



Technical Data

Triple Sugar Iron Agar

M021I

Intended Use:

Recommended for identification of members of *Enterobacteriaceae* especially *Salmonella* species. The composition and performance criteria of this medium are as per the specifications laid down in ISO 6579-1:2017/Amd.1:2020(E), ISO 21567:2004, APHA.

Composition**

ISO 6579-1:2017 ISO21567:2004

Ingredients	g / L
Peptone	20.000
Meat extract	3.000
Yeast extract	3.000
Lactose	10.000
Sucrose	10.000
Glucose	1.000
Iron(III) citrate	0.300
Sodium chloride(NaCl)	5.000
Sodium thiosulphate	0.300
Phenol red	0.024
Agar	9-18
pH after sterilization(at 25°C)	7.4±0.2

Triple Sugar Iron Agar

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Ingredients	g / L
Peptone	20.000
HM extract #	3.000
Yeast extract	3.000
Lactose	10.000
Sucrose	10.000
Glucose(Dextrose)	1.000
Iron(III) citrate	0.300
Sodium chloride	5.000
Sodium thiosulphate	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

Equivalent to Meat extract

Directions

Suspend 64.62 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 sloped form with a butt of depth about 2.5cm-5cm.

Note: For better results, the medium can be sterilized by autoclaving at 10 lbs pressure (115°C) for 15 minutes.

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 *Salmonella* (5,6) and in the identification of gram-negative bacilli (5,7) and FDA BAM (11). ISO Committee has recommended a slight modification in the original medium for the identification of *Salmonella* (8,9,10). Peptone, yeast extract and HM extract provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and dextrose (glucose) are the fermentable carbohydrates. Sodium thiosulphate and ferrous ions make H₂S 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 enables no reversion of pH in that region and thus bacteria exhibit an acid slant and acid butt. Gas production (CO₂) 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 H₂S 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-H₂S gas production

Type of specimen

Food and Dairy products.

Specimen Collection and Handling:

Processing: ISO 6579-1:2017/Amd.1:2020(E) (9)

Mark suspected colonies on each plate. Streak the selected colonies onto the surface of a non-selective agar medium. Incubate plates at 34-38°C for 24h±3h. Streak the selected colony on the biochemical confirmation media i.e. TSI agar slant and stab the butt. Incubate between 34°C and 38°C for 24h±3h.

ISO 21567:2004 (10)

Streak the selected colonies onto the surface of nutrient agar plates. Incubate the plates at 37±1°C for 18h to 24h. Stab the butt of TSI agar and streak the agar slope. Incubate at 37±1°C for 24±3h.

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 H₂S producing *Salmonella* may not be H₂S positive on TSI Agar.
2. Some bacteria may show H₂S 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 H₂S 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.

Reaction

Reaction of 6.45% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

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

Organism	Growth	Slant	Butt	Gas	H ₂ S
<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 medium	acidic reaction, yellowing of the medium	negative reaction	positive, blackening of medium
<i>Salmonella</i> Paratyphi A ATCC 9150	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
<i>Salmonella</i> Typhi ATCC 6539	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative 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>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
<i>Shigella flexneri</i> ATCC 12022	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative 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 20-30°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 (12,13).

Reference

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8. International Organization for Standardization (ISO) 2017, Draft ISO/DIS 6579.
9. ISO6579-1:2017/Amd.1:2020(E), Microbiology of the food chain-Horizontal method for the detection, enumeration and serotyping of *Salmonella*, Part1, Detection of *Salmonella*, Amd 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSR/V and SC.
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12. Isenberg H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
13. 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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