TM 1955 – TRIPLE SUGAR, IRON AGAR (AGAR MEDIUM M) (as per BP/EP)

INTENDED USE

For identification of gram-negative enteric bacilli on the basis of glucose, lactose and sucrose fermentation and hydrogen sulphide production.

PRODUCT SUMMARY AND EXPLANATION

Triple Sugar Iron Agar, cited as Agar Medium M, is recommended for identification and differentiation of Enterobacteria by European Pharmacopoeia, 2008. It was originally proposed by Sulkin and Willett and modified by Hajna for identifying *Enterobacteriaceae*.

Organisms that ferment glucose monohydrate produce a variety of acids, turning the colour of the medium from red to yellow. More amounts 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.

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

Some members of the Enterobacteriaceae and H_2S producing Salmonella may not be H_2S positive on TSI Agar. Some bacteria may show H_2S production on Kligler Iron Agar but not on TSI Agar. This can happen because utilization of sucrose in TSI Agar suppresses the enzymic pathway that result in H_2S production.

COMPOSITION

Ingredients	Gms / Ltr
Beef extract	3.000
Peptones (Casein and Beef)	20.000
Yeast extract	3.000
Lactose monohydrate	10.000
Sucrose	10.000
Glucose monohydrate	1.000
Ferric ammonium citrate	0.300
Sodium chloride	5.000
Sodium thiosulphate	0.300
Phenol red	0.025
Agar	12.000

PRINCIPLE

Peptones (casein and beef), yeast extract and beef extract provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose (monohydrate), sucrose and Glucose (monohydrate) in the medium are the fermentable carbohydrates. Sodium thiosulphate and ferric ions make H2S 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.

INSTRUCTION FOR USE

- Disssolve 64.03 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified /distilled water.
- Heat to boiling to dissolve the medium completely.
- Mix well and distribute into test tubes.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes or as per validated cycle.
- Allow the medium to set in sloped form with a butt about 1 inch long.

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	Blackening of medium	30-35°C	24-48 Hours
Enterobacter aerogenes	13048	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	No blackening of medium	30-35°C	24-48 Hours
Escherichia coli	25922	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	No blackening of medium	30-35°C	24-48 Hours
Klebsiella pneumoniae	13883	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	No blackening of medium	30-35°C	24-48 Hours
Proteus vulgaris	13315	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Blackening of medium	30-35°C	24-48 Hours

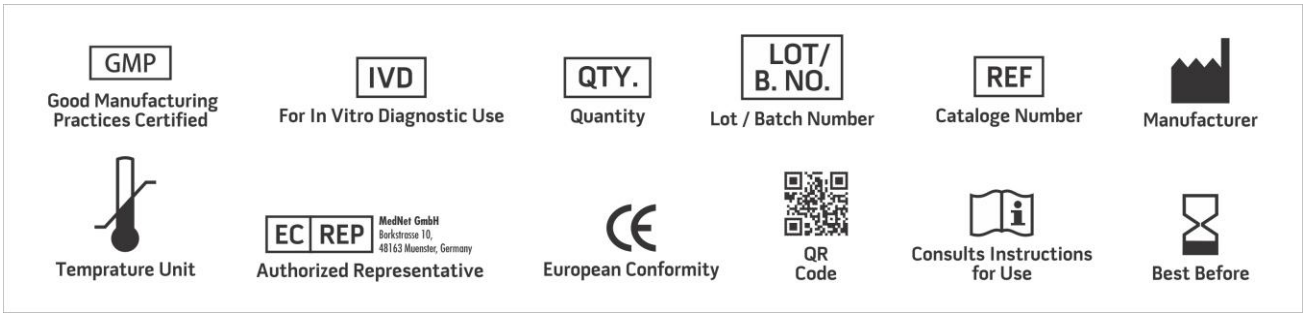
Salmonella Paratyphi A	9150	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Positive reaction	No blackening of medium	30-35°C	24-48 Hours
Salmonella Typhi	6539	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Blackening of medium	30-35°C	24-48 Hours
Salmonella Typhimurium	14028	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Blackening of medium	30-35°C	24-48 Hours
Shigella flexneri	12022	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	No blackening of medium	30-35°C	24-48 Hours
Escherichia coli	8739	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction	30-35°C	24-48 Hours
Klebsiella pneumoniae	10031	50-100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction	30-35°C	24-48 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 25-30°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
1. European Pharmacopoeia 2008, European Dept. for the quality of Medicines.
2. Sulkin E.S. and Willett J.C., 1940, J. Lab. Clin. Med., 25:649.
3. Hajna A.A., 1945, J. Bacteriol, 49:516.



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

***For Lab Use Only**

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