

## TM 1819-TRIPLE SUGAR IRON AGAR (ISO 6785:2001 / ISO 6579:2017)

### INTENDED USE

For identification of gram-negative enteric bacteria on the basis of sugar fermentation & H<sub>2</sub>S production.

### PRODUCT SUMMARY AND EXPLANATION

Triple Sugar Iron Agar was originally proposed by Sulkin and Willett and modified by Hajna for identifying Enterobacteriaceae. This medium is a modification of the Kligler Agar where sucrose was added to differentiate *Proteus* and *Hafnia* (sucrose positive) from *Salmonella* and *Shigella* (sucrose negative). It complies with the specifications given by EN ISO 6785, ISO 6579 and APHA.

### COMPOSITION

Ingredients	Gms / Ltr
Agar	12.000
Lactose	10.000
Peptic digest of animal tissue	10.000
Casein enzymatic hydrolysate	10.000
Sucrose	10.000
Sodium chloride	5.000
Yeast extract	3.000
Beef extract	3.000
Dextrose	1.000
Ferrous ammonium citrate	0.300
Sodium thiosulphate	0.300
Phenol red	0.024

### PRINCIPLE

The Medium is composed of Peptic digest of animal tissue, Beef extract and Yeast extract which provides nitrogen, carbon and vitamins required for bacterial growth. Triple Sugar Iron Agar consists of three carbohydrates; Dextrose, Lactose and Sucrose. When carbohydrates are fermented, acid production is detected by Phenol red pH indicator. Sodium thiosulphate is reduced to Hydrogen sulphide and Hydrogen sulphide reacts with iron salt yielding typical black iron sulphide. Ferrous ammonium citrate is a Hydrogen sulphide (H<sub>2</sub>S) indicator and gives a typical black precipitate. Sodium chloride maintains osmotic balance of the medium. Agar is used as a solidifying agent.

### INSTRUCTION FOR USE

- Dissolve 64.62 grams in 1000ml distilled water.
- Gently heat to boiling with swirling to dissolve the medium completely.
- Dispense into tubes as desired.
- Sterilize by autoclaving at 15 psi (121°C) for 15 minutes
- Allow the medium to set in sloped form with a butt of depth about 2.5cm-5cm.

**Note:** For better results, the medium can be sterilized by autoclaving at 10 psi pressure (115°C) for 15 minutes

### QUALITY CONTROL SPECIFICATIONS

<b>Appearance of Dehydrated powder</b>	:	Light yellow to pink, homogeneous free flowing powder
<b>Appearance of Prepared medium</b>	:	Pinkish red coloured, clear to slightly opalescent gel
<b>pH (at 25°C)</b>	:	7.4± 0.2

### INTERPRETATION

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Slant	Butt	Gas	H <sub>2</sub> S	Incub* Temp	Incub* Period
<i>Citrobacter freundii</i>	8090	50-100	Luxuriant	Acidic Reaction	Acidic Reaction	Positive Reaction	Blackening of medium	35-37°C	18-24 Hours
# <i>Klebsiella aerogenes</i>	13048	50-100	Luxuriant	Acidic Reaction	Acidic Reaction	Positive Reaction	No Blackening of medium	35-37°C	18-24 Hours
<i>Escherichia coli</i>	25922	50-100	Luxuriant	Acidic Reaction	Acidic Reaction	Positive Reaction	No Blackening of medium	35-37°C	18-24 Hours
<i>Klebsiella pneumoniae</i>	13883	50-100	Luxuriant	Acidic Reaction	Acidic Reaction	Positive Reaction	No Blackening of medium	35-37°C	18-24 Hours
<i>Proteus vulgaris</i>	13315	50-100	Luxuriant	Alkaline Reaction	Acidic Reaction	Negative Reaction	Blackening of medium	35-37°C	18-24 Hours
<i>Salmonella Paratyphi A</i>	9150	50-100	Luxuriant	Alkaline Reaction	Acidic Reaction	Positive Reaction	No Blackening of medium	35-37°C	18-24 Hours
<i>Salmonella Typhi</i>	6539	50-100	Luxuriant	Alkaline Reaction	Acidic Reaction	Negative Reaction	Blackening of medium	35-37°C	18-24 Hours
<i>Salmonella Typhimurium</i>	14028	50-100	Luxuriant	Alkaline Reaction	Acidic Reaction	Positive Reaction	Blackening of medium	35-37°C	18-24 Hours
<i>Shigella flexneri</i>	12022	50-100	Luxuriant	Alkaline Reaction	Acidic Reaction	Negative Reaction	No Blackening of medium	35-37°C	18-24 Hours
<i>Escherichia coli</i>	8739	50-100	Luxuriant	Acidic Reaction	Acidic Reaction	Positive Reaction	No Blackening of medium	35-37°C	18-24 Hours
<i>Klebsiella pneumoniae</i>	10031	50-100	Luxuriant	Acidic Reaction	Acidic Reaction	Positive Reaction	No Blackening of medium	35-37°C	18-24 Hours
<i>Shigella flexneri</i>	12022	50-100	Luxuriant	Alkaline Reaction	Acidic Reaction	Negative Reaction	No Blackening of medium	35-37°C	18-24 Hours

Acidic Reaction =Yellowing of the medium, Alkaline Reaction =Red colour of the medium

Blackening of the medium = Positive Reaction, No Blackening of the medium = Negative Reaction

Incub\*=Incubation

# Formerly known as *Enterobacter aerogenes*

### PACKAGING

In 100 & 500 gm packaging size.

### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 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 powder show evidence of microbial contamination, discoloration, drying, or other signs of deterioration.

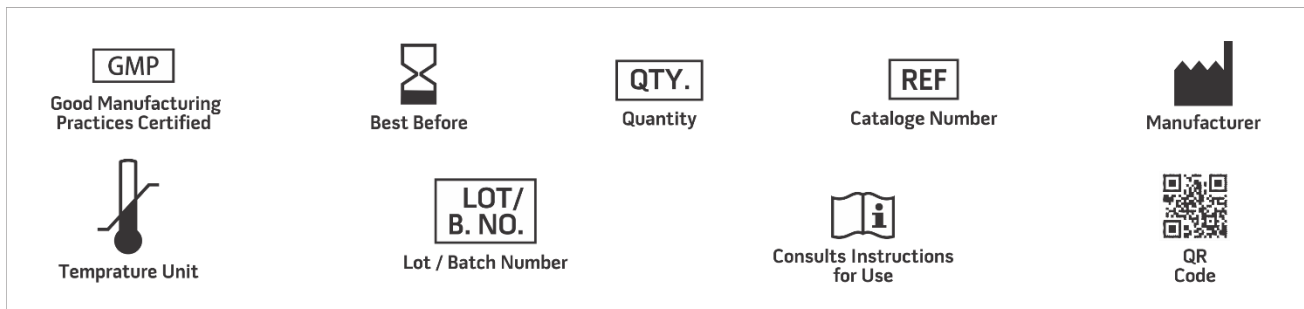
### DISPOSAL

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



## REFERENCES

1. Russell, F. F. The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. J. Med. Res. 25:217. (1911).
2. Kligler, I. J. A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am. J. Public Health 7:1042-1044. (1917).
3. Kligler, I. J. Modifications of culture media used in the isolation and differentiation of typhoid, dysentery, and allied bacilli. J. Exp. Med. 28:319-322. (1918).
4. Sulkin, S. E., and J. C. Willett. A triple sugar-ferrous sulphate medium for use in identification of enteric organisms. J. Lab. Clin. Med. 25:649-653. (1940).
5. ISO 6579 Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Salmonella* spp Standard Methods for the Examination of Dairy Products. APHA, (1972)



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

**\*For Lab Use Only**

**Revision: 9<sup>th</sup> July 2020**