Triple Sugar Iron Agar • TSI Agar

Intended Use

This medium conforms with specifications of *The United States Pharmacopeia* (*USP*).

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] It is recommended in the *USP* for use in performing Microbial Limit Tests.[3]

Hajna developed the formulation for TSI Agar by adding sucrose to the double sugar (dextrose and lactose) formulation of Kligler Iron Agar.[4] 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

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

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, slightly opalescent.
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 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 choleraesuis subsp. *choleraesuis* serotype Enteritidis	13076	Good	K	A	+	+
Shigella flexneri	12022	Good	K	A	–	–

A = Acid K = Alkaline

Identity Specifications

BBL™ TSI Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain many minute to very small tan specks.
Solution:	5.94% solution, soluble in purified water upon boiling. Solution is medium to dark, red-orange to orange-red, clear to slightly hazy.
Prepared Appearance:	Medium to dark, red-orange to orange-red, clear to slightly hazy.
Reaction of 5.94% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ TSI Agar

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

ORGANISM	ATCC™	RECOVERY	SLANT	BUTT	GAS	H_2S
Escherichia coli	25922	Good	A	A	+	–
Pseudomonas aeruginosa	27853	Good	K	K	–	–
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	Good	K	A	+/–	+
Shigella flexneri	12022	Good	K	A	–	–

A = Acid K = Alkaline

Formulae

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

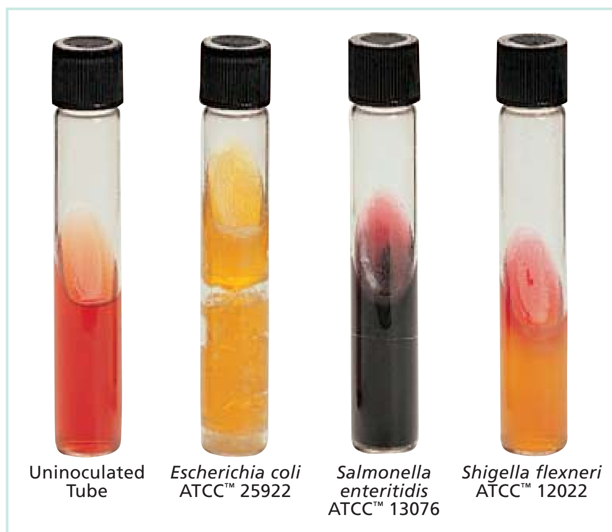
BBL™ TSI Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Peptic Digest of Animal Tissue	10.0	g
Dextrose	1.0	g
Lactose	10.0	g
Sucrose	10.0	g
Ferrous Ammonium Sulfate	0.2	g
Sodium Chloride	5.0	g
Sodium Thiosulfate	0.2	g
Agar	13.0	g
Phenol Red	25.0	mg

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Triple Sugar Iron Agar – 65 g;
   BBL™ TSI Agar – 59.4 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense into tubes and autoclave at 118-121°C (per label directions) 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.



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

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.[5-7]

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[8] showed that the utilization of sucrose could suppress the enzymatic mechanisms responsible for H_2S production. Padron and Dockstader[9] 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]

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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. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
3. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
4. Hajna. 1945. J. Bacteriol. *49*:516.
5. Ewing. 1985. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
6. Murray, Baron, Pfaller, Tenover and Yolken (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
8. Bulmash and Fulton. 1964. J. Bacteriol. *88*:1813.
9. Padron and Dockstader. 1972. Appl. Microbiol. *23*:1107.

Availability

Difco™ Triple Sugar Iron Agar

AOAC BAM BS10 CCAM CMPH COMPF EP MCM7 SMD SMWW USDA USP

Cat. No. 226540 Dehydrated – 500 g

BBL™ TSI Agar

AOAC BAM BS10 CCAM CMPH COMPF EP MCM7 SMD SMWW USDA USP

Cat. No. 211749 Dehydrated – 500 g
221038 Prepared Slants – Pkg. of 10*
221039 Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*

Tryptic Nitrate Medium

Intended Use

Tryptic Nitrate Medium is used for differentiating microorganisms based on nitrate reduction.

Summary and Explanation

Tryptic Nitrate Medium is a differential, semi-solid, general purpose medium that supports growth of aerobes as well as facultative and obligate anaerobes.[1] The formulation includes potassium nitrate which can be reduced by certain organisms to either nitrite or nitrogen gas. Nitrate reduction can be detected by various test methods and is used in differentiating organisms from clinical samples, foods and dairy products.[1-5]

Principles of the Procedure

Peptone is a source of nitrogen, amino acids and vitamins. Dextrose provides carbohydrates. Potassium nitrate provides the basis for nitrate reduction. Disodium phosphate is a buffering agent. The low agar content, which allows varying degrees of anaerobiosis in the medium, supports growth of organisms with various oxygen requirements.

User Quality Control

Identity Specifications

Difco™ Tryptic Nitrate Medium

Dehydrated Appearance: Beige, free flowing, homogeneous.

Solution: 2.5% solution, soluble in purified water upon boiling. Solution is light amber, clear to slightly opalescent.

Prepared Appearance: Light amber, slightly opalescent.

Reaction of 2.5% Solution at 25°C: pH 7.2 ± 0.2

Cultural Response

Difco™ Tryptic Nitrate Medium

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours; incubate *Clostridium sporogenes* anaerobically. Test for nitrate reduction using **Difco™/BBL™** Nitrate A, B and C Reagents.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	NITRATE REDUCTION
Clostridium sporogenes	11437	10^2-10^3	Good	–
Escherichia coli	25922	10^2-10^3	Good	+
Staphylococcus aureus	25923	10^2-10^3	Good	+

Uninoculated Tube | *Escherichia coli* ATCC™ 25922 | *Clostridium sporogenes* ATCC™ 11437