



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

MAP021

(M021B/ME021)

### Intended Use:

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

### Composition\*\*

Ingredients	g / L
HMC Peptones #	20.000
Yeast extract	3.000
HM Peptone B ##	3.000
Lactose monohydrate	10.000
Sucrose	10.000
Glucose monohydrate	1.000
Sodium chloride	5.000
Ferric ammonium citrate	0.300
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

Key : #- Equivalent to Peptone (Casein and Beef); ## - Equivalent to Beef extract

### Directions

Suspend 64.02 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Cool to 45-50°C. 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 British Pharmacopoeia and European Pharmacopoeia (1,2). It was originally proposed by Sulkin and Willett (3) and modified by Hajna (4) for identifying *Enterobacteriaceae*.

HMC Peptone, yeast extract and HM peptone B 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.

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Bubbles or cracks present-gas production

Black precipitate present-H<sub>2</sub>S gas production

## Type of specimen

Pharmaceutical samples

## Specimen Collection and Handling:

For pharmaceutical samples, follow appropriate techniques for sample collection and processing as per guidelines (1,2). 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<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.

## 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 1.2% Agar gel.

### Colour and Clarity of prepared medium

Pinkish red coloured clear to slightly opalescent gel forms in tubes as slants.

### pH

7.4±0.2

### Cultural Response

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

Organism (CFU)	Growth	Slant	Butt	Gas	H <sub>2</sub> S
<i>Salmonella</i> Abony NCTC 6017	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	positive, blackening of medium
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	positive, blackening of medium
<i>Citrobacter freundii</i> ATCC 8090	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	positive, blackening of medium
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
<i>Escherichia coli</i> ATCC 25922 (00013*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium

<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
## <i>Proteus hauseri</i> ATCC 13315	luxuriant	alkaline reaction, red colour of the	acidic reaction, yellowing of the medium	negative reaction	positive, blackening of medium
<i>Shigella flexneri</i> ATCC 12022 (00126*)	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	negative, no blackening of medium
<i>Escherichia coli</i> ATCC 8739 (00012*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
<i>Klebsiella pneumoniae</i> ATCC 10031	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium

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 (5,6).

### Reference

1. The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
2. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
3. Sulkin E.S. and Willett J.C., 1940, J. Lab. Clin. Med., 25:649.
4. Hajna A.A., 1945, J. Bacteriol, 49:516.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. 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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### Disclaimer :

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