TRIPLE SUGAR IRON (TSI) TEST

Principle and Purpose

Triple Sugar Iron (TSI) agar is a differential medium that can assess the ability of a microbe to ferment sucrose, glucose, and lactose in addition to produce gas and/or hydrogen sulfide. Such data generated from cultures on TSI agar can be used to help identify enteric bacteria.

TSI medium contains sucrose and lactose as well as glucose in a ten-fold lower concentration. Phenol red is incorporated as a pH indicator which serves to detect acid production resulting from carbohydrate fermentation. In addition, TSI agar contains ferrous ammonium sulfate which helps reveal hydrogen sulfide production. The medium is inoculated by streaking the agar surface, then stabbing the butt of the agar through the slant surface. This presents two different growth conditions: aerobic respiration occurs on the agar slant surface, whereas the butt is anaerobic and thereby favors fermentation. Hence, two reaction areas exist in the same tube.

When the low level of the glucose present in TSI is fermented, only a corresponding small amount of acid is produced which turns the phenol red in the butt yellow (Fig. 1). Should glucose fermentation not occur, the butt remains alkaline, i.e., red/pink in color. If the higher concentration of sucrose and/or lactose is fermented, sufficient acid is produced that then turns the agar slant yellow as well. Non-fermentation of sucrose or lactose causes the slant to remain red/pink (alkaline). By convention, the production of acid is indicated by the letter 'A', whereas alkaline conditions are designated with the letter 'K'.

Any gas generated from a carbohydrate results in bubbles and/or cracks appear in the medium (Fig. 1). If copious amounts of gas are produced, the agar may be pushed up from the bottom of the tube (even up into the lid of the tube!). The production of hydrogen sulfide is detected by the presence of a black precipitate which forms when the sulfur released from protein degradation combines with the iron atoms of ferrous ammonium sulfate in the medium.

Figure 1 depicts results of TSI agar inoculated with selected bacteria. On this medium, *S. enterica* produced an alkaline slant (K) and an acidic butt (A) with concurrent gas (note the bubbles) and hydrogen sulfide production. This indicates that only glucose was fermented. In contrast, *E. coli* only generated gas and the entire medium was acidic (A), thereby indicating

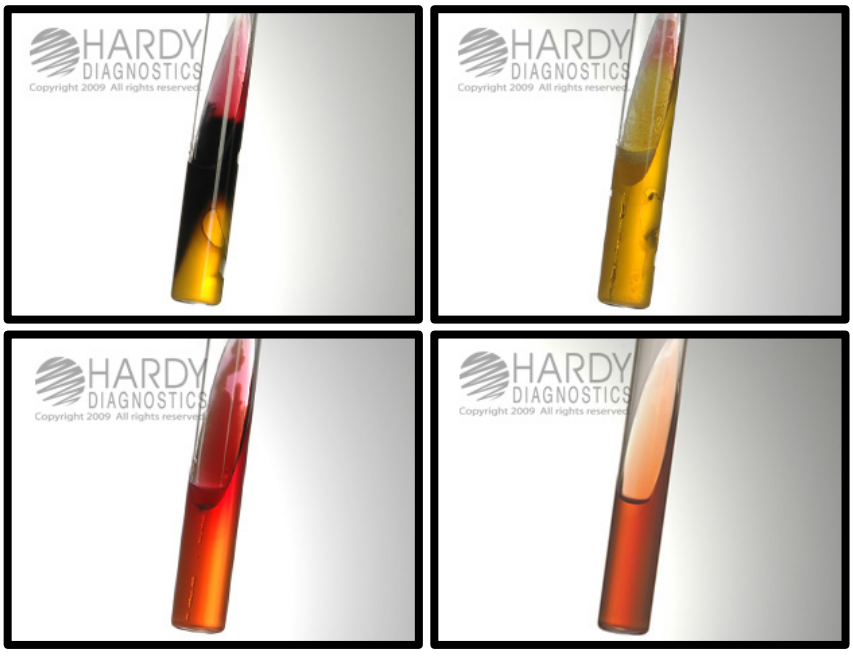

Figure 1. TSI agar reactions of selected bacterial species. S*almonella enterica* (upper left image), *Escherichia coli* (upper right image), and *Pseudomonas aeruginosa* were grown on TSI slants for 24 hours at 35°C. The lower right image depicts an uninoculated (control) TSI agar slant.

that glucose as well as sucrose and/or lactose was fermented. Finally, *P. aeruginosa* did not ferment any carbohydrate and both the slant and butt of the TSI medium remained alkaline (K).

In this exercise, students will examine the reaction of selected bacterial species grown on TSI agar. A commercially available medium will be used for this purpose (Hardy Diagnostics). A video demonstrating the use of TSI media and the interpretation of results is available at the following URL: https://www.micro.iastate.edu/video/microbiology-013-triple-sugar-iron-test. The methodology shown in this video is similar, but not necessarily identical to that described in the exercise presented herein.

Learning Objectives

Upon completion of this exercise, a student should be able to:

- Understand the underlying biochemical basis of the triple sugar iron test;
- Properly conduct the triple sugar iron test; and
- Accurately interpret the results of this test.

Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms – The following organisms should be provided as 24-48 hour TSA slant cultures:

- *Alcaligenes faecalis* (ATCC 18750) [abbreviated as *A. faecalis*]
- *Shigella flexneri* (ATCC 12022) [abbreviated as *S. flexneri*]
- *Escherichia coli* (ATCC 25922) [abbreviated as *E. coli*]
- *Proteus hauseri* (ATCC 13315) [abbreviated as *P. hauseri*]

Media and Reagents

- Triple Sugar Iron (TSI) Agar Slant (Cat. No. R32; Hardy Diagnostic, Santa Maria, CA; https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/TripleSugarIronTSIAgar.htm)

Procedures

Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

1) Obtain five (5) TSI agar tubes and allow them to warm to room temperature before use.
2) Label one of the tubes as 'Control', a second as '*E. coli*', another as '*S. flexneri*', a fourth as '*A. faealis*', and the remaining tube as '*P. hauseri*'. Be sure to add other identifying information as appropriate.
3) Using a microbiological needle and aseptic technique, inoculate the labeled tube of medium with cells from the TSA slant culture matched to the appropriate bacterium. Streak the agar slant in a serpentine manner, then stab the tube butt through the slant three-quarters of the length of the agar.

   Do not inoculate the 'control' tube.

Note: Failure to stab the butt makes the test invalid. Also, the stab must maintain the structural integrity of the agar, i.e., do not twist/move the needle as it is moved in and out of the agar.

4) Incubate all the tubes at 37°C for 18-24 hours. Be sure that the screw-cap lid is loosened, but not to the degree at which it can fall off.

Note: Unloosened caps may create an adverse environment within the tube leading to erroneous results.

5) Remove the tubes from the incubator and examine any reactions that may have occurred.

Record your observations on the report sheet attached to this exercise.

Note: TSI Agar must be observed within the 18-24 hour incubation period. Making any observation prior to 18 hours and after 24 hours may result in a false-positive rand false-negative reaction, respectively.

Interpretation of Results

- An alkaline/acid (red slant/yellow butt; K/A) reaction: only glucose (dextrose) is fermented
- An acid/acid (yellow slant/yellow butt; A/A) reaction: dextrose, lactose and/or sucrose is fermented.
- An alkaline/alkaline (red slant, red butt; K/K) reaction: no carbohydrate fermentation
- Blackening of the medium: hydrogen sulfide (H_2S) is produced
- Bubbles or cracks in the agar: gas (G) is produced
- Large amounts of hydrogen sulfide may mask acid production in the butt of the medium. Because hydrogen sulfide production requires an acidic environment, any butt portion wholly masked by black precipitate should be considered acidic (A).
- TSI is not as sensitive in detecting hydrogen sulfide in comparison to Sulfide Indole Motility (SIM) Medium. In TSI, organisms that weakly produce hydrogen sulfide may show only a trace of hydrogen sulfide activity or none at all.

Student Name: _____

COMPLETE THE FOLLOWING TABLE BASED UPON YOUR OBSERVATIONS

Observations	**Bacteria Tested**			
	Escherichia coli	*Alcaligenes faecalis*	*Shigella flexneri*	*Proteus hauseri*
Slant (A or K?)				
Butt (A or K?)				
Gas (G or 0)				
H_2S (+ or -)				

Discussion Question:

1. Why does TSI contain less glucose than sucrose or lactose?

2. Does an acidic slant indicate that the microbe is a coliform? Explain your answer.