# Triple Sugar Iron Agar Medium

## Intended Use

Triple Sugar Iron Agar was originally proposed by Sulkin and Willett and modified by Hajna for identifying Enterobacteriaceae

Triple Sugar Iron Agar Medium is recommended for identification of gram-negative enteric bacilli based on dextrose, lactose and sucrose fermentation and hydrogen sulphide production in accordance with US Pharmacopoeia.

## Typical Composition (g/litre)

Beef extract 3.0 ; Peptone 20.0 ; Yeast extract 3.000 ; Lactose 10.0 ; Sucrose 10.0 ; Dextrose monohydrate 1.0 ; Ferrous sulphate 0.2 ; Sodium chloride 5.0 ; Sodium thiosulphate 0.3 ; Phenol red 0.024 ; Agar 12.0

## Mode of Action

Peptone, yeast extract and beef extract provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and dextrose monohydrate are the fermentable carbohydrates. Sodium thiosulphate and ferric or ferrous ions make H2S indicator system. Sodium thiosulphate is also an inactivator of halogen and can minimize its toxicity in the testing sample, if any during microbial limit tests. Phenol red is the pH indicator.

## Preparation

Suspend 64.42 grams in 1litre purified/ distilled water. Heat to boiling to dissolve the medium completely. Mix well, distribute into test tubes, and sterilize by maintaining at 10lbs pressure (115°C) for 30 minutes. Allow the medium to solidify to give slant-agar tubes.

pH: 7.4 ± 0.2 at 25 °C

## Storage

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

## Experimental Procedure and Evaluation

Streak the pure culture under investigation on the sloped surface and inoculate the butt of the same tube by a central stab. Incubation: up to 48 hours at 35 °C aerobically.

The reactions can be summarized as follows:

Alkaline slant / acid butt - only glucose 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

## Quality Control

| Organism | Slant | Butt | Gas | $H_2S$ |
|---|---|---|---|---|
| Escherichia coli ATCC 8739 | Acidic | Acidic | + | - |
| Escherichia coli NCTC 9002 | Acidic | Acidic | + | - |
| Citrobacter freundii ATCC 8090 | Acidic | Acidic | + | + |
| Enterobacter aerogenes ATCC 13048 | Acidic | Acidic | + | - |
| Klebsiella pneumoniae ATCC 13883 | Acidic | Acidic | + | - |
| Proteus vulgaris ATCC 13315 | Alkaline | Acidic | - | + |
| Salmonella Paratyphi A ATCC 9150 | Alkaline | Acidic | + | - |
| Salmonella Typhi ATCC 6539 | Alkaline | Acidic | - | + |
| Salmonella Typhimurium ATCC 14028 | Alkaline | Acidic | + | + |
| Klebsiella pneumoniae ATCC 10031 | Acidic | Acidic | + | - |
| Shigella flexneri ATCC 12022 | Alkaline | Acidic | - | - |

## Reference


1. Sulkin E.S. and Willett J.C., 1940, J. Lab. Clin. Med., 25:649.
2. Hajna A.A., 1945, J. Bacteriol, 49:516.
3. The Indian Pharmacopoeia 1996, Govt. of India, 1996. The Controller of Publication, Delhi.
4. 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.
5. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
6. DIN 10160. DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung. Nachweis von Salmonellen. Referenzverfahren. - DIN 10181.
7. International Organization for Standardization: Meat and meat products. - Detection of Salmonella (Reference method). - International Standard ISO 3565 (1975).