



## Triple Sugar Iron Agar

M021S

Triple Sugar Iron Agar is used for the identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production.

### Composition\*\*

Ingredients	Gms / Litre
Peptone	20.000
Yeast extract	3.000
Meat extract	3.000
Lactose	10.000
Sucrose	10.000
Dextrose	1.000
Sodium chloride	5.000
Ferrous sulphate, heptahydrate	0.200
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

### Directions

Suspend 64.32 grams (the equivalent weight of dehydrated media per litre) in 1000 ml 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 sloped form with a butt about 1 inch long.

### Principle And Interpretation

Triple Sugar Iron Agar was originally proposed by Sulkin and Willett (1) and modified by Hajna (2) for identifying *Enterobacteriaceae*. This medium complies with the recommendation of APHA, for the examination of meat and food products (3), for the examination of milk and dairy products (4) and for microbial limit test for confirming the presence of *Salmonellae* (5, 6) and in the identification of gram-negative bacilli (6, 7).

ISO Committee (8) and BIS (11) has recommended a slight modification for the identification of *Salmonellae*. BIS has recommended the medium (M021S) for detection of *Escherichia coli* (9) and *Vibrio* s (10).

Peptone, 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<sub>2</sub>S 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. Thus no reversion of pH in that region is possible and thus bacteria exhibit an acid slant and acid butt. Gas production (CO<sub>2</sub>) 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<sub>2</sub>S 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- H<sub>2</sub>S gas production

Some members of the *Enterobacteriaceae* and some H<sub>2</sub>S producing *Salmonella* may not be H<sub>2</sub>S positive on TSI Agar. Some bacteria may show H<sub>2</sub>S 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<sub>2</sub>S production.

## Quality Control

### Appearance

Light yellow to pink coloured 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 6.43% w/v aqueous solution at 25°C. pH : 7.4±0.2

### Cultural Response

M021S: Cultural characteristics observed after an incubation at 35 - 37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Slant	Butt	Gas	H <sub>2</sub> S
<b>Cultural Response</b> <i>Citrobacter freundii</i> ATCC 8090	50-100	luxuriant	acidic reaction,yellowing of the medium	acidic reaction,yellowing of the medium	positive reaction	positive reaction,blackening of medium
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	acidic reaction,yellowing of the medium	acidic reaction,yellowing of the medium	positive reaction	negative reaction,no blackening of medium
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	acidic reaction,yellowing of the medium	acidic reaction,yellowing of the medium	positive reaction	negative reaction,no blackening of medium
<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	luxuriant	acidic reaction,yellowing of the medium	acidic reaction,yellowing of the medium	positive reaction	negative reaction,no blackening of medium
<i>Proteus vulgaris</i> ATCC 13315	50-100	luxuriant	alkaline reaction,red colour of the medium	acidic reaction,yellowing of the medium	negative reaction	positive reaction,blackening of medium
<i>Salmonella Paratyphi A</i> ATCC 9150	50-100	luxuriant	alkaline reaction,red colour of the medium	acidic reaction,yellowing of the medium	positive reaction	negative reaction,no blackening of medium
<i>Salmonella Typhi</i> ATCC 6539	50-100	luxuriant	alkaline reaction,red colour of the medium	acidic reaction,yellowing of the medium	negative reaction	positive reaction,blackening of medium
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	alkaline reaction,red	acidic reaction,yellowing of the medium	positive reaction	positive reaction,blackening of medium

<i>Shigella flexneri</i> ATCC 12022	50-100	luxuriant	colour of the medium alkaline reaction,red colour of the medium	acidic reaction,yellowing of the medium	negative reaction	negative reaction,no blackening of medium
<i>Vibrio cholerae</i> ATCC 15748	50-100	luxuriant	alkaline reaction,red colour of the medium	acidic reaction,yellowing of the medium	negative reaction	negative reaction,no blackening of medium

## 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. Sulkin E.S. and Willett J.C., 1940, J. Lab. Clin. Med.,
2. Hajna A.A., 1945, J. Bacteriol, 49:516.
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5. Finegold and Baron, 1986, Bailey and Scotts Diagnostic Microbiology, 7th ed., The C.V. Mosby Co., St. Louis.
6. Greenberg A. E., Trussell R. R. and Clesceri L. S. (Eds.), 1985, Standard Methods for the Examination of Water and Wastewater, 16th 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.
9. Bureau of Indian Standards IS : 5887 (Part I) 1976, reaffirmed 1986.
10. Bureau of Indian Standards IS : 5887 (Part V) 1976, reaffirmed 1996.
11. Bureau of Indian Standards IS : 5887 (Part III) 1999.

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