CM 22478 - TRIPLE SUGAR IRON AGAR SLANT

INTENDED USE

Todifferentiategram-negative enteric bacilli based on carbohydrate fermentation.

PRODUCTSUMMARYANDEXPLANATION

TripleSugar Iron Agar was originally proposed by Sulkin and Willett and modified by Hajna for identifying Enterobacteriaceae. This medium complies with the recommendation of APHA, for the examination of meat and food products, for the examination of milk and dairy products and for microbial limit test for confirming the presence of Salmonellae and in the identification of gram-negative bacilli.

The triple sugar- iron agar test is designed to differentiate among the different groups or genera of the Enterobacteriaceae, which are all gram negative bacilli capable of fermenting glucose with the production of acid, and to distinguish them from other gram negative intestinal bacilli. This differentiation is based on the differences in carbohydrate fermentation patterns and hydrogen sulfide production by the various groups of intestinal organisms. Carbohydrate fermentation is indicated by the presence of gas and a visible color change of the pH indicator, phenol red. The production of hydrogen sulphide in the medium is indicated by the formation of a black precipitate that will blacken the medium in the butt of the tube. More amount of acids are liberated in butt (fermentation) than in the slant (respiration). Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone 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.

COMPOSITION

Ingredients	Gms / Ltr
Agar	15.000
Peptic digest of animal tissue	10.000
Casein enzymatic hydrolysate	10.000
Lactose	10.000
Sucrose	10.000
Sodium chloride	5.000
Yeast extract	3.000
Beef extract	3.000
Dextrose	1.000
Sodium thiosulphate	0.300
Ferric ammonium citrate	0.300
Phenol red	0.024

PRINCIPLE

Casein enzymatic hydrolysate,peptic digest of animal tissue, 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 thefermentable carbohydrates. Sodium thiosulphate and ferrous ions make H_2S indicator system. Phenol red is the pHindicator. The acid base indicator Phenol red is incorporated for detecting carbohydrate fermentation that is indicatedby the change in color of the carbohydrate medium from orange red to yellow in the presence of acids.

INSTRUCTION FOR USE

1. Usingfreshlygrown bacterial cultures, inoculate the tubes with an inoculating needle by stabbing the butt and streakingback and forth along the surface of the slant.
2. Incubate tubeswith loosened caps at 35 ± 2 °C in an aerobic atmosphere.
3. Examine tubesafter 18–24 h for growth and reactions.

QUALITY CONTROL SPECIFICATIONS

Appearance	:	Red coloured, clear to slightly opalescent gel forms in tubes as slants
Quantity of Medium	:	8 ml of medium in glass tube.
pH (at 25°C)	:	7.4±0.2

INTERPRETATION

Culturalcharacteristics observed after an incubation at 35-37°C for 18 - 24 hours.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Slant	Butt	Gas	H_2S
# Klebsiella aerogenes	13048	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction, no blackening of medium
Escherichia coli	25922	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction, no blackening of medium
Klebsiella pneumoniae	13883	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction, no blackening of medium
Proteus vulgaris	6380	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Positive reaction, blackening of medium
Shigella flexneri	12022	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Negative reaction, no blackening of medium
Salmonella typhi	19430	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Positive reaction, blackening of medium

Formerly known as *Enterobacter aerogenes*

PACKAGING:

Kitof10Ready-To-Use Slants containing 8 ml medium in each glass tube.

STORAGE

Onreceipt, store tubes in the dark at 2 – 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.
Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

DISPOSAL

Usermustensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

REFERENCES

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4. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
5. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.
6. Eaton A. D., Clesceri L. S. and Greenberg A. W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
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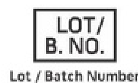

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For LabUse Only**