TRIPLE SUGAR IRON (TSI) AGAR USP
TRIPLE SUGAR IRON (TSI) AGAR ISO

Powdered and ready to use media for the differentiation of *Enterobacteriaceae*

TSI USP - Typical formula (g/l)

Tryptone	10.000
Peptone	10.000
Lactose	10.000
Sucrose	10.000
Glucose	1.000
Ferrous Ammonium Sulphate	0.200
Sodium Chloride	5.000
Sodium Thiosulphate	0.200
Agar	14.000
Phenol Red	0.025

TSI ISO - Typical formula (g/l)

Beef Extract	3.000
Yeast Extract	3.000
Peptone	20.000
Lactose	10.000
Sucrose	10.000
Glucose	1.000
Iron (III) Ammonium Citrate	0.300
Sodium Chloride	5.000
Sodium Thiosulphate	0.300
Agar	12.500
Phenol Red	0.024

DIRECTIONS FOR POWDERED MEDIA

Suspend 60.4g of TSI USP or 65 g of TSI ISO in 1000ml of cold distilled water. Heat to boiling with frequent agitation, distribute into test tubes and sterilise by autoclaving for 15 minutes at 121°C. Allow to solidify in a slanting position so that a butt of 2 cm is formed.
TSI USP: Final pH 7.3 ± 0.2
TSI ISO: Final pH 7.4 ± 0.2

DESCRIPTION

Triple Sugar Iron Agar USP is prepared according to the formulation recommended by USP. Triple Sugar Iron Agar ISO is prepared according to the formulation recommended by ISO 6579 and by EP. The media, with slight differences in the formulations (peptones, ferric compounds and thiosulphate concentration) are used for the differentiation of *Enterobacteriaceae* cultivated on selective or moderately selective media on the basis of lactose, glucose and sucrose fermentation, and the production of hydrogen sulphide and other gases. For details regarding the biochemical reactions involved and its working principle, see the technical sheet of Kligler Iron Agar; the only difference between the two media lies in the addition of sucrose as a third carbohydrate in Triple Sugar Iron Agar. The growth characteristics of *Enterobacteriaceae* on TSI media are slightly different from those on Kligler Iron Agar, because some non-lactose-fermenting bacteria may ferment sucrose, giving a different slope reaction.

TECHNIQUE

To perform the test, inoculate a test tube of TSI Media by stabbing the butt with a needle, which has touched the surface of the centre of a colony, and streak the slope after stabbing. After 18-48 hours of incubation, examine for the following: only acid, or acid and gas in the butt; acid or alkaline or no change on the slope; H_2S production. Acidification is shown by a yellow colour, gas production by the formation of small bubbles in the agar; H_2S production by blackening of the medium.
The table below shows the growth characteristics of some microorganisms on TSI Media.

Microorganisms	Slope	Butt	Gas	H_2S
Escherichia	A(K)	A	+(–)	–
Shigella	K	A	–	–
S. typhi	K	A	–	+ (–)
Salmonella spp.	K	A	+(–)	+++(–)
Citrobacter	K(A)	A	+	+++
Edwardsiella	K	A	+	+++
Klebsiella	A	A	++	–
Enterobacter spp.	A	A	++	–
Serratia	K o A	A	–	–
Proteus vulgaris	A(K)	A	+	+++
P.mirabilis	K(A)	A	+	+++
P. morganii	K	A	(–)+	–
P. rettgeri	K	A	–	–
Providencia	K	A	+ o –	–

K = alkaline (red)
A = acid (yellow)
Gas + = bubbles in the agar slope
H_2S + = blackening
Symbols enclosed in parenthesis indicate occasional reactions

USER QUALITY ASSURANCE (37°C-3 days)
E.coli ATCC 25922: yellow slope and butt, gas positive, H_2S negative
S.typhimurium ATCC 14028: red slope, yellow butt, gas and H_2S positive

STORAGE
Dehydrated medium: 10-30°C
User prepared tubes : 7 days at 2-8°C

REFERENCES
• European Pharmacopoeia, 3rd ed. 2001 Supplement
• ISO 6579:1993 - Microbiology - General guidance on methods for the detection of Salmonella. 1993-09-01
• U.S. Pharmacopoeia 24, NF 19 (2000)

PACKAGING

4021412	**Triple Sugar Iron Agar USP**	**500g (8.3 l)**
402141S2	**Triple Sugar Iron Agar ISO**	**500g (7.7 l)**
552141	**Triple Sugar Iron Agar USP**	**20 ready to use slanted tubes**