

44940 Triple Sugar Iron Agar (TSI Agar)

For the identification of Enterobacteriaceae acc. to Sulkin and Willett (1940), modified acc. to Hajna (1945).

Composition:

Ingredients	Grams/Litre
Meat extract	3.0
Yeast extract	3.0
Mixed peptone	20.0
Lactose	10.0
Sucrose	10.0
Glucose	1.0
Sodium chloride	5.0
Ferrous sulfate	0.2
Sodium thiosulfate	0.3
Phenol red	0.025
Agar	12.0
Final pH 7.4 +/- 0.2 (at 25°C)	

Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions:

Dissolve 64.5 g in 1 litre distilled water. Warm to dissolve, mix well and pour into tubes, sterilize by autoclaving at 121°C for 15 minutes. Allow the medium to set in slant form.

Note: Use the medium in slanted tubes with good depth and short slant. Inoculate by streaking on surface and stabbing deeply. It is advisable to use tubes with cotton plugs, in order to allow a redoxidation of the indicator. If screw caps are used, they must be loose.

Principle and Interpretation:

Casein peptone, Mixed peptone and Yeast extract act as a source of nitrogen, sulfur, carbon, vitamins and minerals. Lactose, Sucrose and Glucose are the fermentable carbohydrate. Due to the building of acid during fermentation the pH falls. In case of oxidative decarboxylation of peptone alkaline products are formed and the pH rises. This is indicated by the phenol red, which changes its colour in acidic surroundings from red-orange to yellow, on alkalization it turns deep red. Sodium chloride is for the osmotic balance. Gas production (CO_2) is detected by the presence of cracks or bubbles in the medium, when the accumulated gas escapes.

The H_2S -positive bacteria reduce the sulfite in the culture medium to sulfide, which reacts with iron. Due to this reaction a black precipitate (FeS) is formed.

Triple Sugar Iron Agar should be used in parallel with Urea Broth (Prod No 51463) to distinguish between *Salmonella* and *Proteus* species.

Some members of the Enterobacteriaceae and H_2S producing *Salmonella* may not be H_2S positive on Triple Sugar Iron Agar. Some bacteria may show H_2S production on Kligler Agar (Prod No 60787) but not on Triple Sugar Iron Agar. This can happen because of sucrose in the medium, which suppresses the enzymatic pathway that results in H_2S production.



Organism	Butt	Slant surface	H ₂ S	
<i>Enterobacter aerogenes</i>	AG	A	-	
<i>Enterobacter cloacae</i>	AG	A	-	
<i>Proteus vulgaris</i>	AG	A	+	
<i>Proteus mirabilis</i>	AG (A**)	NC or ALK	+	dirty black
<i>Morganella morganii</i>	AG	ALK	-	green
<i>Providencia rettgeri</i>	A (ALK)	NC or ALK	-	
<i>Shigella dysenteriae</i>	A	NC or ALK	-	
<i>Shigella sonnei</i>	A	NC or ALK	-	
<i>Shigella schmitzii</i>	A	NC or ALK	-	
<i>Shigella boydii</i>	A	NC or ALK	-	
<i>Shigella flexneri</i>	A	NC or ALK	-	
<i>Dispar</i>	A	A	-	
<i>Salmonella typhi</i>	A	NC or ALK	+	
<i>Salmonella typhosa</i>	A	NC or ALK	+	
<i>Salmonella entericia</i>	AG	NC or ALK	+	
<i>Salmonella pullorum</i>	AG	NC or ALK	+	
<i>Salmonella gallinarum</i>	A	NC or ALK	+	
<i>Salmonella paratyphi A</i>	AG	NC or ALK	-	
<i>Salmonella paratyphi B</i>	AG	NC or ALK	+	
<i>Salmonella enteritidis</i>	AG	NC or ALK	+	
<i>Salmonella typhimurium</i>	AG	NC or ALK	+	
<i>Escherichia coli</i>	AG	A	-	
<i>Citrobacter freundii</i>	AG	A	+	
<i>Klebsiella pneumoniae</i>	A or AG	NC or ALK	-	
<i>Pseudomonas aeruginosa</i>	ALK	NC or ALK*	-	
<i>Alkaligenes faecalis</i>	ALK	NC or ALK	-	

AG = acid (yellow) and gas formation

A = acid (yellow)

NC = no change

ALK = alkaline (red)

+= hydrogen sulphide (black)

- = no hydrogen sulphide (no black)

* may be pigment production

** some strains show may no gas production

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Grow th	Slant surface	Butt	Gas	H ₂ S
<i>Enterococcus faecalis</i> (29212)	++	yellow	red	-	-
<i>Escherichia coli</i> (25922)	+++	yellow	yellow	+	-
<i>Citrobacter freundii</i> (8090)	+++	yellow	yellow	+	+
<i>Proteus mirabilis</i> (7002)	+++	red	yellow	+	+
<i>Salmonella abony</i> (NCTC 6017)	+++	red	yellow	-/+	+
<i>Salmonella typhimurium</i> (14024)	+++	red	yellow	+	+
<i>Shigella sonnei</i> (9290)	+++	red	red	-	-
<i>Shigella flexneri</i> (12022)	+++	red	red	-	-

The vibrant M, Millipore, and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. Detailed information on trademarks is available via publicly accessible resources.
© 2018 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

The life science business of Merck KGaA, Darmstadt, Germany
operates as MilliporeSigma in the US and Canada.



References:

1. M. Cristina, et al., Salmonella screening studies, *J. Microbiol. Methods* 8, 227 (1988)
2. E.S. Sulkin, J.C. Willett, A Triple Sugar-Ferrous Sulphate Medium for use in identification of enteric organisms, *J. Lab. Clin. Med.* 25, 649 (1940)
3. A.A. Hajna, *J. Bact.* 49, 516 (1945); H.J. Kolmos, *J. Schmidt, Acta path. microbiol. immunol. scand. Sect. B* 95, 85 (1987)
4. C. Vanderzant, D. Splitstoesser, (Eds.), *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. APHA, Washington D.C. (1992)
5. R. Marshall (Ed.), *Standard Methods for the Examination of Dairy Products*, 16th ed., APHA, Washington, D.C. (1992)
6. Finegold and Baron, Bailey and Scotts Diagnostic Microbiology, 7th ed., The C.V. Mosby Co., St. Louis (1986)
7. A.E. Greenberg, R.R Trussell, L.S. Clesceri (Eds.), *Standard Methods for the Examination of Water and Wastewater*, 16th ed., APHA, Washington, D.C. (1985)
8. J. MacFaddin, *Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria*, Vol. 1, Williams and Wilkins, Baltimore (1985)
9. International Organization for Standardization (ISO), *Draft ISO/DIS 6579* (1993)
10. Bundesgesundheitsamt: *Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG*. Beuth Verlag Berlin, Köln
11. Deutsches Arzneibuch, 10. Auflage, Chapter VIII, 10
12. DIN Deutsches Institut für Normung: *Untersuchung von Fleisch und Fleischerzeugnissen, Nachweis von Salmonellen (Referenzverfahren)*, DIN 10160
13. DIN Deutsches Institut für Normung e.V.: *Mikrobiologische Milchuntersuchung. Nachweis von Salmonellen. Referenzverfahren*
14. DIN 10181. *European Pharmacopeia II*, Chapter VIII, 10
15. International Organization for Standardization: *Meat and meat products. - Detection of Salmonella (Reference method). - International Standard ISO 3565* (1975).
16. United States Pharmacopeia XXIII, Chapter "Microbial Limit Tests" (1995)
17. J.M. Bulmash, M. Fulton, *J. Bact.*, 88. 1813 (1966)
18. P.R. Edwards W.H. Ewing, *Identification of Enterobacteriaceae*, 3rd Ed., Burgess Publishing Co. Minneapolis, USA (1972)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

The vibrant M, Millipore, and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. Detailed information on trademarks is available via publicly accessible resources.
© 2018 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

The life science business of Merck KGaA, Darmstadt, Germany
operates as MilliporeSigma in the US and Canada.

