Mueller Kauffman Tetrathionate Novobiocin Broth Base

M1496I

Mueller Kauffman Tetrathionate Novobiocin Broth Base is used for improved enrichment and isolation of Salmonellae.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	4.300
Casein enzymic hydrolysate	8.600
Ox bile	4.750
Sodium chloride	2.600
Calcium carbonate	38.700
Sodium thiosulphate, pentahydrate	47.800
Brilliant green	0.0095
Final pH (at 25°C)	8.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 89.42 grams of dehydrated medium in 1000 ml distilled water. Heat the medium just to boiling. DO NOT AUTOCLAVE. Cool to 45-50°C and just before use aseptically add 20 ml of iodine solution (20 gram iodine and 25 gram potassium iodide in 100 ml sterile distilled water) along with rehydrated contents of 1 vial of MKTT Novobiocin Supplement (FD203). Mix well to disperse calcium carbonate uniformly before dispensing in sterile tubes.

Note: Due to presence of calcium carbonate, the prepared media 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 sub cultured 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 (7).

Mueller (1) recommended Tetrathionate Broth as a selective medium for the isolation of *Salmonella* . Kauffman (2) 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 (3). It is also a recommended selective broth for isolating *Salmonella* from animal feces and sewage-polluted water (4). 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 (5).

Mueller Kauffman Tetrathionate Novobiocin Broth Base contains casein enzymic hydrolysate and peptic digest of animal tissue as sources of carbon, nitrogen, vitamins and minerals. Ox 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. If desired, 4 mg of novobiocin per litre of broth can be added to suppress *Proteus* species

(6).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 (8). 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.

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.

Quality Control

Appearance
Cream to greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light green coloured opalescent solution forms with heavy white precipitate

Reaction
Reaction of 8.93% w/v aqueous solution at 25°C. pH : 8.2±0.2

pH
8.00-8.40

Cultural Response
M1496I: Cultural characteristics observed after an incubation at 43°C for 18-48 hours with added 20ml iodine solution and MKTT Novobiocin Supplement (FD203), when subcultured on Soyabean Casein Digest Agar (M290).

Organism	Inoculum	Recovery
Escherichia coli ATCC 25922	50-100	none-poor
Proteus vulgaris ATCC 13315	50-100	none-poor
Shigella flexneri ATCC 12022	>=10^3	inhibited
Salmonella Enteritidis ATCC 13076	50-100	excellent
Salmonella Enteritidis ATCC 13076	50-100	
Salmonella Paratyphi A ATCC 9150	50-100	excellent
Salmonella Paratyphi B ATCC 8759	50-100	excellent
Salmonella Typhi ATCC 6539	>=10^3	inhibited
Salmonella Typhimurium ATCC 14028	50-100	excellent

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

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7.Speck M. L., (Ed.), 1984, Compendium of Methods for the Microbiological Examination of Foods, 2nd Ed., American Public Health Association, Washington, D.C.
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