 Recommended for the identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production.

**Triple Sugar Iron Agar**

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| --- | --- |
| REF: LV.1/SC01.100.0100 100 gram REF: LV.1/SC01.250.0250 250 gram |  REF: LV.1/SC01.500.0500 500 gram  |

# CLINICAL SIGNIFICANCE

# Triple Sugar Iron Agar was originally proposed by Sulkin and Willett and modified by Hajna for identifying Enterobacteriaceae. This medium complies with the recommendation of APHA, for the examination of meat and food products, for the examination of milk and dairy products and for microbial limit test for confirming the presence of Salmonella and in the identification of gram negative bacilli. ISO Committee has recommended a slight modification in the original medium for the identification of Salmonella.

# METHOD PRINCIPLE

# 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 (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.

# MEDIA COMPOSITION

|  |  |
| --- | --- |
| **Item**  | **Formula in g/L**  |
| beef extractPeptone Yeast extractLactoseSucroseDextroseSodium chlorideSodium thiosulphateFerrous citratePhenol redAgar  | 32031010150.30.30.02412 |

##

## Final pH 7.4 ± 0.2 at 25°C

# PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

* Wearing personnel protective equipment (overall, gloves, glasses,..).
* Do not pipette by mouth.
* In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries, seek medical advice immediately.
* Handle specimens and inoculated culture bottles as though capable of transmitting infectious agents. All inoculated culture bottles, specimen collection needles, and blood drawing devices should be decontaminated according to country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment.

For further information, refer to the TSI Agar material safety data sheet.

# MEDIA STORAGE AND STABILITY

**Lab.Vie**. TSI Agar should be stored between 10-30°C in a firmly 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 avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a 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.

##  PROCEDURE

## Dissolve 64.52 grams in 1 liter of distilled water.

## Adjust pH to 7.4 ± 0.2 at 25°C.

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

## Deterioration

**Lab.Vie**. TSI Agar is Light yellow to pink homogeneous free flowing powder prepared medium is Pinkish red coloured clear to slightly opalescent gel forms in tubes as slants.. If there are any physical changes or signs of deterioration (shrinking, cracking, or discoloration), and contaminations for media, discard the medium.

**SPECIMEN COLLECTION AND PRESERVATION**

# For clinical samples follow appropriate techniques for handling specimens as per established guidelines (11,12). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9,10,13). After use, contaminated materials must be sterilized by autoclaving before discarding.

# TYPE OF SPECIMEN

# Isolated microorganism from water, food, or clinical sample. EQUIPMENT REQUIRED NOT PROVIDED

# Sterile cups

# Sterile tubes

# Incubator

# Autoclave

# QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact the technical support.

# PERFORMANCE CHARACTERISTICS

The following organisms are used by us as part of the quality assurance of the product. The total inoculum challenge for each test organism per bottle is 10 to 50 colony forming units (CFU’s).

|  |  |  |
| --- | --- | --- |
| **Microorganism**  | **Growth**  | **Colony Color**  |
| *Escherichia coli ATCC 25922*  | Luxuriant  | Red with black centers  |
| *Pseudomonas aeruginosa ATCC 27853* | Luxuriant  | Red with black centers  |
| *Salmonella Typhi ATCC 6539* | Luxuriant  | Red with black centers  |
| *Staphylococcus aureus subsp.aureus ATCC 25923*  | Luxuriant  | Red with black centers  |
| *Streptococcus pyogenes ATCC 19615* | Luxuriant  | Red  |
| *Salmonella Enteritidis* *ATCC 13067* | Luxuriant  | Red with black centers  |
| *Salmonella Typhimurium ATCC 14028* | Good  | Red  |
| *Yersinia enterocolitica ATCC 9610* | Luxuriant  | Red  |
| *Yersinia enterocolitica ATCC 23715* | Inhibited  | -  |

# REFERENCES

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C. 2. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.

3. Hajna A.A., 1945, J. Bacteriol, 49:516.

4. MacFaddin J., 1985, Media for Isolation-Cultivation-IdentificationMaintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

6. Sulkin E.S. and Willett J.C., 1940, J. Lab. Clin. Med., 25:649

7. International Organization for Standardization (ISO) 2017, Draft ISO/DIS 6579.

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| **SYMBOLS IN PRODUCT LABELLING**  |
|   IVD For in-vitro diagnostic use  |   Number of <n> test in the pack  |
|  LOT Batch Code/Lot number  | A black and white triangle with a exclamation mark  Description automatically generated Caution  |
|  REF Catalogue Number  | Do not use if package is damaged   |
|   Temperature Limitation   Expiration Date   Manufactured by  |  Consult Instruction for use      |

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