Agar Medium M (Triple Sugar, Iron Agar)

M021B

Triple Sugar, Iron Agar is used for the identification and differentiation of gram-negative enteric bacilli on basis of glucose, lactose and sucrose fermentation and hydrogen sulphide production in accordance with British Pharmacopoeia, 2009.

Composition**

Ingredients	Gms / Litre
Peptones (Casein and Beef)	20.000
Yeast extract	3.000
Beef extract	3.000
Lactose monohydrate	10.000
Sucrose	10.000
Glucose monohydrate	1.000
Sodium chloride	5.000
Ferric ammonium citrate	0.300
Sodium thiosulphate	0.300
Phenol red	0.025
Agar	12.000
pH after sterilization (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 64.02 grams of dehydrated medium in 1000 ml purified /distilled water. Heat to boiling for 1 minute with shaking to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the medium to set in sloped form with a butt about 1 inch long.

Principle And Interpretation

Triple Sugar Iron Agar cited as Agar Medium M, is recommended for identification and differentiation of *Enterobacteria* by British Pharmacopoeia, (1) It was originally proposed by Sulkin and Willett (2) and modified by Hajna (3) for identifying *Enterobacteriaceae* .

Peptones (casein and beef), yeast extract and beef extract provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose (monohydrate), sucrose and Glucose (monohydrate) in the medium are the fermentable carbohydrates. Sodium thiosulphate and ferric 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 glucose monohydrate produce a variety of acids, turning the colour of the medium from red to yellow. More amounts of acids are liberated in butt (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 glucose fermenter but is unable to ferment lactose and/or sucrose. Bacteria that ferment lactose or sucrose (or both), in addition to glucose, 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 glucose fermented

Acid slant / acid butt - glucose and sucrose fermented or glucose and lactose fermented or all the three sugars, glucose, 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 1.2% Agar gel.

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

Reaction
After sterilization, reaction of 6.40%w/v aqueous solution. pH : 7.4±0.2

pH
7.20-7.60

Growth Promotion Test
As per British Pharmacopoeia

Cultural Response
M021B: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth	Slant	Butt	Gas	H2S
Cultural Response						
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
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
Shigella flexneri ATCC 12022	50-100	Luxuriant	Alkaline reaction, red	Acidic reaction, yellowing of the medium	Negative reaction	No blackening of medium

			colour of the 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 in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. British Pharmacopoeia, 2009, The Stationery office British Pharmacopoeia.
2. Sulkin E.S. and Willett J.C., 1940, J. Lab. Clin. Med., 25:649.
3. Hajna A.A., 1945, J. Bacteriol, 49:516.

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