

Triple sugar iron agar (ISO)

Code 84698.0500

Also known as

TSI agar

Intended use

For the differentiation of *Enterobacteriaceae* and for the confirmation test of *Salmonella* (ISO 6579).

Formula* - Composition in g/L

Meat extract.....	3.000
Yeast extract.....	3.000
Peptone.....	20.000
Lactose.....	10.000
Sucrose.....	10.000
Glucose.....	1.000
Iron (III) citrate.....	0.300
Sodium chloride.....	5.000
Sodium thiosulphate.....	0.300
Agar.....	12.500
Phenol Red.....	0.024

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

Final pH 7.4 ± 0.2 at 25 °C.

Instructions for preparation

Dissolve 65 g in 1 litre of purified water by bringing to the boil with frequent shaking. Dispense the medium into test tubes in quantities of 10 ml. Sterilise in the autoclave at 121 °C for 15 minutes.

Principle of the method and general information

Triple sugar iron agar is recommended for the differentiation of *Enterobacteriaceae* by their ability to ferment glucose, lactose and sucrose and to produce hydrogen sulphide. The current formulation meets the requirements of ISO 6579 for performing the identification test for *Salmonella* colonies confirmation. Peptones provide nitrogen, carbon, essential amino acids, vitamins and minerals required for organism growth. Lactose, sucrose and glucose are fermentable carbohydrates and phenol red dye acts as an indicator of pH modifications. Iron (III) citrate serves as the indicator by turning the butt black in the presence of free hydrogen sulphide gas produced by the reduction of sodium thiosulphate.

Instruction for use

For laboratory use only.

Inoculate a test tube of Triple sugar iron agar 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 at 37°C, examine for the following:

- only acid, or acid and gas in the butt;
- acid or alkaline or no change on the slope;
- H₂S production.

Acidification is shown by a yellow colour, gas production by the formation of small bubbles in the agar, H₂S production by blackening of the medium.

Interpret the changes in the medium as follows.

Butt

- yellow glucose positive (fermentation of glucose)
- red or unchanged glucose negative (no fermentation of glucose)
- black formation of hydrogen sulphide
- bubbles or cracks gas formation from glucose

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Slant surface

- yellow lactose and/or sucrose positive (lactose and/or sucrose used)
- red or unchanged lactose and sucrose negative (neither lactose nor sucrose used)

Typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and (in about 90 % of the cases) formation of hydrogen sulfide (blackening of the agar)

Limitations

- Sucrose is included in the formula to eliminate certain sucrose-fermenting, non-lactose fermenting bacteria (e.g. *Proteus* and *Citrobacter* spp.)
- Padron and Dockstader found not all H₂S positive *Salmonella* are positive on TSI.
- To inoculate Triple sugar iron agar use a needle, do not use the loop. While stabbing butt, mechanical splitting of medium occurs, causing a false positive result for gas production.
- Fishtail slant and stab but; order may be reversed according to preference.

Quality Control

Physical characteristics:

Appearance of powder	Pinkish, fine, homogeneous, hygroscopic powder
Appearance of prepared medium	Red-orange, limpid
pH (25°C)	7.4 ± 0.2

Microbiological characteristics:

Test strains	Incubation T° / t / At.	Inoculation method	Growth characteristics			
			slant	butt	gas	H ₂ S
<i>E. coli</i> ATCC 25922	37°C / 24 h / AE	ST	A	A	+	-
<i>P. rettgeri</i> ATCC 39944	37°C / 24 h / AE	ST	K	A	-	-
<i>S. Enteritidis</i> NCTC 5188	37°C / 24 h / AE	ST	K	A	+	+
<i>S. Typhimurium</i> ATCC 14028	37°C / 24 h / AE	ST	K	A	+	+
<i>S. Gallinarum</i> CB 506	37°C / 24 h / AE	ST	K	A	+	+
<i>C. freundii</i> ATCC 8090	37°C / 24 h / AE	ST	A	A	+	+

Notes

AE: aerobic incubation

Inoculation method: ST, stabbing the butt and streaking the slant surface.

A: acid reaction (yellow colour); K: alkaline reaction (red-violet colour); GAS: bubbles formation; H₂S: blackening of the medium; NC: no colour change. ATCC is a registered trade mark of American Type Culture Collection; CB: strain obtained from Laboratory culture collection.

References

- ISO 6579:2002 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *Salmonella* spp.
- MacFaddin, J. F. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
- Padron, A.P. and W.B. Dockstader. 1972. Appl. Microbiol. 23, 1107

Storage conditions

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+10°C to 30 °C and <60% RH).

Ordering information

84698.0500 Triple sugar iron agar (ISO) Bottle of 500 g