sucrose in TSI Agar suppresses the enzymic pathway that result in H2S production.

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.3% Agar gel.

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

pH
7.10-7.50

Growth Promotion Test
Growth promotion is carried as per United States Pharmacopoeia

Cultural Response
Cultural characteristics observed after an incubation at 30-35°C for 24-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Slant	Butt	Gas	H2S
Cultural response						
Salmonella Abony NCTC 6017	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Positive reaction	blackening of medium
Salmonella Typhimurium ATCC 14028	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Positive reaction	blackening of medium
Citrobacter freundii ATCC 8090	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Blackening of medium
Enterobacter aerogenes ATCC 13048	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	No blackening of medium
Klebsiella pneumoniae ATCC 13883	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	No blackening of medium
Proteus vulgaris ATCC 13315	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Blackening of medium
Salmonella Paratyphi A ATCC 9150	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Positive reaction	No blackening of medium
Salmonella Typhi ATCC 6539	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Blackening of medium

Shigella flexneri ATCC 12022	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	No blackening of medium
Escherichia coli ATCC 8739	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction
Klebsiella pneumoniae ATCC 10031	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction

Storage and Shelf Life

Store below 30°C and the prepared medium between 2 - 8°C. Use before expiry date on the label.

Reference

1. Sulkin, E.S. and Willet J.C., 1940, J. Lab. Clin. Med., 25:649.
2. Hajna A.A., 1945, J. Bacteriol 49:516.
3. The United States Pharmacopoeia, 2009 United States Pharmacopeial Convention, Rockville, Md.

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