

CM 21,263 - MULLER KAUFFMAN TETRATHIONATE BROTH BASE

INTENDED USE

Forenrichment and isolation of Salmonellae by surpassing Proteus species.

PRODUCT SUMMARY AND EXPLANATION

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 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.

Mueller recommended Tetrathionate Broth as a selective medium for the isolation of Salmonella. Kauffman 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. It is also a recommended selective broth for isolating Salmonella from animal feces and sewage-polluted water. 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. Mueller Kauffman Tetrathionate Broth Base conforms to ISO specifications.

If desired, 4 mg of novobiocin per litre of broth can be added to suppress Proteus species. 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. After an incubation for 18-24 hours and 48 hours, subculture on Brilliant Green Agar, Modified. 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.

COMPOSITION

Ingredients	Gms / Ltr
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

PRINCIPLE

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 anions constitute the principle selective agent in these enrichment media.

INSTRUCTION FOR USE

Dissolve 82.05 grams in 1000 ml 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 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 SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Appearance of prepared medium : With added brilliant green and iodine solution-Light green coloured opalescent solution forms with heavy white precipitate.

pH (at 25°C) : 7.8±0.2

INTERPRETATION

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period
Salmonella Typhimurium	14028	50-100	Luxuriant	43°C	18-24 Hours
Salmonella Enteritidis	13076	50-100	Luxuriant	43°C	18-24 Hours
Salmonella Paratyphi A	9150	50-100	Luxuriant	43°C	18-24 Hours
Salmonella Paratyphi B	8759	50-100	Luxuriant	43°C	18-24 Hours
Salmonella Typhi	6539	>=10 ³	Inhibited	43°C	18-24 Hours
Escherichia coli	25922	50-100	Luxuriant	43°C	18-24 Hours



Proteus vulgaris	13315	50-100	Luxuriant	43°C	18-24 Hours
Shigella flexneri	12022	$\geq 10^3$	Inhibited	43°C	18-24 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°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 they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

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

REFERENCES

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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.



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

*For Lab Use Only

