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Triple Sugar Iron Agar (T.S.I.)

DM224

Intended Use
A medium for the differentiation of Enterobacterales based on hydrogen sulphide production and fermentation of lactose, sucrose and dextrose.

Contents
See pack label.

Formulation*

Material:	Concentration in medium:
Peptone mixture	18.0g/litre
Yeast extract	3.0g/litre
Meat extract	4.0g/litre
Lactose	10.0g/litre
Sucrose	10.0g/litre
Dextrose	1.0g/litre
Sodium chloride	5.0g/litre
Ferric ammonium citrate	0.3g/litre
Sodium thiosulphate	0.3g/litre
Phenol red	0.025g/litre
Agar	14.0g/litre
Final pH: 7.4 ± 0.2	

Storage and shelf life
All dehydrated culture media containers should be kept tightly closed and stored in a dry place at 10 to 25°C until the expiry date shown on the pack label.

Precautions
For *in vitro* diagnostic use only. Observe approved hazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to Product Safety Data sheet (available on request or via MAST® website).

Materials required but not provided
Standard microbiological supplies and equipment such as loops, MAST® selective supplements, swabs, applicator sticks, incinerators and incubators, etc., as well as serological and biochemical reagents and additives such as blood.

Procedure
1. Refer to pack label for quantities and volumes required. Prepare MAST® Triple Sugar Iron Agar (T.S.I.) (DM224D) by suspending the powder in distilled or deionised water. For sachet packs, dissolve the entire contents of the sachet in the volume shown on the label.
2. Boil to dissolve completely.
3. Mix well and distribute into suitable containers (i.e. tubes or bottles).
4. Autoclave at 121°C (15 p.s.i.) for 15 minutes.
5. Allow to set as slopes with butts approximately 3.5cm long.
6. From clinical specimens or food samples obtain a pure culture of the organism to be tested.
7. Using an inoculating needle, inoculate slopes by stabbing the centre of the medium into the deep of the tube to within 3 to 5mm from the bottom.
8. Withdraw the inoculating needle, and streak the surface of the slant.
9. Loosen the lid/closure on the container before incubating.
10. A Urea Agar slope (DM228D) should be inoculated in parallel to aid differentiation of *Proteus* spp and certain other organisms.
11. Incubate for 18 to 48 hours at 35 to 37°C.

Interpretation of results
After incubation read tubes for acid production of slope/butt, gas and hydrogen sulphide reactions. An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose, lactose and/or sucrose. An alkaline slant-alkaline butt (red/red) indicates that neither dextrose nor lactose was fermented. Cracks or bubbles in the medium indicate gas production. A black precipitate in the butt indicates hydrogen sulphide production.

Quality control
Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate expected performance. Do not use the product if the result with the control organism is incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain.

Test Organisms	Slope	Butt	Gas	H_2S
Escherichia coli ATCC® 25922	A(K)	A	+(-)	-
Shigella sonnei ATCC® 25931	K	A	-	-
Proteus mirabilis ATCC® 29906	K(A)	A	+	+++
Klebsiella pneumoniae ATCC® 13883	A	A	++	-

A= Acid, K= Alkaline, () = indicates occasional reactions.

References
Bibliography available on request.

IFU354 GB 08/20 V5

*Formulation may change to meet performance criteria