TM 887 – TRIPLE SUGAR IRON AGAR (as per USP)

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 Medium was originally proposed by Sulkin and Willett and modified by Hajna for identifying *Enterobacteriaceae*. This medium is in accordance with United States Pharmacopoeia and is recommended in pharmaceutical testing for identification of Gram-negative bacilli.

Organisms that ferment dextrose produce a variety of acids, varying the colour of the medium from red to yellow. More amounts of acids are liberated in butt region (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 dextrose fermenter but is unable to ferment lactose and/or sucrose. Bacteria that ferment lactose or sucrose (or both), in addition to dextrose, 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 dextrose fermented

Acid slant / acid butt - dextrose and sucrose fermented or dextrose and lactose fermented or all the three sugars, dextrose, 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 H2S 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
Peptone	10.000
Tryptone	10.000
Lactose	10.000
Sucrose	10.000
Dextrose	1.000
Ferrous ammonium sulphate	0.200
Sodium chloride	5.000
Sodium thiosulphate	0.200
Phenol red	0.025
Agar	13.000

PRINCIPLE

Tryptone and peptone provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and dextrose are the fermentable carbohydrates. Sodium thiosulphate helps in reactivation of sulphur containing compounds and prevents the desiccation of these compounds during storage. It also forms the substrate for enzyme thiosulphate reductase, which breaks it to form H_2S. Sodium thiosulphate and ferric or ferrous 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.

INSTRUCTION FOR USE

- Dissolve 59.42 grams 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.
- Allow the medium to set in form of a slope with a butt about 1inch 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) : 7.3±0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Slant	Butt	Gas	H_2S	Incubation Temperature	Incubation Period
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
Citrobacter freundii	8090	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
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
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	Acidic reaction, yellowing	Negative reaction	No blackenin	30-35°C	24-48 Hours

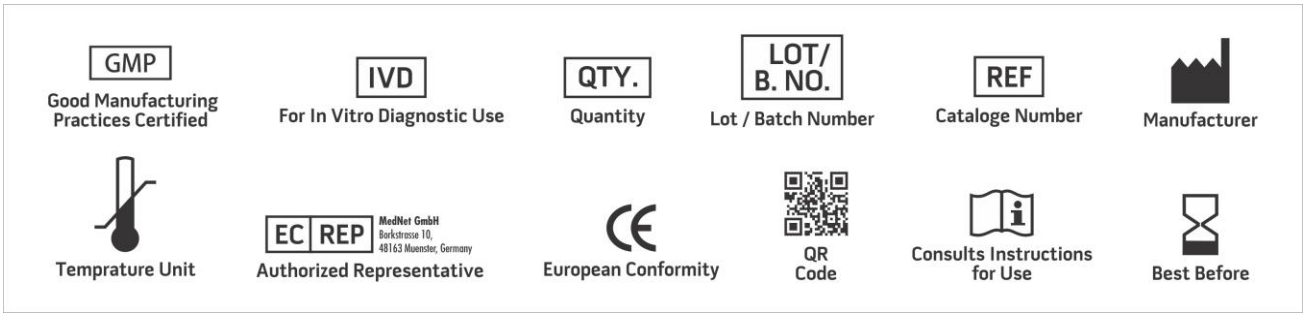
				of the medium	of the medium		g of medium		
Salmonella Typhi	6539	50-100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Blackening of medium	30-35°C	24-48 Hours
Shigella flexneri	12022	50-100	Luxuriant	Alkaline reaction, red colour of the 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. Sulkin, E.S. and Willet J.C., 1940, J. Lab. Clin. Med., 25:649.
2. Hajna A.A., 1945, J. Bacteriol 49:516.
3. The United States Pharmacopoeia, 2009 United States Pharmacopeial Convention, Rockville, Md.



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

***For Lab Use Only**

Revision: 08 Nov., 2019