Triple Sugar Iron Agar (TSI) ISO

Cat #: MM-M-172

For the biochemical confirmation of Salmonella

Principles and uses:

Triple Sugar Iron Agar (TSI) is recommended by ISO 6579 and ISO 19250 for the biochemical confirmation of Salmonella.
Peptone and the beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. The three carbohydrates (glucose, sucrose and lactose) are the fermentable carbohydrates providing carbon and energy. When these are fermented the acid production is indicated by the phenol red indicator, being the color changes yellow for acid production and red for alkalinization. Sodium thiosulfate is reduced to hydrogen sulfide, which reacts with the iron salt to give the black iron sulfide. The ferric ammonium citrate is a H2S indicator. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.
The glucose concentration in the medium is one-tenth the concentration of lactose or sucrose in order to facilitate the detection of organisms that only ferment glucose. The fermentation of glucose produces a small amount of acid in the inclination of the tube, which is rapidly oxidized and the medium remains red or revert to an alkaline pH. On the other hand, the same acid reaction in the butt of the tube keep the acid pH (yellow) due to the lower oxygen tension.
When all glucose is used, organisms able to ferment lactose or glucose will begin to utilize them. In order to enhance the free exchange of air in the slant of the tube, the tube cap must be closed loosely.
The addition of 1% Sucrose in the TSI Agar allows differentiating between Proteus and Salmonella. The fermentation of the sucrose by Proteus turns the color of the Phenol red indicator in the slant from red to yellow. Dextrose positive and lactose negative members of the genus Salmonella, all cause a reddening of the slant and acidify the depths of the agar tubes.

Formula per Litre:

Glucose	1g	Bacteriological agar	12g
Lactose	10g	Beef extract	3g
Peptone	20g	Phenol red	0.024g
Sodium chloride	5g	Sodium thiosulfate	0.3g
Sucrose	10g	Yeast extract	3g
Ferric Citrate	0.3g		

Preparation:

Suspend 64.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121 ºC for 15 minutes. Allow to cool in a slanted position in order to obtain butts of 1.5-2.0 cm. depth.

Instructions for use:

Streak the agar slant surface and stub the butt.
Incubate at 37 ºC for 24±3 h according to ISO 6579 and 36±2 ºC for 24±3 h according to ISO 19250.
Interpret the changes in the medium as follows: Butt:

- Yellow: (glucose positive).
- Red or unchanged: (glucose negative).
- Black: (formation of hydrogen sulphide). Bubbles or cracks: (gas formation from glucose).

Slant surface:

- Yellow: (lactose and/or sucrose positive).
- Red or unchanged: (lactose and/or sucrosenegative).

Quality control:

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Pink	Red	7.4 ± 0.2

Microbiological test:

According to ISO 6579:

Incubation conditions: (37ºC /24±3 h).
According to ISO 19250: (36±2=ºC /24±3 h).

Microorganisms	Specification	Characteristic reaction
Shigella flexneri ATCC 12022	Good growth	Slant (Red), Butt (Yellow), H2S (-), Gas (-).
Salmonella enteritidis ATCC 13076	Good growth	Slant (Yellow), Butt (Yellow), H2S (+), Gas (+).
Proteus vulgaris ATCC 13315	Good growth	Slant (Yellow), Butt (Yellow), H2S (+), Gas (+).
Escherichia coli ATCC 25922	Good growth	Slant (Yellow), Butt (Yellow), H2S (-), Gas (+).
Pseudomonas aeruginosa ATCC 9027	Good growth	Slant (Red), Butt (Red), H2S (-), Gas (-).

Storage:

Temperature: 2ºC - 25ºC

Bibliography:

ISO 6579 Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Salmonella spp Standard Methods for the Examination of Dairy Products. APHA, 1972.
Food and Drug Administration. Bacteriological Analytical Manual, 1976.
Vanderzant, C. and D.F. Splitt stresser (ed) 1992. Conpendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington D.C.
European Pharmacopoeia. 4th Edition. 2002.
ISO 19250 water quality-dectetion of Salmonella spp.