

## TRIPLE SUGAR IRON AGAR (TSI) ISO 6579, ISO 19250

**CAT N°: 1172**

For the biochemical confirmation of *Salmonella*

### FORMULA IN g/l

Peptone	20.00	D-Glucose	1.00
Lactose	10.00	Ferric Ammonium Citrate	0.30
Sucrose	10.00	Sodium Thiosulfate	0.30
Sodium Chloride	5.00	Phenol Red	0.024
Beef Extract	3.00	Bacteriological Agar	12.00
Yeast Extract	3.00		

**Final pH 7.4 ± 0.2 at 25°C**



*Escherichia coli*  
ATCC 25922

### 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°C for 15 minutes. Allow to cool in a slanted position in order to obtain butts of 1.5 - 2.0 cm. depth. The prepared medium should be stored at 2-8°C. The color is red.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

### 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 H<sub>2</sub>S 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 sucrose 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, lactose negative members of the genus *Salmonella* all cause a reddening of the slant and acidify the depths of the agar tubes. Inoculate with sample and incubate at 37 ± 1°C for 24 ± 3 hours, according to ISO 6579 and 36 ± 2°C for

24 ± 3 hours, according to ISO 19250.

## MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37 ± 1°C and observed after 24 ± 3 hours.

Microorganisms	Growth	Slant	Depth	H <sub>2</sub> S	Gas
<i>Escherichia coli</i> ATCC 25922	Good	Yellow	Yellow	-	+
<i>Proteus vulgaris</i> ATCC 13315	Good	Yellow	Yellow	+	+
* <i>Salmonella enteritidis</i> ATCC 13076	Good	Red	Yellow	+	+
<i>Shigella flexneri</i> ATCC 12022	Good	Red	Yellow	-	-
<i>Pseudomonas aeruginosa</i> ATCC 9027	Good	Red	Red	-	-

\* According to ISO 19250 incubate at 36 ± 2°C for 24 ± 3 hours .

## 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. Compendium 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*.



## STORAGE

Once opened keep powdered medium closed to avoid hydration.

