Triple Sugar Iron Agar Medium

MU021

Intended Use:

Recommended for identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production in accordance with USP.

Composition**

Ingredients	g / L
Peptone	10.000
Tryptone	10.000
Lactose	10.000
Sucrose	10.000
Dextrose (Glucose)	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 15 lbs pressure (121°C) for 15 minutes. Allow the medium to set in slope form 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 H_2S. Sodium thiosulphate and ferric or ferrous ions make H_2S 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 (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 (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 - H_2S gas production

Some members of the *Enterobacteriaceae* and H_2S producing Salmonella may not be H_2S positive on TSI Agar. Some bacteria may show H_2S 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.

Type of specimen

Pure bacterial isolate

Specimen Collection and Handling:

For pharmaceutical products, follow appropriate techniques for sample processing in case of viscous materials as mentioned under sterility. (3) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Some members of the *Enterobacteriaceae* and H_2S producing *Salmonella* may not be H_2S positive on TSI Agar. Some bacteria may show H_2S 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.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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.

Organism	Inoculum (CFU)	Growth	Slant	Butt	Gas	H2S
Salmonella Abony NCTC 6017 (00029*)	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 (00031*)	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
#*Klebsiella aerogenes* ATCC 13048 (00175*)	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 (00097*)	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	No blackening of medium

## *Proteus hauseri* 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 (00126*)	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 (00012*)	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

Key : (*) Corresponding WDCM numbers.

(#) Formerly known as *Enterobacter aerogenes* ## Formerly known as *Proteus vulgaris*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

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-National Formulatory (USP-NF), 2022.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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