

TRIPLE SUGAR IRON AGAR (TSI) EUROPEAN PHARMACOPOEIA

CAT N°: 1046

For the identification and differentiation of Enterobacteria

FORMULA IN g/l

Peptones (Beef & Casein)	20.00	Glucose Monohydrate	1.00
Lactose Monohydrate	10.00	Ferric Ammonium Citrate	0.30
Sucrose	10.00	Sodium Thiosulfate	0.30
Sodium Chloride	5.00	Phenol Red	0.025
Beef Extract	3.00	Bacteriological Agar	12.00
Yeast Extract	3.00		

Final pH 7.4 ± 0.2 at 25°C

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 a differential medium used to differentiate enteric Gram-negative Enterobacteria on the basis of carbohydrate fermentation and H₂S production. It is used as an aid in the identification of pathogenic and saprophytic Enterobacteria isolated from routine bacteriological analysis of material samples such as feces. This medium is used to initiate the identification of Enterobacteria in some FDA schemas.

Peptone mixture and the Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. TSI contains three carbohydrates (Dextrose, Sucrose and Lactose) as sources of 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 alkalization. Sodium thiosulfate is reduced to Hydrogen sulfide, which reacts with the iron salt to give the black iron sulfide. Ferric ammonium citrate is a H₂S indicator. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

The mode of action is similar to Kligler Iron Agar (Cat. 1042) which contains two sugars. The addition of 1% Sucrose in TSI Agar allows to differentiate between *Proteus* and *Salmonella*. The fermentation of 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.

The European Pharmacopoeia recommends Triple Sugar Iron (TSI) Agar as one of the media used for the confirmation of *Salmonella*. The Eur. Pharmacopoeia recommends transferring separately a few of the suspect colonies to TSI Agar using surface and deep inoculation. The presence of salmonellae is provisionally confirmed if in the deep inoculation, but not in the surface culture, there is a change of color from red to yellow and, usually, a formation of gas, with or without production of Hydrogen sulphide in the agar. Precise confirmation may be carried out by the appropriate biochemical and serological tests. The product passes the test if colonies of the type described do not appear, or if the confirmatory biochemical and serological tests are negative.

Inoculate with sample and incubate at 35 ± 2 for 18 - 72 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of $35 \pm 2^\circ\text{C}$ and observed after 18-72 hours.

Microorganisms	Growth	Slant	Depth	H ₂ S	Gas
<i>Escherichia coli</i> ATCC 25922	Good	Yellow	Yellow	-	+
<i>Proteus vulgaris</i> ATCC 13315	Good	Yellow	Yellow	+	+
<i>Salmonella typhimurium</i> ATCC 14028	Good	Red	Yellow	+	+
<i>Shigella flexneri</i> ATCC 12022	Good	Red	Yellow	-	-

BIBLIOGRAPHY

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. 6th Edition. 2007.



STORAGE

Once opened keep powdered medium closed to avoid hydration.

