

## 44940 Triple Sugar Iron Agar (TSI Agar)

For the identification of Enterobacteriaceae acc. to Sulkin and Willett (1940), modified acc. to Hajna (1945). Recommended by the "Schweizerisches Lebensmittelbuch" 5<sup>th</sup> ed., chapter 56A.

### 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 37°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.

### 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 carbohydrates. Due to the building of acid during fermentation the pH falls. In case of oxidative decarboxylation of peptone alkaline products are built 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<sub>2</sub>) is detected by the presence of cracks or bubbles in the medium, when the accumulated gas escapes.

The H<sub>2</sub>S-positive bacteria reduce the sulfite in the culture medium to sulfide, which reacts with iron. Due to this reaction a black precipitate (FeS<sub>2</sub>) is formed.

Triple Sugar Iron Agar should be used in parallel with Urea Broth (Fluka 51463) to distinguish between Salmonella and Proteus species.

Some members of the Enterobacteriaceae and H<sub>2</sub>S producing Salmonella may not be H<sub>2</sub>S positive on Triple Sugar Iron Agar. Some bacteria may show H<sub>2</sub>S production on Kligler Agar (Fluka 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<sub>2</sub>S production.

Organism	Butt	Slant surface	H <sub>2</sub> S
Enterobacter aerogenes	AG	A	-
Enterobacter cloacae	AG	A	-
Escherichia coli	AG	A	-
Citrobacter	AG	NC	+
Klebsiella	AG	A	-
Proteus vulgaris	AG	A	+
Proteus mirabilis	A or AG	A*	+
Proteus morganii	A or AG	NC or ALK	-
Proteus rettgeri	A (ALK)	NC	-
Shigella dysenteriae	A	NC or ALK	-
Shigella sonnei	A	NC or ALK	-
Shigella schmitzii	A	NC	-
Shigella boydii	A	NC	-

<i>Shigella flexneri</i>	A	NC	-
<i>Alkaescens</i>	A	ALK or A*	-
<i>Dispar</i>	A	A	-
<i>Salmonella typhi</i>	A	NC or ALK	+
<i>Salmonella typhosa</i>	A	NC	+
<i>Salmonella choleraesuis</i>	AG	NC	-
<i>Salmonella pullorum</i>	AG	NC	+
<i>Salmonella gallinarum</i>	A	NC	+
<i>Salmonella paratyphi</i>	AG	NC or ALK	-
<i>Salmonella enteritidis</i>	AG	NC or ALK	+
<i>Salmonella typhimurium</i>	AG	NC or ALK	+
<i>Klebsiella pneumoniae</i>	A or AG	NC	-
<i>Pseudomonas aeruginosa</i>	NC	NC (may be pigment production)	-
<i>Alteromonas faecalis</i>	NC	NC	-

AG = acid (yellow) and gas formation

A = acid (yellow)

NC = no change

ALK = alkaline (red)

+ = hydrogen sulphide (black)

- = no hydrogen sulphide (no black)

\* On Kligler Agar (Fluka 60787): NC

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

Organisms (ATCC)	Growth	Slant surface	Butt	Gas	H <sub>2</sub> S
<i>Citrobacter freundii</i> (8090)	+++	acidic, yellow	acidic, yellow, black	+	+
<i>Enterobacter aerogenes</i> (13048)	+++	acidic, yellow	acidic, yellow	+	-
<i>Escherichia coli</i> (25922)	+++	acidic, yellow	acidic, yellow	+	-
<i>Klebsiella pneumoniae</i> (13883)	+++	acidic, yellow	acidic, yellow	+	-
<i>Proteus mirabilis</i> (14153)	+++	alkaline, red, black	acidic, yellow, black	+	+
<i>Proteus vulgaris</i> (13315)	+++	alkaline, red	acidic, yellow, black	-	+
<i>Salmonella paratyphi A</i> (9150)	+++	alkaline, red	acidic, yellow	+	-
<i>Salmonella typhi</i> (6539)	+++	alkaline, red	acidic, yellow, black	-	+
<i>Salmonella typhimurium</i> (14028)	+++	alkaline, red	acidic, yellow, black	+	+
<i>Shigella flexneri</i> (12022)	+++	alkaline, red	acidic, yellow	-	-

#### 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. Splittstoesser, (Eds.), *Compendium of Methods for the Microbiological Examination of Foods*, 3<sup>rd</sup> ed. APHA, Washington D.C. (1992)
5. R. Marshall (Ed.), *Standard Methods for the Examination of Dairy Products*, 16<sup>th</sup> ed., APHA, Washington, D.C. (1992)
6. Finegold and Baron, *Bailey and Scotts Diagnostic Microbiology*, 7<sup>th</sup> 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*, 16<sup>th</sup> 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*, 3<sup>rd</sup> Ed., Burgess Publishing Co. Minneapolis, USA (1972)