Department of Microbiology
Triple Sugar Iron (TSI) Agar Procedure

I. Purpose and Test Principle

Triple Sugar Iron (TSI) Agar contains three sugars, dextrose, lactose and sucrose. The medium contains phenol red as a pH indicator, and ferrous sulfate for detection of hydrogen sulfide production. Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow due to the acidification of the medium.

To facilitate the detection of organisms that only ferment dextrose, the dextrose concentration is one-tenth the concentration of lactose or sucrose. A small amount of acid produced in the slant of the tube during dextrose fermentation. Oxidative metabolism continues in the slant after the low concentration of dextrose has been depleted, producing an alkaline pH from aerobic breakdown of peptone. This causes the medium in the upper slant portion of the tube to revert to an alkaline pH and turn red. However, the butt of the tube remains acid and yellow 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. Since these carbohydrates are in higher concentration, a large amount of acid is produced leading to a yellow slant and butt. If the slant and the butt remain neutral, the organism is not capable of fermenting any of the three carbohydrates. Gas production from sugar fermentation is indicated by bubbles, fracturing of the medium, or displacement of the medium. Hydrogen sulfide is produced by the action of the bacteria with sodium thiosulfate. This is detected by the reduction of ferric ions to produce a black precipitate.

While this medium is primarily used as a screening test for fecal pathogens, it can be a useful biochemical test for *Erysipelothrix* and other H_2S-producing organisms. This medium may also be useful for the determination of the ability of some fastidious organisms to ferment glucose, since they may not react in OF medium and appear to be non-glucose fermenting.

II. Specimen Information

Suspect colonies must be isolated to assure a pure inoculum and accurate results.

III. Reagents & Equipment

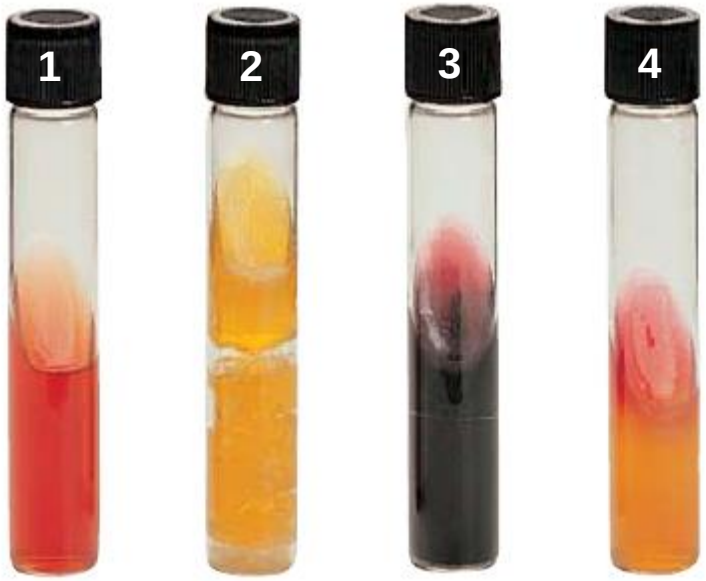
- TSI slants - On receipt, store tubes in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tube media stored as labeled, until just prior to use, may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.
- Sterile inoculating needle
- Aerobic, non-CO_2 incubator set at 35 ± 2°C

IV. Procedure

A. Remove medium from refrigerator and allow warming to room temperature. Examine the medium for cracks in the agar. Do not use if cracks are apparent.
B. Using a sterile inoculating needle, touch the center of a well-isolated colony.
C. Stab the TSI medium to within 3 to 5 mm from the bottom of the tube.
D. Withdraw the needle, and streak the entire surface of the agar slant.
E. **Place cap loosely on tube. Do not tighten cap.**
F. Incubate aerobically in a non-CO_2 incubator at 35 ± 2°C for 18 to 24 h.
G. Examine the reaction in the slant and the butt. Observe for gas and hydrogen sulfide production.
H. Do not interpret sugar fermentation reactions after 24 h.
I. Tubes may be incubated for up to 3 d to detect H_2S production for organisms such as *Erysipelothrix*.

V. Interpretation

A. Observations
   1. Acid reaction: yellow
   2. Alkaline reaction: red
   3. H2S production: black color throughout the medium, a black ring at the junction of the butt and the slant, or a black precipitate in the butt.
   4. Gas production: cracks or displacement of media
B. Interpretation and Examples



1. Uninoculated tube
2. Acid slant/acid butt, + gas, - H_2S
3. Alkaline slant/acid butt, - gas, + H_2S
4. Alkaline slant/acid butt, - gas, - H_2S

VI. Quality Control

Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration. TSI Agar is listed as an exempt medium in CLSI M22-A3 (2004).

VII. Limitations

A. It is important to incubate tubes with the cap loose. If the tube is tightly closed, an acid reaction on the slant caused solely by dextrose fermentation will result, rather than reversion to alkaline by oxidation.
B. Do not read the test before 18 h, since false readings of acid in the slant may result.
C. Copious amounts of H_2S may mask the glucose reaction. If this exists, glucose has been fermented, even though the reaction cannot be observed.
D. The sugar reactions of acid/acid may be obtained with *Yersinia enterocolitica* since it can ferment sucrose but not lactose.
E. H_2S production may be inhibited on TSI for organisms that utilize sucrose and suppress the enzyme mechanism that results in production of H_2S.

VIII. References

A. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.
B. Package insert: BBL TSI Agar Slants. L007520. Rev. 08, January 2011.
C. Clinical and Laboratory Standards Institute M22-A3. Volume 24, Number 19. June 2004. Quality Control for Commercially Prepared Microbial Culture Media; Approved Standard – Third Edition

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