Triple Sugar Iron Agar • TSI Agar

Intended Use

Triple Sugar Iron Agar (TSI Agar) is used for the differentiation of gram-negative enteric bacilli based on carbohydrate fermentation and the production of hydrogen sulfide.

Summary and Explanation

TSI Agar is used for the determination of carbohydrate fermentation and hydrogen sulfide production in the identification of gram-negative bacilli.[1,2]

Hajna developed the formulation for TSI Agar by adding sucrose to the double sugar (dextrose and lactose) formulation of Kligler Iron Agar.[3] 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.

Carbohydrate fermentation is detected by the presence of gas and a visible color change (from red to yellow) of the pH indicator, phenol red. The production of hydrogen sulfide is indicated by the presence of a precipitate that blackens the medium in the butt of the tube.

Principles of the Procedure

TSI Agar contains three sugars (dextrose, lactose and sucrose), phenol red for detecting carbohydrate fermentation and ferrous ammonium sulfate for detection of hydrogen sulfide production (indicated by blackening in the butt of the tube).

Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow. To facilitate the detection of organisms that only ferment dextrose, the dextrose concentration is one-tenth the concentration of lactose or sucrose. The small amount of acid produced in the slant of the tube during dextrose fermentation oxidizes rapidly, causing the medium to remain red or revert to an alkaline pH. In contrast, the acid reaction (yellow) is maintained in the butt of the tube because it is under lower oxygen tension.

After depletion of the limited dextrose, organisms able to do so will begin to utilize the lactose or sucrose.[2]

To enhance the alkaline condition of the slant, free exchange of air must be permitted by closing the tube cap loosely. If the tube is tightly closed, an acid reaction (caused solely by dextrose fermentation) will also involve the slant.

User Quality Control

Identity Specifications

Difco™ Triple Sugar Iron Agar

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in purified water upon boiling. Solution is red, very slightly opalescent, may contain up to a small amount of dark brown precipitate.
Prepared Appearance:	Red, slightly opalescent.
Reaction of 6.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Difco™ Triple Sugar Iron Agar

Prepare the medium per label directions. Inoculate with fresh cultures by the stab and streak method and incubate with caps loosened at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	RECOVERY	SLANT	BUTT	GAS	H_2S
Escherichia coli	25922	Good	A	A	+	–
Pseudomonas aeruginosa	9027	Good	K	K	–	–
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	Good	K	A	+	+
Shigella flexneri	12022	Good	K	A	–	–

A = Acid K = Alkaline

Formula

Difco™ Triple Sugar Iron Agar

Approximate Formula* Per Liter

Beef Extract	3.0	g
Yeast Extract	3.0	g
Pancreatic Digest of Casein	15.0	g
Proteose Peptone No. 3	5.0	g
Dextrose	1.0	g
Lactose	10.0	g
Sucrose	10.0	g
Ferrous Sulfate	0.2	g
Sodium Chloride	5.0	g
Sodium Thiosulfate	0.3	g
Agar	12.0	g
Phenol Red	24.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 65 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense into tubes and autoclave at 121°C for 15 minutes.
4. Cool in a slanted position so that deep butts are formed.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

To inoculate, carefully touch only the center of an isolated colony on an enteric plated medium with a cool, sterile needle, stab into the medium in the butt of the tube, and then streak back and forth along the surface of the slant. Several colonies from each primary plate should be studied separately, since mixed infections may occur.

Incubate with caps loosened at 35°C and examine after 18-24 hours for carbohydrate fermentation, gas production and hydrogen sulfide production. Any combination of these reactions may be observed. Do not incubate longer than 24 hours because the acid reaction in the slant of lactose and sucrose fermenters may revert to an alkaline reaction.

Expected Results

Compare reactions produced by the unknown isolate with those produced by the known control organisms.

Carbohydrate fermentation is indicated by a yellow coloration of the medium. If the medium in the butt of the tube becomes yellow (acidic), but the medium in the slant becomes red (alkaline), the organism being tested only ferments dextrose (glucose).

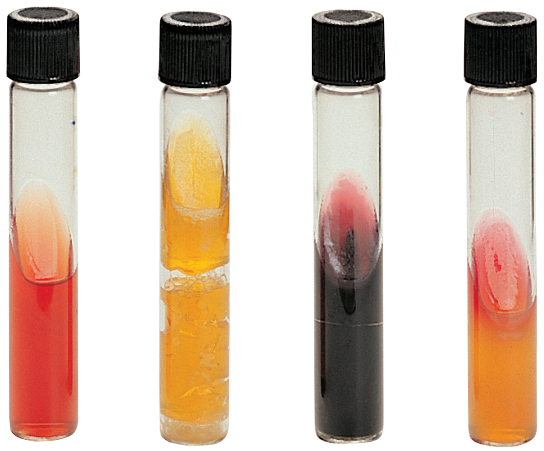
A yellow (acidic) color in the slant and butt indicates that the organism being tested ferments dextrose, lactose and/or sucrose.

A red (alkaline) color in the slant and butt indicates that the organism being tested is a nonfermenter.

Hydrogen sulfide production results in a black precipitate in the butt of the tube.

Gas production is indicated by splitting and cracking of the medium.

For final identification, perform biochemical tests and other identification procedures with a pure culture of the organism. Consult appropriate references for further information.[4-6]



Escherichia coli ATCC™ 25922 | *Salmonella* Enteritidis ATCC™ 13076 | *Shigella flexneri* ATCC™ 12022 | Uninoculated Tube

Limitations of the Procedure

1. Hydrogen sulfide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton[7] showed that the utilization of sucrose could suppress the enzymatic mechanisms responsible for H_2S production. Padron and Dockstader[8] found that not all H_2S-positive *Salmonella* are positive on TSI.
2. Sucrose is added to TSI to eliminate some sucrose-fermenting lactose-nonfermenters such as *Proteus* and *Citrobacter* spp.[1]
3. Further biochemical tests and serological typing must be performed for definite identification and confirmation of organisms.
4. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar. While stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production.[1]
5. A pure culture is essential when inoculating Triple Sugar Iron Agar. If inoculated with a mixed culture, irregular observations may occur.
6. Tubes should be incubated with caps loosened. This allows a free exchange of air, which is necessary to enhance the alkaline condition on the slant.[1]

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
3. Hajna. 1945. J. Bacteriol. *49*:516.
4. Ewing. 1985. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
5. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
6. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
7. Bulmash and Fulton. 1964. J. Bacteriol. *88*:1813.
8. Padron and Dockstader. 1972. Appl. Microbiol. *23*:1107.

Availability

Difco™ Triple Sugar Iron Agar

AOAC | BAM | BS12 | CCAM | CMPH2 | COMPF | EP | ISO | MCM9 | SMD | SMWW | USDA

Cat. No. 226540 Dehydrated – 500 g

BBL™ TSI Agar

AOAC | BAM | BS12 | CCAM | CMPH2 | COMPF | EP | ISO | MCM9 | SMD | SMWW | USDA

Cat. No. 221038 Prepared Slants – Pkg. of 10*
221039 Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*