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TECHNICAL PRODUCT INFORMATION

Triple Sugar Iron Agar

Catalog No.: T1950 16 x 125 mm
Catalog No.: T1941 w/Slip-on Cap
Catalog No.: T1952 13 x 100 mm

INTENDED USE:

Triple sugar Iron Agar is used for the differentiation of microorganisms on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulfide production.

HISTORY/SUMMARY:

In 1911, Russell[1] described a combination of two sugars in an agar medium to differentiate gram negative intestinal microorganisms. Since then, a number of experimenters have improved upon this original medium to obtain larger amounts of information on the activity of enteric organisms from the one medium.
Kligler[2,3] added lead acetate to Russell's medium and reported successful differentiation of typhoid, paratyphoid and dysentary. Krunwiede and Kohn[4] added sucrose to Russell's formulation producing earlier detection of some coliforms.
Sulkin and Wellett[5], in 1940, proposed a formulation with three sugars, ferrous sulfate other components, and bromothymol blue as the indicator of fermentation.
Difco Laboratories[5] and Hajna experimented with a very similar medium with the added sucrose, but the indicator was phenol red.

The formulation used by NEL in the manufacture of Triple Sugar Iron Agar is essentially the formula of Sulkin and Wellett except for the peptones and the indicator. Ewin and Martin[8] recommend the use of TSI in conjunction with LIA and MIO medium for screening enteric organisms.

PRINCIPLES:

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Yeast Enriched Peptone provide the nitrogen, carbon, and vitamins required for organism growth. TSI Agar contains three carbohydrates dextrose, lactose and sucrose. A color change from a reddish salmon to a pale yellow indicates carbohydrate fermentation; this acid production is detected by the Phenol Red pH indicator. The production of gasses as by-products of fermentation will cause the agar to bubble or to break off and separate in the butt.

Sodium Thiosulfate is reduced to hydrogen sulfide, which reacts with an iron salt producing the black iron sulfide. Ferric Ammonium Citrate is the hydrogen sulfide (H_2S) indicator; this reaction is mainly in the butt of the tube. Sodium Chloride sustains the osmotic balance of the medium. Agar is the solidifying agent.

The nitrogenous components of the medium will neutralize small amounts of acid produced on the slant as a result of aerobic respiration of bacteria unless very large amounts of acid diffuse from the butt to the slant as a result of anaerobic fermentation of more than one sugar. Organisms which ferment only glucose do not produce enough acidity to turn the slant to yellow. Organisms which ferment the more abundant sucrose or lactose or both will generate enough acidity to diffuse to the slant and change it to yellow.

FORMULA:

INGREDIENTS PER LITER OF PURIFIED WATER	AMOUNT
Beef Extract	3.0 g
Yeast Extract	3.0 g
Gelatin/Meat Peptone	15.0 g
Casein/Meat Peptone	5.0 g
Lactose	10.0 g
Sucrose	10.0 g
Dextrose	1.0 g
Ferric Ammonium Citrate	0.2 g
Sodium Chloride	5.0 g
Sodium Thiosulfate	0.3 g
Agar	12.0 g
Phenol Red	24.0 mg

Final pH: 7.4 ± 0.2 @ 25□C

PRECAUTIONS:
This medium is for IN VITRO DIAGNOSTIC USE. It supports the growth of pathogens and should be handled with caution by adequately trained personnel under the supervision of a microbiologist. Media showing signs of deterioration or contamination must not be used. Media must be brought to room temperature before use.

STORAGE:
This media should be stored at 2-8°C, do not use beyond its expiration date.

SPECIMEN COLLECTION:
Triple Sugar Iron Agar must not be used as a medium for primary isolation of microorganisms from clinical specimens. Prior to its use inoculation of specimens on adequate media is necessary. Standard procedure for inoculation of clinical specimens must be followed.

PROCEDURE:
1. Using a straight inoculating needle, select an isolated colony from the culture plate.
2. Remove tube cap, stab needle into the butt of the medium.
3. Withdraw inoculating needle to the slant and streak half way up the slant surface.
4. Replace cap loosely on the tube.
5. Incubate aerobically overnight (18–24 hours) at 35 ± 2°C, observe and record reactions.
6. Good growth must occur in the butt and slant, or equivocal reactions will result.

NOTE:
1. On occasion, equivocal reactions may result and may be due to the selection of mixed organisms. Whenever there is a question, the purity of the culture should be confirmed by repeat testing with a pure isolate of the organism.
2. It is recommended to streak only half way up the slant to avoid reversion of sugar to an alkaline reaction (pink/red) in the thin tip of the slant.

Some of the typical reactions of Enterobacteriaceae on TSI are described as follows:

Organisms	Slant	Butt	H_2S	Gas
Escherichia coli	A (K)	A	-	+ (-)
Shigella	K	A	-	-
Salmonella sp.	K	A	+ (-)	+
Salmonella typhosa	K	A	+ (-)	-
Klebsiella	A	A	-	+
Enterobacter	A	A	-	+
Serratia	K or A	A	-	-
Proteus vulgaris	A (K)	A	+	+
Proteus mirabilis	K (A)	A	+	+
Providencia	K	A	-	-
Citrobacter	K	A	+ (-)	+
Edwardsiella	K	A	+	+

KEY:

A = Acid
K = Alkaline
+ = Positive
- = Negative
+/- = usually negative, positive, growth

QUALITY CONTROL:
It is recommended the user confirm the performance characteristics of this medium. Careful selection of organisms must be made to assure maximum testing success. Incubation temperatures and other environmental conditions must be controlled to further warrant effective test results.
Media is classified as Exempt in CLSI M22-A3.[9]

LIMITATIONS:
Although TSI provides valuable information in the presumptive identification of Enterobacteriaceae, a number of other tests must be performed before complete identification of some species is accomplished. If Salmonella or Shigella are isolated, serologic testing must be done in conjunction with biochemical testing.

A number of mutant species might deviate from the typical reactions above described, the use of a wide variety of carbohydrates and other tests then become necessary. The biochemical reactions must be considered as a whole when trying to identify these less common strains.

PERFORMANCE CHARACTERISTICS:
NEL approval for each lot of TSI Agar is based on results obtained on a number of tests, among them bacteriological performance with challenge organisms.

REFERENCES:

1) Journal Medical Research. 25:217, 1911
2) American Journal Public Health, 7:1042, 1917
3) Journal Experimental Medicine, 28:319, 1918
4) Journal Medical Research, 37:225, 1917
5) Journal Laboratory Clinical Medicine, 25:649, 1940
6) BBL & Difco Manual, Manual of Clinical Microbiology Media, 2003 Edition
7) Journal Bacteriology, 49:516, 1945
8) Manual of Clinical Microbiology, ASM, 2nd Edition, 1974
9) CLSI, M22-A3 Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard 3rd Edition, June 2004

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