PRODUCT SPECIFICATION SHEET

Fluid Tetrathionate Medium w/o Iodine and BG (Tetrathionate Broth Base w/o Iodine and BG) (DM353)

Intended Use

Fluid Tetrathionate Medium w/o Iodine and BG (Tetrathionate Broth Base w/o Iodine and BG) (DM353) is recommended for selective enrichment and isolation of *Salmonellae* from foods and other pathological specimen.

Product Summary and Explanation

Salmonellosis is one of the most important and most frequently reported human food borne diseases worldwide.[1] Outbreaks have been associated with the consumption of pork and pork products,[2, 3] broiler chickens,[4] and other animals. Environmental sources include animal feed, litter and dust from hen houses, and animal faeces. The process of isolating *Salmonella* from food is often difficult. 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, accompanied by larger numbers of other contaminating bacteria and the frequently poor physiological state of these pathogens following exposure to stressful conditions during food processing or storage. *Salmonella* present in food samples may be sublethally damaged during various stages of food processing where they may be exposed to low temperatures, heat drying, radiations, various chemicals.[5] These damaged cells are able to cause spoilage, and if ingested cause diseases under favourable conditions. Therefore it is important to resuscitate these damaged bacteria before enumeration. Pre-enrichment is necessary to permit the detection of low numbers of *Salmonella* or injured *Salmonella*. 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*.

Fluid Tetrathionate Medium (with added iodine and brilliant green) is recommended for the selective enrichment of Salmonella including *Salmonella typhi* from faeces, urine, food and other material of sanitary importance. The medium, originally formulated by Mueller[6] is recommended by APHA[7-9] for enrichment of Salmonella. Due to the addition of iodine and potassium iodide, tetrathionate is formed in the medium. Organisms possessing the enzyme tetrathionate reductase grow in this medium.

Principles of the Procedure

Fluid Tetrathionate Medium w/o Iodine and BG contains casein enzymic hydrolysate and peptic digest of animal tissue which are the sources of carbon, nitrogen, vitamins and minerals and other essential growth nutrients. Bile salts inhibit accompanying gram-positive microorganisms. The selectivity of the medium depends on the ability of thiosulphate and tetrathionate in combination to suppress commensal coliform organisms. Calcium carbonate neutralizes the acidic tetrathionate decomposition products. Brilliant green also helps to select *Salmonella* by inhibiting the accompanying bacteria. For further confirmation, streak the enriched cultures after incubation, on plates of Brilliant Green Agar (DM044), MacConkey Agar (DM143) and Bismuth Sulphite Agar (DM039).

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	2.50
Peptic digest of animal tissue	2.50
Bile salts	1.00
Calcium carbonate	10.00
Sodium thiosulphate	30.00
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Due to the presence of calcium carbonate, the prepared medium forms opalescent solution with white precipitate.

Directions

1. Suspend 46 grams of the medium in one liter of distilled water.
2. Heat just to boiling.

3. DO NOT AUTOCLAVE.
4. Cool below 45°C and add 20 ml iodine solution (iodine - 6 grams and potassium iodide - 5 grams in 20 ml distilled water) and 10 ml of 0.1% brilliant green solution.
5. Mix well and dispense in 10 ml quantities.
6. This complete medium should be used on the day of preparation otherwise sterilized broth base may be stored for some time.
7. Do not heat after the addition of iodine solution.
8. Use the medium immediately after addition of iodine.

Quality Control Specifications

Dehydrated Appearance	White to cream homogeneous free flowing powder
Prepared Medium	Complete medium with added brilliant green and iodine solution - Light green coloured, opalescent solution with heavy white precipitate, which on standing the precipitate settles down
Reaction of Solution	Not Applicable
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed with added brilliant green and iodine solution when sub cultured on MacConkey Agar (DM143) after enrichment in Tetrathionate medium, after an incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Recovery	Colour of colony
1.	*Escherichia coli ATCC 25922*	50 – 100	little or no increase in number	pink-red with bile precipitate
2.	*Salmonella Choleraesuis ATCC 12011*	50 – 100	good-excellent	colourless
3.	*Salmonella Typhi ATCC 6539*	50 – 100	good-excellent	colourless
4.	*Salmonella Typhimurium ATCC 14028*	50 – 100	good-excellent	colourless
5.	*Escherichia coli NCTC 9002*	50 – 100	little or no increase in number	pink-red with bile precipitate
6.	*Escherichia coli ATCC 8739*	50 – 100	little or no increase in number	pink-red with bile precipitate

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Aseptically inoculate test specimen into Fluid Tetrathionate medium (with added iodine and brilliant green).
2. Incubate at 35-37°C for 18-24 hours.
3. Following the incubation, isolate onto selective media plates.
4. Refer standard procedures for enrichment and isolation.

Results

1. After incubation, growth is evidenced by the presence of turbidity compared to an uninoculated control.
2. Strict aerobes tend to grow in a thin layer at the surface of the broth; obligate anaerobes will grow only in that portion of the broth below the upper oxidized layer.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Anaerobes can be overgrown by more rapidly growing facultative organisms.
2. If plating medium reveals no growth examine and Gram stain.
3. Never rely on broth cultures exclusively for isolation of anaerobes. Some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.
4. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Fluid Tetrathionate Medium w/o Iodine and BG (Tetrathionate Broth Base w/o Iodine and BG)
Product Code : DM353
Available Pack sizes : 100gm/ 500gm

References

1. Baird Parker. 1990. The Lancet. *336*:1231.
2. Van Pelt and Valkenburgh. 2001. Zoonoses and zoonotic agents in humans, food, animals and feed in the Netherlands. <http://www.keuringsdienstvanwaren.nl>.
3. Hald et al. 1999. 3rd International Symposium of the Epidemiology and Control of *Salmonella* in Pork, Washington, D.C. 197.
4. Cherry et al, 1972, Appl. Microbiol., 24:334
5. Mueller, 1923, Compt. Rend. Sco. Biol., 89:434.
6. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods For The Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
7. Eaton A. D., Clesceri L. S. and Greenberg A. W., (Eds.), 2005, Standard Methods for the Examination of Water and
8. Wastewater, 21st Ed., APHA, Washington, D.C.
9. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.

Further Information

For further information please contact your local MICROMASTER Representative.

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