TSI (TRIPLE SUGAR IRON)

Principle

To determine the ability of an organism to attack a specific carbohydrate incorporated in a basal growth medium, with or without the production of gas, along with the determination of possible hydrogen sulfide (H_2S) production. This test is used, in conjunction with others, for the identification of enteric pathogens.

Materials

TSI Slant
Inoculating wire or sterile glass pasteur pipette.

Procedure

1. Using the an inoculating wire, dip into the previously inoculated TSB.
2. Stab the butt of the TSI to within 1/4 inch from bottom, draw out and fishtail over slant. Do not tighten cap.
3. Incubate O_2, 35^{o}C X 18-24 hours.

Interpretation

Carbohydrate utilization:

1. Fermentation of glucose only
   (a) slant: red colour (alkaline reaction)
   (b) butt: yellow colour (acid reaction)

2. Fermentation of glucose and sucrose and/or lactose
   (a) slant: yellow colour (acid reaction)
   (b) butt: yellow colour (acid reaction)

3. Neither glucose nor lactose nor sucrose fermented
   (a) slant: red colour (alkaline reaction)
   (b) butt: (i) aerobic organism
   (a) No growth
   (b) No colour change
   (ii) facultative organism
   red colour (alkaline reaction)

Gas production:

1. Aerogenic:
   (a) Gas production: CO_2 and H_2
   (b) Evident by one of the following:
      (i) a single gas bubble
      (ii) bubbles in the medium
      (iii) splitting of medium
      (iv) complete displacement of the medium from bottom of the tube leaving a clear area
      (v) slight indentation of medium from the side of the tube

2. Anaerogenic:
   No gas production

H_2S production:

The presence of a black precipitate (ferrous sulfide) is evident by:
(i) A black colour spread throughout the entire butt masking the acidity; may even be a slight evidence on the slant
(ii) A black ring near the top of the butt area
(iii) A black precipitate scattered throughout the butt but not entirely masking the acidity present

Summary:

The ways of recording the TSI reactions are listed below. Remember that the slant is first, followed by the butt reaction.

acid/acid	+/+
acid/acid/gas	+/+ with gas
acid/acid/gas/H_2S	+/+ with H_2S
alkaline/acid	-/+
alkaline/acid/gas	-/+ with gas
alkaline/acid/gas/H_2S	-/+ with gas and H_2S
alkaline/acid/H_2S	-/+ with H_2S
alkaline/alkaline	-/-

Precautions

1. The TSI tube should be read within 18-24 hr. If read earlier, a false +/+ reaction may occur; if after 24 hr, a false -/-reaction may occur.

2. An H_2S organism may produce so much black precipitate that the acidity in the butt is completely masked. If H_2S is produced, an acid condition exists in the butt.

3. There is no inhibitor in this medium, therefore any organism may grow. Be certain that the organism tested is a catalase positive, gram negative bacillus.

4. *S. typhi* usually produces a ring of H_2S near the surface of the butt. Occasionally the amount of H_2S produced is so small that it will not be detected in TSI, but will show up in SIM media.

5. Some organisms produce such an abundance of gas that the medium may be completely displaced by gas, resulting in the medium being blown up into the cap of the tube. Use caution to avoid contamination.

6. Do not tighten the cap of a TSI tube. A free exchange of air is necessary to enhance the alkaline reaction of the slant.

Quality Control

Test the media each time it is prepared using the following organisms:

E. coli:	(ATCC 25922):	+/+
P. mirabilis:	(ATCC 12453):	-/+/H_2S
P. aeruginosa:	(ATCC 27853):	-/-

References

1. MacFaddin JF, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore MD, 1980, p183-194.