Triple Sugar Iron Agar

Intended Use
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.

Summary
Sulkin and Willett originally developed Triple Sugar Iron Agar, which was later modified by Hajna by adding sucrose to the double sugar (dextrose and lactose) formulation of Kligler Iron Agar. The addition of sucrose increased the sensitivity of the medium by facilitating the detection of sucrose fermenting bacilli as well as lactose and/or dextrose fermenters. Acid and gas production is an indication of carbohydrate fermentation, which gives a visible colour change from red to yellow due to change in the phenol red indicator. The production of hydrogen sulphide is indicated by the presence of a precipitate that blackens the medium in the butt of the tube. The medium complies with the recommendations of APHA for examination of food, dairy, water and wastewater and for microbial limit test in confirming the presence of *Salmonella* and in the identification of Gram-negative bacilli. Triple Sugar Iron Agar is also included in the Bacteriological Analytical Manual for food and cosmetics testing.

Principle
Tryptone, peptone, yeast extract and cara beef extract provide nitrogenous compounds, sulphur, trace elements, vitamin B complex, etc. while sodium chloride maintains the osmotic equilibrium. Lactose, sucrose and dextrose are the fermentable carbohydrates. Sodium thiosulphate and ferrous sulphate make the H_2S indicator system. Phenol red is the pH indicator.

Formula*

Ingredients	**g/L**
Lactose	10.0
Sucrose	10.0
Peptone	10.0
Tryptone	10.0
Sodium Chloride	5.0
Yeast Extract	3.0
Cara Beef Extract#	3.0
Dextrose	1.0
Sodium Thiosulphate	0.3
Ferrous Sulphate	0.2
Phenol Red	0.024
Agar	12.0
Final pH (at 25°C)	7.4 ± 0.2

*Adjusted to suit performance parameters.
#Equivalent to Beef Extract

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Food and dairy sample; Water and waste water sample; Clinical samples

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 64.52 g of powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely
4. Dispense in desired containers as per requirements.
5. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
6. Allow the medium to set in sloped form with a butt about 1-inch long.

Quality Control

Dehydrated Appearance: Light yellow to pink coloured, homogenous, free flowing powder.
Prepared Appearance: Pinkish red to reddish brown coloured, clear to slightly opalescent gel forms in tubes as slants.
Cultural Response: Cultural characteristics observed after an incubation of 18-24 hours at 30°C-35°C.

Organism (ATCC)	Growth	Slant	Butt	Gas	H_2S
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (14028)	Good	K	A	+	+
Escherichia coli (25922)	Good	A	A	+	-
Shigella flexneri serotype 2b (12022)	Good	K	A	-	-
Pseudomonas aeruginosa Strain Boston 41501 (27853)	Good	K	K	-	-

Key:
A = Acidic (Colour of the media changed to yellow)
K = Alkaline (No change in colour of the media or the colour of the media changed to pink)
+ = Positive (For H_2S positive colour of the media changed to black and for gas positive bubbles / gap / disruption observed into the media)
- = No reaction (No change in colour of the media)

Interpretation of Results

1. Carbohydrate fermentation is indicated by the production of gas and a change in the colour of the pH indicator from red to yellow. More amounts of acids are liberated in the butt (fermentation) than in the slant (respiration).
2. Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amount of acid present in the butt, therefore, if the medium in the butt of the tube becomes yellow (acidic) while the medium in the slant becomes red (alkaline) the organism being tested only ferments dextrose (glucose).
3. A yellow colour in the slant and butt indicates that the organism being tested ferments dextrose, lactose and/or sucrose.
4. A red colour in the slant and butt indicates that the organism being tested is a non-fermenter.
5. Hydrogen sulphide results in a black precipitate in the butt of the tube because reduction of thiosulphate proceeds in an acid environment.
6. Some members of the *Enterobacteriaceae* and H_2S producing Salmonella may not be H_2S positive on Triple Sugar Iron Agar (may be H_2S positive on Kligler Iron Agar) because utilization of sucrose in TSI. Agar suppresses the enzyme pathway that results in H_2S production.
7. Splitting and cracking of the medium indicates gas production.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. It is important to stab the butt of the medium. Failure to stab the butt invalidates this test. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar because while stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production. Caps must be loosened during this test or erroneous results will occur.
2. Triple Sugar Iron Agar must be read within the 18-24 hour stated incubation period. A false positive reaction may be observed if read too early. A false-negative reaction may be observed if read later than 24 hours.
3. Hydrogen sulphide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton showed that the utilization of sucrose could suppress the enzymatic mechanism responsible for H_2S production. Not all H_2S positive *Salmonellae* are positive on Triple Sugar Iron Agar.
4. Sucrose is added to Triple Sugar Iron Agar to eliminate some sucrose fermenting, lactose non-fermenters such as *Proteus* species.

Warranty

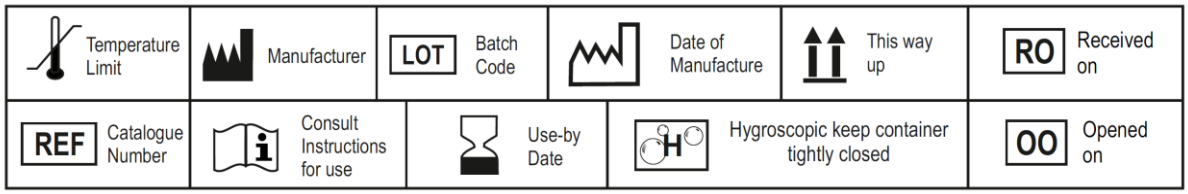
This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

References

1. Downes and Ito (ed.) 2001, Compendium Of Methods For The Microbiological Examination Of Foods, 4th edition, APHA Washington DC.
2. H. Wehr and J. Frank, 2004, Std. Methods for The Examination of Dairy Products, 17th Edition; APHA, Washington, DC.
3. Greenberg AE; Clesceri LS and Eaton AD (Eds), 1998, Std Methods for The Examination of Water and Wastewater, 20th edition, APHA, Washington, DC.
4. IP, 1996, Ministry of Health and Family Welfare, Govt. of India, Vol. 2.
5. US Pharmacopeial Convention, Inc. 2001. The United States Pharmacopoeia 25/NF 20-2002. The US Pharmacopeial Convention, Inc; Rockville, Md.
6. US Food and Drug Adm; 1998, Bacteriological Analytical Manual, 8th Ed; Rev. A, AOAC, International, Gaithersburg, Md.
7. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201200170100	Dehydrated Culture Media	100 g
201200170500	Dehydrated Culture Media	500 g
203200440025	Ready Prepared slant	25 Slants



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Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.