TMV 1625 – TRIPLE SUGAR IRON AGAR (VEG)

INTENDED USE

For identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and H_2S production.

PRODUCT SUMMARY AND EXPLANATION

Triple Sugar Iron Agar, is recommended for identification and differentiation of *Enterobacteria* by British Pharmacopoeia. It was originally proposed by Sulkin and Willett and modified by Hajna for identifying *Enterobacteriaceae*. This medium complies with the composition as specified in British Pharmacopoeia. It can also be used 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 *Salmonella* and in the identification of gram-negative bacilli.

Organisms that ferment glucose produce a variety of acids, turning the colour of the medium from red to yellow. More amount of acids is 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. Triple Sugar Iron Agar should be used in parallel with Urea Agar/ Broth to distinguish between *Salmonella* and *Proteus* species. The reactions can be summarized as follows:

Alkaline slant / acid butt-only glucose fermented

Acid slant / acid butt-glucose and sucrose fermented or glucose and lactose fermented or all the three sugars, glucose, lactose and sucrose fermented.

Bubbles or cracks present-gas production

Black precipitate present- H_2S gas production

COMPOSITION

Ingredients	Gms / Ltr
Veg. peptone	10.000
Veg. hydrolysate	10.000
Yeast extract	3.000
Veg. extract	3.000
Lactose monohydrate	10.000
Sucrose	10.000
Glucose monohydrate	1.000
Sodium chloride	5.000
Ferric ammonium citrate	0.200
Sodium thiosulphate	0.300
Phenol red	0.024
Agar	12.000

PRINCIPLE

Veg. hydrolysate Veg. peptones, yeast extract and Veg. extract provide nitrogenous and carbonaceous 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 ferrous ions make H_2S indicator system. Phenol red is the pH indicator.

INSTRUCTION FOR USE

- Dissolve 64.52 grams in 1000 ml distilled water.
- Heat to boiling to dissolve the medium completely.
- Mix well and distribute into test tubes. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes.
- Allow the medium to set in sloped form with a butt about 1inch long.

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QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Light yellow to pink homogeneous free flowing powder.
Appearance of prepared medium : Pinkish red coloured clear to slightly opalescent gel forms in tubes as slants.
pH (at 25°C) : 7.4±0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Slant	Butt	Gas	H_2S	Incubation Temperature	Incubation Period
Citrobacter freundii	8090	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Positive reaction	35-37°C	18-24 Hours
Klebsiella aerogenes	13048	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction	35-37°C	18-24 Hours
Klebsiella pneumoniae	13883	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction	35-37°C	18-24 Hours
Proteus vulgaris	13315	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Positive reaction	35-37°C	18-24 Hours
Escherichia coli	25922	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction	35-37°C	18-24 Hours

Salmonella Paratyphi A	9150	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction	35-37°C	18-24 Hours
Salmonella Typhi	6539	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Positive reaction	35-37°C	18-24 Hours
Salmonella Typhimurium	14028	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Positive reaction	35-37°C	18-24 Hours
Shigella flexneri	12022	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Negative reaction	35-37°C	18-24 Hours
Escherichia coli	8739	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction	35-37°C	18-24 Hours
Klebsiella pneumoniae	10031	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction	35-37°C	18-24 Hours

PACKAGING:
In pack size of 100 gm and 500 gm bottles.

STORAGE
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 10-25°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES
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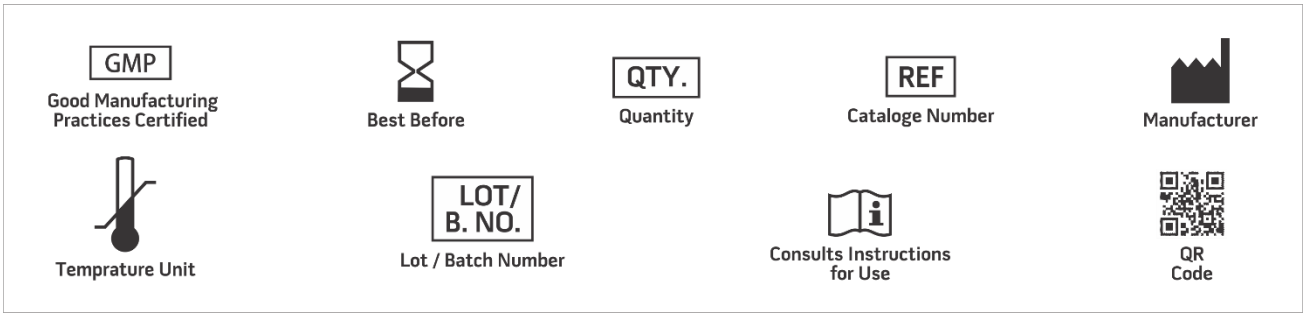

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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