Specification

Solid differential medium for the identification of enterobacteria according to ISO standards 6579, 6785 and 10272. **Formula * in g/L**

Peptone			
Meat extract	3.000	Iron(III) citrate	0.300
Yeast extract	3.000	Sodium thiosulphate	
Lactose		Phenol red	
Sucrose		Agar	
Dextrose		5	
Sodium chloride	5.000	Final pH 7.4 ±0.2 at 25 °C	

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 64,6 g of powder in 1 L of distilled water and bring to the boil. Dispense into tubes and sterilize at 121°C for 15 minutes. Leave to solidify with short slants and good butts.

Description

TSI Agar is a modification of the classical Kliger's agar. 1% sucrose has been added to this medium to differentiate *Proteus* and *Hafnia* (sucrose positive) from *Salmonella* and *Shigella* (sucrose negative).

Sugar degradation with acid formation is detected by turning an indicator (phenol red) to yellow, whereas alkalinization turns it to purple. When only glucose is degraded, the acid production is weak and is evaporated on the surface, so the indicator may be re-oxidised producing an alkaline surface (red) and an acid butt (yellow). If lactose or sucrose is degraded, acid production is intense and the entire medium (surface and butt) turns yellow. Gas production is detected by the formation of bubbles and occasionally cracks in the agar.

Hydrogen sulfide production, from thiosulfate or sulphured amino-acids from peptones, is detected by the formation of black FeS precipitate when the medium reacts with iron salts.

Use the medium in slanted tubes with a good butt and a short slant. Inoculate by streaking on the surface and stabbing deeply. It is advisable to use tubes with cotton plugs, in order to allow a re-oxidation of the indicator. If screw caps are used, they must be loose. See the following page for the table of reading (observations) and interpretation of results in TSI Agar.

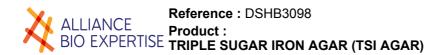
Quality control

Incubation temperature: 37°C ±1

Incubation time: 24 ± 3h

Inoculum: Stab the butt and streak the slant. ≥ 10³ CFU (specificity) according to ISO 11133:2014/Amd 1:2018 & Adm 2:2020

Microorganism	Growth	Remarks
Shigella flexneri ATCC [®] 12022	Good to very good	Slant:K; Butt:A; G(-); H2S (-)
Proteus mirabilis ATCC [®] 43071	Good to very good	Slant:K; Butt:A; G(-); H2S (+)
Escherichia coli ATCC [®] 25922	Good to very good	Slant:A; Butt:A; G(+); H2S (-)
Salmonella typhimurium ATCC [®] 14028	Good to very good	Slant:K Butt:A; G(-); H2S (+)
Salmonella enteritidis ATCC [®] 13076	Good to very good	Slant:K; Butt:A; G(D); H2S (+)
Shigella sonnei ATCC [®] 9290	Good to very good	Slant:K; Butt:A; G(-); H2S (-)
Pseudomonas aeruginosa ATCC® 27853	Good to very good	Slant:K; Butt:K; G(-); H2S (-)



References

- · DIN Standards. Nachweiss von Salmonella. Referenzeverfahren DIN 10160 (Fleisch und Fleischerzenguissen) and DIN 10181 (Milch).
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the microbiological examination of Foods. 4th ed. APHA. Washington DC. USA.
- · EDWARD, S.P. and EWING, W.H. (1962). Identification of Enterobacteriaceae. Burgess. Pub. Co. Minneapolis.
- · EUROPEAN PHARMACOPOEIA (2005) Supp. 5.8 § 2.6.13 Test for specified microorganisms. EDQM. Strasboug E.U.

· FIL-IDF (1991) International Standard 93A. Milk and Milk Products. Detection of Salmonella species.

- HAJNA, A.A. (1945) Triple Sugar-Iron medium for the identification of the intestinal group of bacteria. J.Bact. 49:516 -517.
- · HORWITZ, W. (2000) Official Methods of Analysis. 17th ed. AOAC International. Gaithersburg. Md. USA.
- · ISO 3560 Standard (1975) Reference Method for the Detection of Salmonella in meat and meat products.
- · ISO Standard 6579-1 (2017) Microbiology of food chain Horizontal method for the detection, enumeration and serotyping of Salmonella Part 1 : Detection of Salmonella spp.
- · ISO 6785 Standard (2001) Milk and milk Products Detection of Salmonella spp.
- · ISO 10272 Standard (1995) Microbiology of foods and animal feeding stuffs Horizontal method for the detection of thermotolerant Campylobacter.
- . ISO 11133:2014/ Adm 1:2018/ Adm 2:2020/ Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- · ISO 21567 Standard (2004) Microbiology of food and animal feeding stuffs.- Horizontal method for the detection of Shigella spp.
- · KRUMWIEDE, C. & L. KOHN (1917) A triple sugar modification of the Russell Double Sugar Medium. J. Med. Res. 37:225-229.
- · US PHARMACOPOEIA (2002) <61> Microbial Limit Tests. 25th ed. US Phamacopeial Convention Inc. Rockville. Md. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).