TSI/Agar
(Triple Sugar Iron)

356-4384

DEFINITION
Medium used for the biochemical characterization of enterobacteria in general and of Salmonella in particular in the analysis of food products, in screening for contamination of non-sterile pharmaceutical products and waters.

STANDARDS
FOOD MICROBIOLOGY
- **NF EN ISO 10272-1 (April 2006):** Food microbiology - Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection method. (IC: V 08-026)
- **NF ISO 8914 (May 1991):** Microbiology - General guidelines for the detection of *Vibrio parahaemolyticus* (IC: V 08-024)
- **FIL 93B (1995):** Milk and dairy products - Detection of *Salmonella*
- **NF EN ISO 6579 (December 2002):** Food microbiology - Horizontal method for the detection of *Salmonella* spp.

WATER
- **V45-111 (July 1985):** Fishery products - Detection of *Vibrio parahaemolyticus* in shellfish waters and in live seawater shellfish (IC: V45-111)
- **ISO 19250 (July 2010):** Water quality - Detection and enumeration of *Salmonella*

PRINCIPLE
The principle of the medium relies on the ability or otherwise of *enterobacteria* to ferment glucose (with or without gas production), lactose, and saccharose and to reduce sulfates to sulfides which, in the presence of iron, produces a black precipitate of iron sulfide.

PRESENTATION
Dehydrated
500 g **code 356-4384**

STORAGE
- +15-25°C, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Meat extract	3 g
Yeast extract	3 g
Peptone	20 g
Sodium chloride	5 g
Lactose	10 g
Saccharose	10 g
Glucose	1 g
Ferric ammonium sulfate	300 mg
Phenol red	24 mg
Sodium thiosulfate (anhydrous)	300 mg
Agar	11 g
Distilled water	1,000 ml

Final pH (25°C) = 7.4 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED)
(non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- Test tubes (16 x 160 mm) with autoclave proof stoppers
- Sterile Pasteur pipettes (code 355-0751) or inoculating loops
- Thermostatically-controlled incubator or incubation room, precise to ±1°C
- Autoclave
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM
Always shake well before use.
Dissolve 63.5 g of powder in 1 liter of distilled water, mix until a homogenous suspension is obtained. Heat gently, swirling frequently, then bring to the boil until completely dissolved.
Dispense 10 ml per tube and sterilize in autoclave at 115°C ± 1°C for 15 minutes.
Leave to rest in an inclined position so as to obtain a 2.5 cm deep sediment.

Reconstitution ratio: 63.5 g/l
500 g of powder makes 7.9 liters of medium.

Bio-Rad, 3 boulevard Raymond Poincaré • 92430 Marnes-la-Coquette
Tel. : +33 (0)1 47 95 60 00 • Fax : +33 (0)1 47 41 91 33
www.foodscience.bio-rad.fr

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PROTOCOL

• Preparation of samples

According to the standards applicable to the product concerned.

• Inoculation and incubation

From each dish of selective medium, collect the recommended number of colonies and re-isolate on an ordinary Nutrient Agar (code 356-4485) in order to obtain pure strains.
Incubate at 37°C ± 1°C for 24 hours.

Using the pure strains, streak-inoculate the slope of the medium and stab the pellet with a depth inoculation using a previously flame-sterilized Pasteur pipette or inoculating loop.

READING AND INTERPRETATION

The resulting phenomena can be interpreted as follows:

• Sediment

- Yellow: glucose-positive (fermentation of glucose)
- Red or unaltered: glucose-negative
- Black: formation of hydrogen sulfide
- Bubbles or cracking: production of gas from glucose

• Slope

- Yellow: lactose-positive and/or saccharose-positive (utilization of lactose and/or saccharose)
- Red or unaltered: lactose- and/or saccharose-negative.

Refer to the table in the margin:

[1] The pellet must be yellow (except in the case of H_2S release causing intense blackening), since all enterobacteria ferment glucose.

[2] Apart from *E. agglomerans.*

[3] Only small quantities of gas are producedby *Proteus, Providencia* and *Serratia* strains.

[4] d: variable results depending on the strains.

Table for reading and interpretation

Bacteria	Lactose and/or saccharose slope	Gas sediment [1]	H_2S
Citrobacter	+	+	+ **[-]**
Edwardsiella	-	+	+
Hafnia	- +	+ + **[2]**	- -
Escherichia	+ **[-]**	+	-
E. coli (biotype A.d.)	**-**	-	-
Klebsiella	+	+ **[-]**	-
Levinea	-**or** +	+	-
Proteus • *vulgaris* • *mirabilis* • *morganii* • *rettgeri*	 + **or** - - **or** + - -	 + **or** - + **or** - + **or** - -	 + + - -
Providencia	-	- **[+]**	-
Salmonella sp. *S.typhi* *S.paratyphi A* *S. arizona*	- - - -	+ - + +	+ **traces** - +
Serratia	+ **or**	**-or** + **[3]**	-
Shigella	-	-	-
Yersinia	**d [3]**	-	-

PRECAUTIONS

- The time lapse between the end of preparation of the stock solution (or the 10^{-1} dilution in the case of a solid product) and the moment when the dilutions come into contact with the culture medium must not exceed 15 minutes.
- Comply with Good Laboratory Practice.

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PERFORMANCES/QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Biochemical characteristics observed over 24 hr at 37°C				
	Glc	Lac	Sac	Gaz	H_2S
Escherichia coli ATCC 25922	+	+	+	+	-
Citrobacter freundii ATCC 8090	+	+	-	+	+
Klebsiella pneumoniae ATCC 13883	+	+	+	+	-
Salmonella Enteritidis ATCC 13076	+	-	-	+	+
Shigella sonnei ATCC 25931	+	-	-	-	-
Pseudomonas aeruginosa ATCC 9027	-	-	-	-	-

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.
Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

TSI/*Enterobacteria*/*Salmonella*/Food products/Identification/Triple sugar/Ferric citrate/Medium

BIBLIOGRAPHY

HAJNA A.A. (1945): Triple Sugar Iron medium for the identification of the Intestinal group of bacteria. Journal of bacteriology 49: 516 - 517

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