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Mueller Kauffman Tetrathionate Broth Base

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

REF: BS.1/MK01.100.0100	100 Gram	REF: BS.1/MK01.250.0250	250 Gram
REF: BS.1/MK01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE

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 (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 Broth Base conforms to ISO specifications (9).

METHOD 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 (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. 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.

MEDIA COMPOSITION

Item	Formula per liter of medium
Casein enzymic hydrolysate	7 gm
Papaic digest of soyabean meal	2.3 gm
Sodium chloride	2.3 gm
Calcium carbonate	25 gm
Sodium thiosulphate	40.7 gm
Ox bile	4.75 gm

Final pH 8.0 ± 0.2 at 25°C

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the Shigella Borth material safety data sheet.

STORAGE AND STABILITY

BioScien Mueller Kauffman Tetrathionate Broth should be stored between 10- 30°C in a firmly 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 avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a 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.

PREPARATION

- Suspend 82.05 grams in 1000 ml distilled water.
- Adjust pH to 8.0 ± 0.2 at 25°C
- 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

Deterioration

The color of ***BioScien*** Mueller Kauffman Tetrathionate Broth is Cream to yellow homogeneous free flowing powder. Prepared Media With added brilliant green and iodine solution – Light green coloured opalescent solution forms with heavy white precipitate. If there are any physical changes for powder or signs of deterioration (shrinking, cracking, or discoloration),

and contaminations for hydrated media, discard the medium.

SPECIMEN

- Clinical samples (e.g., faeces, blood, and wounds).
- Food sample (milk and other dairy products)
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EQUIPMENT REQUIRED NOT PROVIDED

Inoculating loops, swabs, collection containers
Incubators
Sterile test tube

Performance characteristics

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	Growth
Salmonella Typhimurium ATCC 14028	Luxuriant
Salmonella Enteritidis ATCC 13076	Luxuriant
Salmonella Paratyphi A ATCC 9150	Luxuriant
Salmonella Paratyphi B ATCC 8759	Luxuriant
Salmonella Typhi ATCC 6539	Inhibited
Escherichia coli ATCC 25922	None - poor
Proteus vulgaris ATCC 13315	None - poor
Shigella flexneri ATCC12022	Inhibited

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control still out of range please contact ***BioScien*** technical support.

REFERENCES

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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.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number	⚠	Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		