Spectrum For Diagnostic Industries

Triple Sugar Iron Agar

REF.	Pack size
651 01 100	100 gm
651 01 500	500 gm

Intended Use

For the identification of gram-negative enteric bacilli such as Escherichia coli isolated from stool , sputum and sterile urine samples on the basis of three sugar fermentations and hydrogen sulphide production.

Background

In 1940,Sulkin and Willett described a triple sugar ferrous sulfate medium for use in identification of enteric organisms. Hajna developed the formulation for TSI Agar by adding sucrose to the double sugar (glucose and lactose) formulation of Kligler Iron Agar. When these carbohydrates are fermented, the resulting production of acid is detected by the phenol red indicator.The color changes that result are yellow for acid production and red for alkalinization.Triple Sugar Iron Agar is recommended for differentiation of enteric, Gram-negative bacilli from dairy samples and food products.

Principle

Peptone,Tryptone, yeast extract and beef extract provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc.Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and Dextrose are the fermentable carbohydrates.Sodium thiosulphate and ferric or ferrous ions make H_2S indicator system. Phenol red is the pH indicator.Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow.

Components	gm/Liter
Beef extract	3.0
Peptone	10.0
Yeast extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous sulphate	0.2
Sodium chloride	5.0
Sodium thiosulphate	0.30
Phenol red	0.024
Sodium Thiosulphate	0.3
Agar	12.0

Final pH (at 25°C) 7.4 ± 0.2

Preparation, Storage and Stability

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.Store the prepared medium at 2-8°C. After the desired amount has been taken out, replace the cap tightly to protect from hydration.

Procedure

1. Suspend 64.52 g in 1 litre of distilled water and mix well.
2. Heat with frequent agitation and boil for one minute to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C for 15 minutes.
4. Allow the medium to set in sloped form with a butt about 1 inch long.

Quality Control Appearance

1- Dehydrated Appearance : Light yellow to pink homogeneous free flowing powder

2- Prepared Appearance : Pinkish red coloured clear to slightly opalescent gel

3- Cultural Response : after 18-24 hours at 30-35°C

Organisms (ATCC)	Growth	slant	Butt	Gas	H_2S
Escherichia coli	Good	A	A	+	-
Proteus mirabilis	Good	K	A	-	+
Pseudomonas aeruginosa	Good	K	K	-	-
Salmonella typhimurium	Good	K	A	+	+
Shigella flexneri	Good	K	A	-	-

Key:
A=Acidic, yellow K=Alkaline, no change

Interpretation of the results

1- An alkaline slant (red),acid butt (yellow) indicates fermentation of dextrose only.
2- An acid slant (yellow),acid butt (yellow) indicates fermentation of dextrose, lactose and/or sucrose.
3- An alkaline slant (red),alkaline butt (red) indicates dextrose or lactose were not fermented (non-fermenter).
4- Cracks, splits, or bubbles in medium indicate gas production.
5- A black precipitate in butt indicates hydrogen sulfide production.

Precautions

1- Do not use inoculating loop to inoculate a tube of Triple Sugar Iron Agar. While stabbing butt, mechanical splitting of medium occurs, causing a false positive result for gas production.
2- TSI agar must be read within the 18-24 hour stated incubation period to avoid false results.
3- Not all H_2S positive Salmonella are positive on TSI.

Bibliography

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3. Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, M.D.
4. Marshall, R.T. (ed.). Standard methods for the examination of dairy products,16th ed. American Public Health Association,Washington,D.C.
5. Padron, A. P. and W. B. Dockstader. 1972. Selective medium for hydrogen sulfide production. Appl. Microbiol. 23:1107.

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