Tetrathionate Broth Base

Intended Use

Tetrathionate Broth Base, with added iodine-iodide solution, is used as a selective enrichment medium for the isolation of *Salmonella* from feces, urine, foods and other materials of sanitary importance.

Summary and Explanation

Tetrathionate Broth Base is used as a selective enrichment for the cultivation of *Salmonella* species that may be present in small numbers and compete with intestinal flora. *Salmonella* organisms may also be injured in food-processing procedures, which include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives and sanitizers.[1] Although injured cells may not form colonies on selective media, they can, if ingested, cause disease.[2]

Tetrathionate Broth was originally described by Mueller who found that the medium selectively inhibited coliforms, thereby permitting enteric pathogens to grow virtually without restriction.[3] Kaufmann modified Mueller's medium and achieved a higher percentage of isolates.[4,5] The medium is specified in standard methods.[6,7]

Principles of the Procedure

Peptones provide nitrogen, vitamins, amino acides and carbon. Oxgall inhibits gram-positive microorganisms. Tetrathionate, which is formed in the medium by the addition of the iodine-iodide solution, inhibits the normal intestinal flora of fecal specimens.[8] Calcium carbonate neutralizes and absorbs toxic metabolites.

Formula

Difco™ Tetrathionate Broth Base

Approximate Formula* Per Liter		
Proteose Peptone	2.5	g
Pancreatic Digest of Casein	2.5	g
Oxgall	1.0	g
Sodium Thiosulfate	30.0	g
Calcium Carbonate	10.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 4.6 g of the powder in 100 mL of purified water.
2. Heat to boiling. Cool to below 60°C.
3. Add 2 mL of iodine solution (6.0 g of iodine crystals and 5.0 g of potassium iodide in 20.0 mL of water). DO NOT REHEAT MEDIUM AFTER ADDING IODINE. DO NOT AUTOCLAVE.
4. Use immediately.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For tubes prepared from dehydrated media with added iodine, inoculate with a swab or loopful of specimen or, where the tube volume permits, add feces, other solid sample or liquid specimen (approximately 10% by volume) and emulsify with an inoculating needle, if necessary. Incubate tubes for 18-24 hours at 35 ± 2°C in an aerobic atmosphere.

User Quality Control

Identity Specifications

Difco™ Tetrathionate Broth Base

Dehydrated Appearance:	White to off-white, may have a slight greenish tint, free-flowing, homogeneous.
Solution:	4.6% solution, partially insoluble in purified water. Solution is a milky white, opaque suspension. Upon standing, appearance is nearly colorless to light yellow supernatant over insoluble white precipitate.
Prepared Appearance:	Nearly colorless to light yellow supernatant over a heavy white precipitate.
Reaction of 4.6% Solution at 25°C:	pH 8.4 ± 0.2

Cultural Response

Difco™ Tetrathionate Broth Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. After incubation, subculture onto MacConkey Agar plates and incubate plated media at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONIES ON MACCONKEY AGAR
Escherichia coli	25922	10^2-10^3	Little or no increase in number of colonies	Pink with bile precipitate
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good	Colorless

For **BBL**™ Tetrathionate Broth Base prepared tubes, prepare an iodine-iodide solution by adding 6.0 g of iodine crystals and 5.0 g of potassium iodide to 20.0 mL of sterile purified water. Immediately before inoculation, add 0.2 mL of the iodine-iodide solution to each 10 mL of medium and inoculate as described above.

Expected Results

Growth is indicated by turbidity in the medium. Subculture to selective and differential enteric plating media for further investigations.

Limitation of the Procedure

Enrichment broths should not be used as the sole isolation medium. They are to be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens, especially when they may be present in small numbers. Consult references for detailed information and recommended procedures.[6,7,9]

References

1. Hartman and Minich. 1981. J. Food Prot. *44*:385.
2. Sorrells, Speck and Warren. 1970. Appl. Microbiol. *19*:39.
3. Mueller. 1923. C. R. Soc. Biol. (Paris) *89*:434.
4. Kaufman. 1930. Zentrabl. Bakteriol. Parsitenkd. Infektionskr. Hyg. Abt. I Orig. *113*:148.
5. Kaufman. 1935. Z. Hyg. Infektionskr. *117*:26.
6. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
7. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
8. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore, Md.
9. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Tetrathionate Broth Base

AOAC BAM CCAM COMPF SMD SMWW

Cat. No.	210430	Dehydrated – 500 g
	210420	Dehydrated – 2 kg

BBL™ Tetrathionate Broth Base

AOAC BAM CCAM COMPF SMD SMWW

Cat. No.	298249	Prepared Tubes, 10 mL (D Tubes) – Pkg. of 10*

Europe

Cat. No.	257329	Prepared Bottles, 5 mL – Pkg. of 50*
	257328	Prepared Bottles, 10 mL – Pkg. of 50*
	254958	Prepared Bottles, 100 mL – Pkg of 25*

**Store at 2-8°C.*