TECHNICAL DATA SHEET

TSI AGAR (ISO 6579-1)

IDENTIFICATION OF *ENTEROBACTERIACEAE*

1 INTENDED USE

TSI (Triple Sugar Iron) Agar is used for the identification of enterobacteria by the rapid detection of the fermentation of lactose, glucose (with or without gas production) and of sucrose, as well as the production of hydrogen sulfide.

The typical composition corresponds to that defined in the standards NF EN ISO 6579-1 and NF EN ISO 19250 for the detection of *Salmonella* spp..

2 HISTORY

Hajna developed the formulation of this agar containing three sugars by adding sucrose to a two-sugar (lactose and glucose) medium of Kligler. The addition of sucrose increases the sensitivity of the medium by leading to the early detection of coliform bacteria which ferment lactose slowly and degrade sucrose more rapidly. The medium also favors the differentiation of certain *Proteus* strains (lactose-negative) which ferment sucrose within 24 hours of incubation.

3 PRINCIPLES

Sugar fermentation results in an acidification which makes phenol red (pH indicator) turn yellow).

The detection of bacteria fermenting only glucose is facilitated by decreasing the concentration of the sugar to 1/10 of that of lactose or sucrose, so that the small quantity of acid produced on the slant during fermentation is rapidly oxidized. This causes a rapid return to the red color or else a more pronounced re-alkalinization. The acid reaction (yellow color), on the contrary, is maintained in the depth of the agar, in the butt of the tube.

Bacteria fermenting lactose or sucrose will make the slant of the tube turn yellow.

Bacteria fermenting none of these sugars will not change the color of the medium.

The production of H_2S is revealed in the butt by the appearance of black iron sulfide, due to the reduction of thiosulfate in the presence of ferric citrate.

The production of gas (H_2, CO_2), resulting from sugar fermentation, is revealed by the appearance of bubbles or by the fragmentation of the agar.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Yeast extract ..... 3,0 g
- Meat extract..... 3,0 g
- Peptone ..... 20,0 g
- Sodium chloride ..... 5,0 g
- Lactose..... 10,0 g
- Sucrose ..... 10,0 g
- Glucose ..... 1,0 g
- Sodium thiosulfate..... 0,3 g
- Iron (III) citrate ..... 0,3 g
- Phenol red ..... 24,0 mg
- Bacteriological agar..... 9,0 g

pH of the ready-to-use media at 25 °C : 7,4 ± 0,2.

5 PREPARATION

- Dissolve 61,6 g of dehydrated media (BK221) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Incline the tubes to obtain a butt 3 cm high and a slant.

✓ **Reconstitution : 61,6 g/L**

✓ **Sterilization : 15 min at 121 °C**

Note : When the medium will not be used within 8 days of its preparation, it is recommended to regenerate it in a boiling water bath and solidify it again in the correct position.

6 INSTRUCTIONS FOR USE

- Inoculate the butt by stabbing and the slant by streaking close together, using a suspected colony taken from a selective isolation medium.
- Incubate at 37 ± 1 °C for 24 ± 3 hours (caps loosened) to favor gas exchanges.

Note : For the analyze of water samples, incubate at 36 ± 2 °C for 24 ± 3 hours.

✓ **Inoculation : Central stab and streak on surface.**

✓ **Incubation : 24 h at 37 °C**

7 RESULTS

The use of one of the sugars in the medium results in acidification (phenol red turns yellow). Alkalinization is shown by a dark red color. Hydrogen sulfide production from thiosulfate is detected by a black color due to the formation of iron sulfide in the presence of ferric citrate.

TSI Agar supplies four types of information:

Glucose fermentation :

- Butt red : glucose not fermented
- Butt yellow : glucose fermented

Lactose and/or sucrose fermentation :

- Slant red : lactose and sucrose not fermented
- Slant yellow : lactose and/or sucrose fermented

Gas production :

- Production of gas bubbles in the butt.

H_2S formation :

- Formation of a black color between the butt and the slant, or along the inoculation stab.

Typical reactions are in the following table :

Microorganisms	Slant	Butt		H_2S
	Lactose / Sucrose	Glucose	Gas	
Salmonella Typhi (2)	-	+	-	+
Salmonella Paratyphi *A* (2)	-	+	+	-
Salmonella Choleraesuis (2)	-	+	+	-
Salmonella Pullorum (2)	-	+	+	+
Salmonella Paratyphi *B* (2)	-	+	+	+
Salmonella Typhimurium (2)	-	+	+	+
Salmonella Enteritidis (2)	-	+	+	+
Salmonella Gallinarum (2)	-	+	-	+
Shigella dysenteriae	-	+	-	-
Shigella flexneri	-	+	-	-
Shigella sonnei	-	+	-	-
Shigella boydii	-	+	-	-
Proteus vulgaris	+	+	[-]	+
Proteus mirabilis	-	+	+	+
Proteus morganii	-	+	+	-
Proteus rettgeri	-	+	-	-

Microorganisms	Slant	Butt		H_2S
	Lactose / Sucrose	Glucose	Gas	
Serratia marcescens	-	+	-	-
Enterobacter hafniae	-	+	+	-
Enterobacter aerogenes	+	+	+	-
Enterobacter cloacae	+	+	+	-
Escherichia coli (1)	+	+	+	-
Citrobacter freundii	+	+	+	+
Klebsiella pneumoniae	+	+	+	-
Alcaligenes faecalis	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-
Yersinia enterocolitica	-	-	-	-

(1) Some strains of *Escherichia coli* ferment lactose only very late in growth.
(2) In the case where interpretation may suggest the presence of salmonellae, it is possible to use TSI medium to detect β-galactosidase, urease and lysine decarboxylase.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : pinkish powder, free-flowing and homogeneous.
Prepared media : orange-red agar.

Typical culture response after 24 hours of incubation at 36 °C (FD T90-461) :

Microorganisms		Growth	Slant	Butt	H_2S	Gas
Salmonella Enteritidis	WDCM 00030	Good, score 2	Red	Yellow	+	+
Pseudomonas aeruginosa	WDCM 00026	Good, score 2	Red	Red	-	-

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
The expiration date is indicated on the label.

Prepared media in tubes not inclined (*) : 180 days at 2-8 °C.
Prepared media in inclined tubes (*) : 8 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :
500 g bottle BK221HA

11 BIBLIOGRAPHY

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Hajna, A.A. 1945. Triple Sugar Iron Medium for the Identification of the Intestinal groups of Bacteria. J. Bact. 49: 516-517.

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NF ISO 19250. Juin 2013. Qualité de l'eau. Recherche de *Salmonella* spp..

FD T90-461. Août 2016. Qualité de l'eau - Microbiologie - Contrôle qualité des milieux de culture.

NF EN ISO 6579-1. Avril 2017. Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche, le dénombrement et le sérotypage des Salmonella - Partie 1 : recherche des Salmonella spp..

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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TSI Agar

Identification of *Enterobacteriaceae*

Results :

Growth obtained after 24 hours of incubation at 37°C.