Triple Sugar Iron Agar

TSI-Agar

Culture medium proposed by SULKIN and WILLETT (1940) and modified by HAJNA (1945) for identifying Enterobacteriaceae.

This medium complies with the recommendations of the International Organization for Standardization (ISO) (1975), DIN Norm 10160 for the examination of meat and DIN Norm 10181 for the examination of milk. Its composition is equivalent to that recommended by the United States Pharmacopeia XXVI (2003), the European Pharmacopeia II and the German examination procedure for food acc. to § 35 LMBG.

Mode of Action

Degradation of sugar and accompanying acid production are detected by the pH indicator phenol red, which changes its colour from red-orange to yellow, on alkalinization it turns deep red. Thiosulfate is reduced to hydrogen sulfide by several species of bacteria, the hydrogen sulfide reacts with an iron salt to give black iron sulfide.

Typical Composition (g/litre)

Peptone from casein 10.0; peptone from meat 10.0; meat extract 3.0; yeast extract 3.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; D(+)glucose 1.0; ammonium iron(III) citrate 0.5; sodium thiosulfate 0.5; phenol red 0.024; agar-agar 12.0.

Preparation

Suspend 65 g/litre, dispense into test tubes, autoclave (15 min at 121 °C). Allow the medium to solidify to give slant-agar tubes.

pH: 7.4 ± 0.2 at 25 °C.

The prepared medium is clear and red.

Experimental Procedure and Evaluation

Streak the pure culture under investigation on the sloped surface and inoculate the butt of the same tube by a central stab.

Incubation: up to 48 hours at 35 °C aerobically.

Microorganisms	Butt	Slant surface	H_2S-production	
S.-typhosa	S	OA	+	Only in the upper part of the butt, often accompanied by ring formation, may take 48 hours
S. paratyphi A	SG			
S. choleraesuis	SG	OA	-	
S. pullorum	SG	OA	+	
S. paratyphi B	SG	OA	+	Butt black
S. typhimurium	SG	OA	+	
S. enteritidis	SG	OA	+	
S. gallinarum	S	OA	+	
Sh. dysenteriae type 1	S	OA	-	
Sh. schmitzii	S	OA	-	
Sh. boydii	S	OA	-	
Sh. flexneri	S	OA	-	
Sh. flexneri type 6 var. Newcastle	S/SG	OA	-	
Alkalescens	S	A/S***	-	
Sh. sonnei	S	S	-	
Dispar	S	S	-	
Ent. aerogenes	SG	S	-	
Ent. cloacae	SG	S	-	
E. coli	SG	S	-	
Citrobacter	SG	S	+	
Klebsiella	SG	S	-	
Pr. vulgaris	SG**	S***	+	
Pr. mirabilis	SG**	A	+	Dirty black-green
Pr. morganii	SG**	OA	-	
Pr. rettgeri	S(A)	OA	-	
K. pneumoniae	S/SG	OA	-	
Ps. aeruginosa	OA	OA*	-	
Al. faecalis	OA	OA		

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Abbreviations:

A	=	Colour changes to red due to alkalinization
O A	=	No change in the original colour of the culture medium or colour changes to red due to alkalinization
S	=	Colour changes to yellow due to acid production
S G	=	Colour changes to yellow and gas is produced
+	=	Blackening due to H_2S production
-	=	No blackening
*		May be due to pigment production
*		Some strains: A, possibly without gas production
*		On KLIGLER (double sugar iron agar): OA

Literature

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. Beuth Verlag Berlin, Köln.

Deutsches Arzneibuch, 10. Auflage, Chapter VIII, 10.

DIN Deutsches Institut für Normung: Untersuchung von Fleisch und Fleischerzeugnissen. - Nachweis von Salmonellen (Referenzverfahren). - DIN 10160.

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung. Nachweis von Salmonellen. Referenzverfahren. - DIN 10181.

European Pharmacopeia II, Chapter VIII, 10.

HAJNA, A.A.: Triple-Sugar Iron Medium for the identification of the intestinal group of bacteria. - J. Bact., 49; 516-517 (1945).

International Organization for Standardization: Meat and meat products. - Detection of Salmonella (Reference method). - International Standard ISO 3565 (1975).

SULKIN, E.S., a. WILLETT, J.C.: A Triple Sugar-Ferrous Sulphate Medium for use in identification of enteric organisms. - J. Lab Clin. Med., 25; 649-653 (1940).

United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 2003.

Ordering Information

Product	Merck Cat. No.	Pack size
Triple Sugar Iron Agar	1.03915.0500	500 g

Quality control

Test strains	Growth	Butt	Slant surface
Escherichia coli ATCC 25922	good / very good	yellow	yellow
Citrobacter freundii ATCC 8090	good / very good	yellow and black	yellow
Enterobacter cloacae ATCC 13047	good / very good	yellow	yellow
Shigella flexneri ATCC 12022	good / very good	yellow	red
Salmonella typhimurium ATCC 14028	good / very good	yellow and black	red
Salmonella enteritidis ATCC 13076	good / very good	yellow and black	red
Proteus mirabilis ATCC 14153	good / very good	yellow and black	red and black
Proteus vulgaris ATCC 13315	good / very good	yellow and black	yellow