Triple Sugar Iron (TSI)

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Tubes with TSI agar inoculated with different microorganisms. Different results for the TSI agar (from left to right): a) organism can utilize several carbohydrates, including lactose and has produced gas; b) organism can only utilize glucose; c) organism has produced H_2S; d) no reaction or agar was not inoculated.

Why do we use it and what does it reveal?

TSI agar is a culture medium used to grow bacteria. This culture medium enables the differentiation of bacterial species based on their ability to ferment three types of sugars (glucose, lactose, and/or sucrose) and release acid and hydrogen sulfide (H_2S) gas. A relevant feature of the TSI medium is the color change based on the bacterial metabolism of sugars and the H_2S production, making it a powerful tool for identifying Gram-Negative enteric bacteria.

What are its key ingredients?

- Lactose, sucrose, glucose: different types of sugars fermented by bacteria.
- Ferric ammonium citrate and sodium thiosulphate: used to detect the production of H_2S gas.
- Sodium chloride: salt that maintains the osmotic balance of the environment.
- Peptic digest of animal tissue, pancreatic digest of casein, yeast extract and beef extract: provide nitrogen, peptides, vitamins and amino acids essential for bacterial growth.
- Phenol red: pH indicator that turns yellow at a pH below 6.4 and red at any pH above 8.2.
- Agar: a solidifier agent such as gelatin to solidify the medium.

How do we make it?

Glucose	1 g
Lactose	10 g
Sucrose	10 g
Ferric ammonium citrate	0.5 g
Sodium thiosulphate	0.3 g
Peptic digest of animal tissue	5 g
Pancreatic digest of casein	15 g
Yeast extract	3 g

Beef extract	3 g
Sodium Chloride	5 g
Phenol red	0.024 g
Agar	12 g
Water	1 L

- Accurately weigh each of the ingredients listed.
- In a large container, combine the ingredients with 1 L of distilled water.
- Heat to boiling to completely dissolve the medium and mix.
- Sterilize the medium by autoclaving at 115 °C for 30 minutes.
- Distribute into sterilized test tubes and set in sloped form with a butt about 2.5 cm long.
- Allow the poured medium to solidify in the tubes. This forms the agar test tubes ready for use.

What do we inoculate it with?

Triple sugar iron agar facilitates the differentiation of microorganisms based on their distinct patterns of carbohydrate fermentation and H_2S gas production. To inoculate test tubes with triple sugar iron agar, a well-isolated colony is stabbed through the centre of the medium to the bottom of the tube and then the surface of the agar slant is streaked. Subsequently, the cap is loosely closed, and the tube is incubated at 35–37 °C in ambient air for 18 to 24 hours.

What can we see after incubation?

If the inoculated bacteria ferment carbohydrates, the colour of the medium changes from red to yellow and gas production (CO_2 and O_2) will produce bubbles or cracks in the agar. If oxidative decarboxylation of peptone occurs, the colour of the medium changes to deep red. The black colours in the butt of the tube indicates H_2S production.

How do we interpret what we see?

Bacterial metabolic activities produce observable changes in the medium. The variations in colour of the medium are due to the action of the pH indicator (phenol red), which changes when the acidity varies. Fermentation of sugars produces acids, so the pH drops and the colour of the medium changes to yellow. However, alkaline products are produced through oxidative decarboxylation of peptone, and the colour of the medium turn to deep red. The slant reflects aerobic reactions, while the butt reflects anaerobic reactions.

Possible outcomes:

- Red slant and red butt: absence of fermentation (alkaline reaction). Example: *Pseudomonas aeruginosa*.
- Red slant and yellow butt: glucose fermentation only. After glucose is exhausted, bacteria degrade proteins, raising the pH in the slant (alkaline/acidic reaction). Example: *Shigella species*.
- Yellow slant and yellow butt: glucose, lactose and/or sucrose fermentation (acidic reaction). Examples: *Escherichia coli* and *Klebsiella pneumoniae*.
- Blackening of medium: presence of H_2S. Examples: *Salmonella species* and *Proteus mirabilis*.
- Gas production: presence of bubbles or cracks in the agar. Examples: *Escherichia coli* and *Enterobacter species*.

How do we confirm our interpretation?

We can confirm the results obtained by carrying out additional biochemical tests, such the IMViC tests (Indole, Methyl Red, Voges–Proskauer, and Citrate), which can help differentiate enteric bacteria or/and Urease test to confirm Proteus species. For definitive identification, molecular techniques like PCR or sequencing can be used to validate the bacterial species after initial biochemical analysis.

How is the interpretation used?

The medium is mainly used to differentiate species of the *Enterobacteriaceae* family based on their ability to ferment glucose, lactose, and/or sucrose and produce gas during fermentation.