

## TRIPLE SUGAR IRON AGAR AND ITS USE

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David B. Fankhauser, Ph.D.

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see *Difco Manual*, 9th Ed, (1953) pp. 166-168 (Table on p. 161.)

[http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Triple\\_Sugar\\_Iron/TSI\\_Use.htm](http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Triple_Sugar_Iron/TSI_Use.htm)

**INTRODUCTION:** This is a differential medium used to distinguish between a number of Gram-negative enteric bacteria based on their physiological ability (or lack thereof) to:

- conduct fermentation to produce acid      turns the butt yellow
- metabolize lactose and/or sucrose      turns butt *and slant* yellow
- produce gas during fermentation      causes the butt to crack
- generate H<sub>2</sub>S.      Turns the butt black

It contains 1.0% each of sucrose and lactose plus 0.1% glucose. If only glucose is fermented, a small amount of acid products are formed, sufficient to turn the methyl red in the butt yellow, but not enough to affect the slant (stays red). The scoring of the slants is as follows:

**color of the slant ( R or Y ) / the color of the butt ( R or Y ) [G if it makes gas, B if it is black]**

Thus fermentation of only glucose without gas would be = R/Y. However, if either sucrose or lactose are fermented, sufficient acid fermentation products will be formed to turn both the butt *and* the slant yellow = Y/Y. If gas is formed during the fermentation, it will show in the butt either as bubbles or as cracking of the agar =\*/G. If *no* fermentation occurs (eg: obligate aerobe), the slant *and* butt remain red = R/R.

The medium also contains ferrous sulfate. If the bacterium forms H<sub>2</sub>S, it will react to form ferrous sulfide, which is seen as a black precipitate in the butt (a black butt).

**SUPPLIES and EQUIPMENT:** TSI agar, 600 mL beaker, heat source, 16x150 mm tubes & closures, "S" shaped TT racks (hold tubes snugly), repipet, ring stands, clamps.

(NOTE: prepare at beginning of class.)

**PREPARATION OF MEDIA:** (for 10 students: 40 tubes)

- Weigh out, dissolve:** 17.8 g dry medium, add to 300 mL water, heat to 95°C to dissolve.
- Aliquot out 6 mL** to 16 x 150 mm tubes with a repipet. Cover with closures.
- Autoclave** 13 lb, 15 min. (Our autoclave only does 15 lb...)
- Slant the test tube rack** so that it has a 3 cm slant with a 2-3 cm butt. Illustrate the slanting of the rack with agar tubes. Let cool until solid. Incubate 48 hr at 37°C to assure sterility.

**INOCULATION:**

- Inoculate the slants with a pure culture by streaking over the entire surface of the slant (zig-zag to cover surface) and then stabbing deep into the butt.
- Incubate at 37°C for 24 hours (48 hr may be necessary to show all H<sub>2</sub>S reactions).

**SCORING THE SLANTS:** Examine the slant and butt, and record data:

<u>SLANT COLOR:</u>	<u>Code letter:</u>	<u>Interpretation:</u>
RED:	R	does not ferment either lactose or sucrose
YELLOW:	Y	ferments lactose and/or sucrose

**BUTT COLOR AND CONDITION:**

RED:	R	no fermentation, the bacterium is an obligate aerobe
YELLOW:	Y	some fermentation has occurred, acid has been produced, it is a facultative anaerobe.
GAS FORMED:	YG	Seen as cracks in the agar, bubbles, or the entire slant may be pushed out of the tube ( <i>caution:</i> these gassy fermenters may have bacteria close to the opening.)
BLACK:	"+"	H <sub>2</sub> S has been produced