Mueller Kauffman Tetrathionate Broth Base

M876

Intended Use:

Recommended for improved enrichment and isolation of *Salmonellae*.

Composition**

Ingredients	Gms / Litre
Tryptone	7.000
Soya peptone	2.300
Sodium chloride	2.300
Calcium carbonate	25.000
Sodium thiosulphate	40.700
Bile #	4.750

**Formula adjusted, standardized to suit performance parameters
Equivalent to Ox bile

Directions

Suspend 82.05 grams in 1000 ml purified / distilled water. Heat the medium just to boiling. **DO NOT AUTOCLAVE.** Cool and just before use aseptically add 19 ml of iodine solution (20 g iodine and 25 g potassium iodide in 100 ml sterile distilled water) and 9.5 ml of 0.1% brilliant green solution. Mix well before dispensing in the sterile tubes to disperse calcium carbonate uniformly.

Note: Due to presence of Calcium Carbonate, the prepared medium forms opalescent solution with white precipitate.

Principle And Interpretation

The examination of various types of food products for *Salmonella* requires methods different from those used in clinical laboratories. The need for such method is due to the generally low numbers of *Salmonellae* in foods and the frequently poor physiological state of these pathogens following exposure to stressful conditions during food processing or storage. Injured *Salmonella* are resuscitated in non-selective broth medium, which facilitates detection of sublethally injured *Salmonella*. The ideal pre-enrichment broth should provide for the repair of cell damage, dilute toxic or inhibitory substances and nutritive enough to favour growth of *Salmonella*. In the analysis of food for *Salmonella*, pre-enrichment cultures are usually incubated at 35-37°C for 18-24 hours and then a portion is subcultured to one or more selective enrichment broths. Normally 1 ml of pre-enrichment culture is inoculated to 9 ml of selective enrichment broth. Selective enrichment media contains selective ingredients that allow the proliferation of *Salmonella* and inhibit the growth of competing non-salmonella microorganisms. Lactose Broth (M1003) is recommended by BAM for pre-enrichment of *Salmonella* from food. Selective enrichment is done in Tetrathionate Broth and Rappaport Vassiliadis Medium. For the detection of foodborne *Salmonella*, various modifications of Tetrathionate Broth have generally found wider applications (1).

Mueller (2) recommended Tetrathionate Broth as a selective medium for the isolation of *Salmonella*. Kauffman (3) modified the formula to include ox bile and brilliant green as selective agents to suppress bacteria such as *Proteus* species. The British Standard Specification specifies Brilliant Green Tetrathionate Broth for isolating *Salmonella* from meat and meat products and from poultry and poultry products (4). It is also a recommended selective broth for isolating *Salmonella* from animal feces and sewage-polluted water (5). Selectivity is conferred by tetrathionate (from the reaction of thiosulphate and iodine). Using more than one selective broth increases the isolation of *Salmonella* from samples with multiple serotypes (6). Mueller Kauffman Tetrathionate Broth Base conforms to ISO specifications (7).

Mueller Kauffman Tetrathionate Broth Base contains tryptone and soya peptone as sources of carbon, nitrogen, vitamins and minerals. Bile and added brilliant green are selective agents, which inhibit gram-positive and other gram-negative organisms. Calcium carbonate is the buffer. Sodium chloride maintains osmotic equilibrium. Sodium thiosulphate is a source of sulfur. The tetrathionate (S_4O_6) anions constitute the principle selective agent in these enrichment media. Organisms other than *Salmonellae*, such as *Morganella morganii* and some *Enterobacteriaceae* may grow in the medium. Therefore, confirmatory tests should be carried out on all presumptive Salmonella colonies that are recovered.

Type of specimen

Food samples

Specimen Collection and Handling:

If desired, 4 mg of novobiocin per litre of broth can be added to suppress *Proteus* species (8). Add approximately 10 grams of sample to 100 ml of broth. Shake well and place the flask in a 45°C water bath for 15 minutes. Remove the flasks and place in an incubator or water bath at 43°C. Several studies have shown increased recovery of *Salmonella* following incubation of selective enrichment at 43°C (9). After an incubation for 18-24 hours and 48 hours, subculture on Brilliant Green Agar, Modified (M016). This medium is not suitable for the growth of *Salmonella* Typhi, *Salmonella* Sendai, and *Salmonella* Pullorum etc. The complete medium is unstable and should be used immediately. It may be stored at 2-8°C in the dark for no more than 7 days.

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. The complete medium is unstable and should be used immediately. It may be stored at 2-8°C in the dark for no more than 7 days.
2. Confirmatory tests should be carried out on all presumptive *Salmonella* colonies that are recovered.

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

With added brilliant green and iodine solution - Light green coloured opalescent solution forms with heavy white precipitate

Reaction

Reaction of 8.20% w/v aqueous solution at 25°C.

Cultural Response

Cultural characteristics observed, when subcultured on Soyabean Casein digest Agar,after an incubation at 43°C for 18-24 hours with added iodine and brilliant green solution.

Organism	Inoculum (CFU)	Recovery
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	excellent
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	excellent
Salmonella Paratyphi A ATCC 9150	50-100	excellent
Salmonella Paratyphi B ATCC 8759	50-100	excellent
Salmonella Typhi ATCC 6539	>=10^4	inhibited
Escherichia coli ATCC 25922 (00013*)	50-100	none-poor
Proteus vulgaris ATCC 13315	50-100	none-poor
Shigella flexneri ATCC 12022 (00126*)	>=10^4	inhibited

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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 (10,11).

Reference

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Revision : 03/2023