Technical Information

Mueller Kauffman Tetrathionate Broth Base

Product Code: DM 1876

Application: - Mueller Kauffman Tetrathionate Broth is used for improved enrichment and isolation of Salmonellae.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	7.000
Papaic digest of soyabean meal	2.300
Sodium chloride	2.300
Calcium carbonate	25.000
Sodium thiosulphate	40.700
Ox bile	4.750
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

The examination of various types of food products for *Salmonella* requires methods quite different from those used in clinical laboratories. The need for such method is due to the low numbers of Salmonellae in foods and the poor physiological state of these pathogens following exposure to stressful conditions during processing or storage of food. Injured *Salmonella* are resuscitated in non-selective broth medium, which facilitates detection of sublethally injured *Salmonella.* The ideal pre-enrichment broth should repair damaged cell, 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 that allows the proliferation of *Salmonella* and inhibits the growth of competing non-salmonella microorganisms. Lactose Broth (DM2003) 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,* different modifications of Tetrathionate Broth have resulted in wider applications [7] of this media.

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 Broth Base conforms to ISO specifications [9].

Mueller Kauffman Tetrathionate Broth Base contains casein enzymic hydrolysate and papaic digest of soyabean meal 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 (DM1016). 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.

Methodology

Suspend 82.05 grams of powder media in 1000 ml distilled water. Shake well and 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 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

Quality Control

Physical 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 8.2% w/v aqueous solution at 25°C pH : 7.8±0.2

pH Range 7.60-8.00

Cultural Response/Characteristics
DM 1876: 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	50-100	Excellent
Salmonella Enteritidis ATCC 13076	50-100	Excellent
Salmonella Paratyphi A ATCC 9150	50-100	Excellent
Salmonella Paratyphi B ATCC 8759	50-100	Excellent
Salmonella Typhi ATCC 6539	>=10^3	inhibited
Escherichia coli ATCC 25922	50-100	none-poor
Proteus vulgaris ATCC 13315	50-100	none-poor
Shigella flexneri ATCC 12022	>=10^3	inhibited

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Mueller L., 1923, C. R. Soc. Biol., (Paris) 89:434.

2. Kauffman F., 1935, Ztschr. F. Hyg., 117:26.

3. International Organization for Standardization, 1974, (Draft International Standard ISO/DIS 3565), Geneva, Switzerland. 4. Public Health Laboratory Service, 1974, Monograph Series No. 8, Public Health Laboratory Service, London, England. 5. Harvey R. W. S. and Price T. S., 1976, J. Hyg. Camb., 77:333.

6. Jeffries L., 1959, J. Clin. Pathol., 12:568.

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

8. DAoust J. Y., 1989, Salmonella in Food borne Bacterial pathogens, (Eds.) Doyle M. P., 327, Marcel Dekker, New York. 9. International Organization for Standardization (ISO), 2002, Draft 6579.

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