



## Agar Medium M (Triple Sugar, Iron Agar)

ME021

Triple Sugar Iron Agar is used for the identification and differentiation of gram negative enteric pathogens on basis of glucose, lactose and sucrose fermentation and hydrogen sulphide production as recommended by European Pharmacopoeia, 2008.

### Composition\*\*

Ingredients	Gms / Litre
Beef extract	3.000
Peptones (Casein and Beef)	20.000
Yeast extract	3.000
Lactose monohydrate	10.000
Sucrose	10.000
Glucose monohydrate	1.000
Ferric ammonium citrate	0.300
Sodium chloride	5.000
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 64.03 grams (the equivalent weight of dehydrated medium per litre) 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 or as per validated cycle. 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 European Pharmacopoeia, 2008 (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 H<sub>2</sub>S 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 (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 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 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 6.4% w/v aqueous solution. pH : 7.4±0.2

### pH

7.20-7.60

### Growth Promotion Test

As per European Pharmacopoeia

### Cultural Response

Cultural characteristics observed after an incubation at 30 - 35°C for 24-48 hours.

### Cultural Response

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	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	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	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	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	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	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	Blackening of medium

<i>Salmonella Typhimurium</i> ATCC 14028	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, Positive yellowing of the medium	Blackening of medium
<i>Shigella flexneri</i> ATCC 12022	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, Negative yellowing of the medium	No blackening of medium
<i>Escherichia coli</i> ATCC 8739	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, Positive yellowing of the medium	Negative reaction
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, Positive yellowing of the medium	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. European Pharmacopoeia 2008, European Dept. for the quality of Medicines.
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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