

Triple Sugar Iron Agar (TSI)

Cat. 1046

For the identification and differentiation of Enterobacteriaceae

Practical information

Applications	Categories
Differentiation	Enterobacteria

Industry: Pharmaceutical/Veterinary / Clinical / Food



Principles and 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 an 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 differentiating 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 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 sulfide in the agar. Precise confirmation may be carried out by the appropriate biochemical and serological tests.

Formula in g/L

Bacteriological agar	12	Ferric ammonium citrate	0,3
Glucose monohydrate	1	Beef extract	3
Phenol red	0,025	Sodium chloride	5
Sodium thiosulfate	0,3	Sucrose	10
Yeast extract	3	Lactose monohydrate	10
Mixture of peptic digest of animal tissue and pancreatic digest of casein (1:1)	20		

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

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 cooling in a slanted position in order to obtain butts of 1,5-2,0 cm. depth.

Instructions for use

For clinical diagnosis, the type of sample is human feces.

- Inoculate the surface and the bottom. Smear the slope and stab the butt of the TSI agar tube with the sample.
- Incubate at 35 ± 2 °C for 18-72 hours.
- Reading and interpretation of the results.

Note: the inoculum must be an isolated colony.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Pink	Red	$7,4\pm 0,2$

Microbiological test

Incubation conditions: (35 ± 2 °C / 18-72 h)

Microorganisms	Specification	Characteristic reaction
<i>Shigella flexneri</i> ATCC 12022	Good growth	Red slant, yellow depth, H ₂ S (-), gas (-)
<i>Proteus vulgaris</i> ATCC 13315	Good growth	Yellow slant, yellow depth, H ₂ S (+), gas (+)
<i>Salmonella typhimurium</i> ATCC 14028	Good growth	Red slant, yellow depth, H ₂ S (+), gas (+)
<i>Escherichia coli</i> ATCC 25922	Good growth	Yellow slant, yellow depth, H ₂ S(-), gas (+)

Storage

Temp. Min.: 2 °C
Temp. Max.: 25 °C

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.