PRODUCT SPECIFICATION SHEET

Triple Sugar Iron Agar (Agar Medium M) (DM254E)

Intended Use

Triple Sugar Iron Agar (Agar Medium M) (DM254E) is recommended for identification of gram-negative enteric bacilli based on their ability to ferment dextrose, lactose and sucrose and produce hydrogen sulphide in compliance with EP.

Product Summary and Explanation

Russell described the use of two sugars in a medium to differentiate Gram-negative organisms of intestinal origin.[1] Lead or iron salts were added to Russell's medium to detect the presence of hydrogen sulfide. Kligler added lead acetate to Russell's Double Sugar Agar, resulting in a medium capable of differentiating typhoid, paratyphoid, and dysentery.[2,3] Kligler Iron Agar is a modification of this medium developed, using Phenol Red as an indicator and iron salts to detect hydrogen sulfide. Sulkin and Willett described a triple sugar ferrous sulfate medium for use in identification of enteric organisms.[4] Triple Sugar Iron Agar is essentially the formula originally described by Sulkin and Willett.[4] Hajna developed the formulation for TSI Agar by adding sucrose to the double sugar (dextrose and lactose) formulation of Kligler Iron Agar.[5] Addition of sucrose increased the sensitivity of the medium by facilitating the detection of sucrose-fermenting bacilli, as well as lactose and/or dextrose fermenters. This medium is cited as Agar Medium M, is recommended for identification and differentiation of *Enterobacteria* by European Pharmacopoeia.[6]

Principles of the Procedure

Triple Sugar Iron Agar (Agar Medium M) contains peptones (casein and Beef) and beef extract which provides nitrogen, carbon, and amino acids required for organism growth. Yeast extract is a source of B complex vitamins. Glucose monohydrate, lactose and sucrose are fermentable sugars. Sodium thiosulphate and ferric ions make H_2S indicator system. Sodium thiosulphates are also inactivators of halogens and can minimize its toxicity in the testing sample, if any during microbial limit tests. Phenol red is the pH indicator. Organisms that ferment glucose monohydrate produce a variety of acids, turning the colour of the medium from red to yellow. Large amounts of acids are liberated in butt (fermentation) than in the slant (respiration). Oxidative decarboxylation of peptones by growing bacteria also form alkaline products and these alkaline products neutralize the large amounts of acid present in the butt. Thus the appearance of an alkaline (red) slant and an acid (yellow) butt after incubation indicates that the organism is a glucose fermenter, but is unable to ferment lactose and/or sucrose. Bacteria that ferment lactose or sucrose (or both), in addition to glucose, produce large amounts of acid enables no reversion of pH in that region and thus bacteria exhibit an acid slant and acid butt. Gas production (CO_2) is detected by the presence of cracks or bubbles in the medium, when the accumulated gas escapes. Thiosulphate is reduced to hydrogen sulphide by several species of bacteria and H_2S combines with ferric ions of ferric salts to produce the insoluble black precipitate of ferrous sulphide. Reduction of thiosulphate proceeds only in an acid environment and blackening usually occurs in the butt of the tube.

Formula / Liter

Ingredients	Gms / Litre
Peptones (casein and Beef)	20.00
Beef extract	3.00
Yeast extract	3.00
Lactose monohydrate	10.00
Sucrose	10.00
Glucose monohydrate	1.00
Sodium chloride	5.00
Ferric ammonium citrate	0.30
Sodium thiosulphate	0.30
Phenol red	0.025
Agar	12.00
Final pH : 7.4±0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 64.02 g of the medium in one liter of purified /distilled water.
2. Heat to boiling to dissolve the medium completely. Mix well and distribute into test tubes.
3. Autoclave at 15lbs pressure (121°C) for 15 minutes or as per validated cycle.
4. Allow the medium to set in sloped form with a butt about 1cm long.

Quality Control Specifications

Dehydrated Appearance	Light yellow to pink, homogeneous, free flowing powder
Prepared Medium	Pinkish red coloured clear to slightly opalescent gel forms in tubes as slants
Reaction of 6.4% Solution	pH 7.4 ± 0.2 at 25°C
Gel Strength	Firm, compared to 1.2% Agar Gel

Growth Promotion Test

Growth Promotion is carried out as per European Pharmacopoeia

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Sr No	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	Slant	Butt	Gas	H_2S
1.	*Citrobacter freundii ATCC 8090*	50-100	Luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	Blackening of medium
2.	*Enterobacter aerogenes ATCC 13048*	50-100	Luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	no blackening of medium
3.	*Klebsiella pneumoniae ATCC 13883*	50-100	Luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	no blackening of medium
4.	*Proteus vulgaris ATCC 13315*	50-100	Luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	Blackening of medium
5.	*Salmonella Paratyphi A ATCC 9150*	50-100	Luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	no blackening of medium
6.	*Salmonella Typhi ATCC 6539*	50-100	Luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	Blackening of medium
7.	*Salmonella Typhimurium ATCC 14028*	50-100	Luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	Blackening of medium
8.	*Shigella flexneri ATCC 12022*	50-100	Luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	no blackening of medium
9.	*Escherichia coli ATCC 8739*	50-100	Luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction
10.	*Escherichia coli ATCC 25922*	50-100	Luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	no blackening of medium
11.	*Klebsiella pneumoniae ATCC 10031*	50-100	Luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. To inoculate, carefully touch only the center of an isolated colony on an enteric plated medium with a cool, sterile needle, stab into the medium in the butt of the tube, and then streak back and forth along the surface of the slant.
2. Several colonies from each primary plate should be studied separately, since mixed infections may occur.
3. Incubate with caps loosened at 35°C and examine after 18-24 hours for carbohydrate fermentation, gas production and hydrogen sulfide production.
4. Any combination of these reactions may be observed.
5. Do not incubate longer than 24 hours because the acid reaction in the slant of lactose and sucrose fermenters may revert to an alkaline reaction.
6. However for specific procedures, refer to appropriate references using Triple Sugar Iron Agar.

Results

1. Reactions produced by the unknown isolate should be compared with those produced by the known control organisms.
2. Carbohydrate fermentation is indicated by a yellow coloration of the medium. If the medium in the butt of the tube becomes yellow (acidic), but the medium in the slant becomes red (alkaline), the organism being tested only ferments dextrose (glucose).
3. A yellow (acidic) color in the slant and butt indicates that the organism being tested ferments dextrose, lactose and/or sucrose.
4. A red (alkaline) color in the slant and butt indicates that the organism being tested is a non-fermenter.
5. Hydrogen sulfide production results in a black precipitate in the butt of the tube.
6. Gas production is indicated by splitting and cracking of the medium.
7. For final identification, perform biochemical tests and other identification procedures with a pure culture of the organism. Consult appropriate references for further information[7-9]

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Padron and Dockstader8 found not all H_2S positive Salmonella are positive on TSI.
2. Sucrose is added to TSI to eliminate some sucrose-fermenting non-lactose fermenters, such as Proteus and Citrobacter spp.[7]
3. 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.[7]
4. Hydrogen sulfide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar.
5. Studies by Bulmash and Fulton[11] showed that the utilization of sucrose could suppress the enzymatic mechanisms responsible for H_2S production.
6. Sucrose is added to TSI to eliminate some sucrose-fermenting lactose-nonfermenters such as *Proteus* and *Citrobacter* spp.[1]
7. Further biochemical tests and serological typing must be performed for definite identification and confirmation of organisms.
8. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar. While stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production.[1]
9. A pure culture is essential when inoculating Triple Sugar Iron Agar. If inoculated with a mixed culture, irregular observations may occur.
10. Tubes should be incubated with caps loosened. This allows a free exchange of air, which is necessary to enhance the alkaline condition on the slant.[1]

Packaging
Product Name: Triple Sugar Iron Agar (Agar Medium M)
Product Code : DM254E
Available Pack sizes : 100gm / 500gm

References

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4. Sulkin, S. E., and J. C. Willett. 1940. A triple sugar-ferrous sulfate medium for use in identification of enteric organisms. J. Lab. Clin. Med. 25:649- 653.
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8. MacFaddin, J. F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
9. Ewing. 1985. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
10. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
11. Bulmash and Fulton. 1964. J. Bacteriol. *88*:1813.

Further Information

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DM254EPSS,Rev.00,Ver.00/01.02.2016