Technical Information

Triple Sugar Iron Agar

Product Code: DM 1021I

Application: Triple Sugar Iron Agar is recommended for identification of members of *Enterobacteriaceae* especially *Salmonella* species.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	20.000
Meat extract	3.000
Yeast extract	3.000
Lactose	10.000
Sucrose	10.000
Glucose	1.000
Ferric citrate	0.300
Sodium chloride	5.000
Sodium thiosulphate, pentahydrate	0.300
Phenol red	0.024
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Triple Sugar Iron Agar originally proposed by Sulkin and Willett [1] was modified by Hajna [2] for identifying members of *Enterobacteriaceae.* This medium complies with the recommendation of APHA, for the examination of meat and food products [3], including of milk and dairy products [4] microbial limit test for confirming the presence of *Salmonellae* [5, 6] and identification of gram-negative bacilli [6, 7]. ISO Committee [8] has also recommended a slight modification in the original medium for the identification of *Salmonellae.*

Peptic digest of animal tissue, yeast extract and meat extract provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and glucose are the fermentable carbohydrates. Sodium thiosulphate and ferric or ferrous ions make H_2S indicator system.

Phenol red is the pH indicator. Organisms that ferment glucose produce a variety of acids, turning the colour of the medium from red to yellow. More amount 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 (CO_2) 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 H_2S 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 (DM1112/DM1111) 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 H_2S 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 H_2S production

Methodology

Suspend 64.51 grams of dehydrated medium in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes. Allow the medium to set in sloped form with a butt about 1 inch long.

Quality Control

Physical 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
Reaction of 6.45% w/v aqueous solution at 25°C. pH : 7.4±0.2
pH Range
7.2-7.6

Cultural Response/Characteristics
DM1021I: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Slant	Butt	Gas	H2S
Citrobacter freundii ATCC 8090	50-100	luxuriant	acidic reaction, yellowing ofthe medium	acidic reaction, yellowing of the medium	positive reaction	positive,blackening of medium
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	acidic reaction, yellowing ofthe medium	acidic reaction,yellowing of the medium	positive reaction	negative, noblackening of medium
Escherichia coli ATCC 25922	50-100	luxuriant	acidic reaction, yellowing ofthe medium	acidic reaction,yellowing of the medium	positive reaction	negative, noblackening of medium
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	acidic reaction, yellowing ofthe medium	acidic reaction,yellowing of the medium	positive reaction	negative, noblackening of medium
Proteus vulgaris ATCC 13315	50-100	luxuriant	alkalinereaction, redcolour of themedium	acidic reaction,yellowing of the medium	negative reaction	positive,blackening of medium
Salmonella Paratyphi A ATCC 9150	50-100	luxuriant	alkalinereaction, redcolour of themedium	acidic reaction,yellowing of the medium	positive reaction	negative, noblackening of medium
Salmonella Typhi ATCC 6539	50-100	luxuriant	alkaline reaction, redcolour of themedium	acidic reaction, yellowing of the medium	negative reaction	positive,blackening of medium
Salmonella Typhimurium ATCC14028	50-100	luxuriant	alkalinereaction, redcolour of themedium	acidic reaction,yellowing of the medium	positive reaction	positive,blackening of medium
Shigella flexneri ATCC 12022	50-100	luxuriant	alkalinereaction, redcolour of themedium	acidic reaction,yellowing of the medium	negative reaction	negative, noblackening of medium

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Sulkin E.S. and Willett J.C., 1940, J. Lab. Clin. Med., 25:649.
2. Hajna A.A., 1945, J. Bacteriol, 49:5 16.
3. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
4. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
5. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis. 6.Eaton A. D., Clesceri L. S. and Greenberg A. W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
7. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
8. International Organization for Standardization (ISO), 1993, Draft ISO/DIS 6579.

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