Tryptone Soya Broth, w/ Ferrous Sulphate

M1875

Intended Use:

Recommended for isolation of *Salmonella* species from food samples in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
Soya peptone	3.000
Sodium Chloride	5.000
Dipotassium hydrogen phosphate	2.500
Glucose (Dextrose)	2.500
Ferrous sulphate	0.035
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.03 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Distribute in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Tryptone Soya Broth, w/ Ferrous Sulphate is used to pre enrich *Salmonella* during isolation from egg specimens in accordance with FDA BAM (3). *Salmonella* constitute the most taxonomically complex group of bacteria among the *Enterobacteriaceae* (7). Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta. Contaminated eggs or foods containing eggs have also been a source of food borne salmonellosis, with a significant proportion of these outbreaks being attributed to *Salmonella* Enteritidis. Since the level of contamination in individual eggs or a pool of such eggs may be low, enrichment to increase cell numbers can take several days. Pre-enrichment of raw blended eggs with medium supplemented with ferrous sulphate, significantly enhance the growth of *Salmonella* (2).

Disinfect eggs with 3:1 solution of 70% alcohol and 5% iodine/potassium iodide solution. Eggs are cracked aseptically by gloved hands and mix samples thoroughly until yolks are completely mixed with the albumen. These are incubated at room temperature (20-24°C) for 96 ± 2 h. After 96 ± 2 h, remove 25 ml of this mix and add to 225 ml Tryptone Soya Broth, w/ Ferrous Sulphate (M1875). After incubation for 24 ± 2 h at 35°C, transfer 0.1 ml mixture to 10 ml Rappaport-Vassiliadis (M880F) medium and another 1 ml mixture to 10 ml tetrathionate (M032F) broth. Vortex and incubate at optimum temperature for 24 ± 2 h depending upon the microbial load and type of the sample. These are further subcultured into XLD Agar (M031F) or Hektoen Enteric Agar (M467F), incubate the plates 24 ± 2 h at 35°C and observe for the appearance of typical salmonellae colonies. Blue-green to blue colonies will be appeared in XLD Agar and pink colonies with or without black centers on HE Agar.

Tryptone and soya peptone provides the nitrogen source, long chain amino acids and vitamins, glucose acts as the carbon source, NaCl maintains the osmotic balance and phosphate acts as the buffering agent. Ferrous Sulphate helps in the recovery of injured *Salmonella* strains (3).

Type of specimen

Food and dairy samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6,8). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Further biochemical and serological tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured clear solution may have slight particles

Reaction

Reaction of 3.0 w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

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

Organism	Inoculum (CFU)	Growth
Salmonella Enteritidis *ATCC 13076* (00030*)	50-100	good-luxuriant
Salmonella Typhi *ATCC 6539*	50-100	good-luxuriant
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	good-luxuriant
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

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