QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

TSI Agar (Triple Sugar Iron Agar) is a differential medium for gram-negative enteric organisms on the basis of their ability to ferment dextrose, lactose and sucrose and to produce sulfides.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Using 18- to 24-h **Trypticase™** Soy Agar slant cultures, inoculate the tubes with an inoculating needle by stabbing the butt and streaking back and forth along the surface of the slant.
   b. Incubate tubes with loosened caps at 35 ± 2 °C in an aerobic atmosphere.
2. Examine tubes after 18–24 h for growth and reactions.
3. Expected Results

Organisms	ATCC®	Slant	Butt	Gas	H_2S
**Escherichia coli*	25922	Acid	Acid	+	–
**Salmonella enterica* subsp. *enterica* serotype Typhimurium	14028	Alkaline	Acid	+/–	+
**Shigella flexneri*	12022	Alkaline	Acid	–	–
**Pseudomonas aeruginosa*	27853	Alkaline	Alkaline	–	–

*Recommended organism strain for User Quality Control.

NOTE: This medium is exempt from User QC testing according to CLSI M22-A3.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under “Product Deterioration.”
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.3 ± 0.2.
4. Incubate uninoculated representative tubes at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

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

V 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.

VI PRINCIPLES OF THE PROCEDURE

TSI Agar contains three sugars (dextrose, lactose and sucrose), phenol red for detecting carbohydrate fermentation, and ferrous 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.[4]

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.

VII REAGENTS

TSI Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	10.0 g	Dextrose	1.0 g
Peptic Digest of Animal Tissue	10.0 g	Ferrous Ammonium Sulfate	0.2 g
Sodium Chloride	5.0 g	Sodium Thiosulfate	0.2 g
Lactose	10.0 g	Phenol Red	0.025 g
Sucrose	10.0 g	Agar	13.0 g

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe established precautions against microbiological hazards throughout all procedures. Prior to discarding, sterilize prepared tubes, specimen containers and other contaminated materials by autoclaving.

Storage Instructions: On receipt, store tubes in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[2,5] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: TSI Agar Slants

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

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 h for carbohydrate fermentation, gas production and hydrogen sulfide production. Any combination of these reactions may be observed. Do not incubate longer than 24 h because the acid reaction in the slant of lactose and sucrose fermenters may revert to an alkaline reaction.

User Quality Control: See “Quality Control Procedures.”

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory’s standard quality control procedures.

X 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.

Consult appropriate references for further information.[2,5-7]

XI LIMITATIONS OF THE PROCEDURE

Some organisms may demonstrate hydrogen sulfide production on Kligler Iron Agar, but not on TSI Agar because utilization of the sucrose in TSI Agar suppresses the enzyme mechanism that results in the production of H_2S. Specifically, H_2S-producing *Salmonella* and some members of the *Enterobacteriaceae* may not be H_2S positive on TSI Agar.[1]

As with Kligler Iron Agar, hydrogen sulfide-producing organisms on TSI Agar may produce so much of the black precipitate, ferrous sulfide, that the acidity produced in the butt is completely masked. However, if H_2S is reduced, an acid condition does exist in the butt even if not observable and should be recorded as such.[1]

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[2,5-7]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of TSI Agar slants are tested for performance characteristics. Representative samples of the lot are tested with **Trypticase** Soy Agar cultures of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella* Typhimurium (ATCC 14028) and *Shigella flexneri* (ATCC 12022) by streaking the slant and stabbing the butt with an inoculating needle. The tubes are incubated with loosened caps at 35 ± 2 °C and read after 18–24 h for growth and reactions. Growth of all organisms is moderate to heavy. The slant of the tube inoculated with *E. coli* shows an acid reaction while the slants of all other inoculated tubes are alkaline. *S. flexneri* produces an acid reaction in the butt, *P. aeruginosa* an alkaline reaction. *E. coli* produces acid and gas in the butt. *Salmonella* Typhimurium produces an acid reaction in the butt along with blackening of the medium; gas may or may not be present.

XIII AVAILABILITY

Cat. No.	Description
221038	**BD BBL™** TSI Agar Slants, Pkg. of 10 size K tubes
221039	**BD BBL™** TSI Agar Slants, Ctn. of 100 size K tubes

XIV REFERENCES

1. MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore.
2. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
3. Hajna, A.A. 1945. Triple-sugar iron agar medium for the identification of the intestinal group of bacteria. J. Bacteriol. *49*:516-517.
4. Baron, E.J., L.R. Peterson and S.M. Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
6. Ewing, W.H. 1985. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York.
7. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

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