



QUALITY CONTROL PROCEDURES

I INTRODUCTION

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.

II PERFORMANCE TEST PROCEDURE

1. Prior to inoculation, add 0.2 mL of potassium iodide solution per 10 mL of medium, prepared by adding 6.0 g of iodine crystals and 5.0 g of potassium iodide to 20.0 mL of sterile purified water.
2. Inoculate representative samples with 0.1 mL of a 0.5 McFarland suspension of the cultures listed below.
3. Subculture to **BBL™ Trypticase™** Soy Agar with 5% Sheep Blood at time 0 and after 18 – 24 h of incubation at 35 ± 2 °C in an aerobic atmosphere.
4. Incubate subcultures at 35 ± 2 °C for 18 – 24 h in an aerobic atmosphere and examine for growth. Refrigerate time 0 plates to compare growth recovery with the 24 h plates.
5. Expected Results

Organisms	ATCC™	Growth on Trypticase Soy Agar with 5% Sheep Blood after Subculture from Tetrathionate Broth	
		0 Time	24 h
* <i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	Fair to moderate growth	Moderate to heavy growth
* <i>Escherichia coli</i>	25922	Fair to moderate growth	No growth to slight growth

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine the tubes for signs of deterioration as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 8.4 ± 0.2 .
4. Incubate uninoculated representative samples at 20 – 25 °C and 30 – 35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV 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.

V SUMMARY AND EXPLANATION

Tetrathionate Broth was originally described by Mueller who found that the medium selectively inhibited coliforms, thereby permitting enteric pathogens to grow virtually without restriction.¹ Kauffman modified Mueller's medium and achieved a higher percentage of isolates.^{2,3} The medium now is formulated according to specifications of the American Public Health Association (APHA), AOAC International (AOAC) and the Food and Drug Administration (FDA).

VI PRINCIPLES OF THE PROCEDURE

Bile salts inhibit 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.⁴

VII REAGENTS

Tetrathionate Broth Base

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein.....	2.5 g	Calcium Carbonate.....	10.0 g
Peptic Digest of Animal Tissue	2.5 g	Sodium Thiosulfate	30.0 g
Bile Salts	1.0 g		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens.

"Standard Precautions"⁵⁻⁸ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize prepared tubes, specimen containers and other contaminated materials by autoclaving.

Storage Instructions: On receipt, store tubes in the dark at 2 – 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, precipitation, evaporation or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be obtained using various techniques. Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for proper delivery to the laboratory. For more information, consult appropriate texts.⁹⁻¹²

IX PROCEDURE

Material Provided: Tetrathionate Broth Base

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Prepare 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 iodine-iodide solution to each tube. 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 12 – 24 h at 35 ± 2 °C in an aerobic atmosphere.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Subculture to selective and differential enteric plating media for further investigations.

XI LIMITATIONS 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 in a specimen. Consult texts for detailed information and recommended procedures.^{10,12,13-17}

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Tetrathionate Broth Base are tested for expected performance characteristics. A 2% potassium iodide solution is added to each tube. Tubes are inoculated with 0.1 mL of 0.5 McFarland *S. typhimurium* ATCC 14028 and *E. coli* ATCC 25922 (organisms are grown in **Trypticase** Soy Broth for 4 h and diluted 100-fold prior to inoculation) and then subcultured to **Trypticase** Soy Agar with 5% Sheep Blood at time 0 and after 18 – 24 h of incubation at 35 ± 2 °C in an aerobic atmosphere. Plates are incubated overnight at 35 ± 2 °C in an aerobic atmosphere and examined for growth. Tubes subcultured at 24 h produce fair to heavy growth of *S. typhimurium*, whereas *E. coli* is partially to completely inhibited.

XIII AVAILABILITY

Cat. No.	Description
298249	BBL™ Tetrathionate Broth Base, Pkg. of 10 size K tubes, 10 mL

XIV REFERENCES

1. Mueller, L. 1923. Un nouveau milieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. C.R. Soc. Biol. (Paris), 89:434-437.
2. Kaufmann, F. 1930. Ein kombiniertes Anreicherungsverfahren für Typhus- und Paratyphusbazillen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig., 113:148-152.
3. Kaufmann, F. 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren für Salmonellabazillen. Z. Hyg. Infektionskr. 117:26-32.
4. MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore.
5. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
6. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. 17:53-80.
7. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.
8. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
9. Isenberg, H.D., F.D. Schoenkecht, and A. von Graevenitz. 1979. Cumitech 9, Collection and processing of bacteriological specimens. Coordinating ed., S.J. Rubin. American Society for Microbiology, Washington, D.C.
10. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby Company, St. Louis.
11. Miller, J.M., and H.T. Holmes. 1999. Specimen collection, transport, and storage, p. 33-63. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
12. Farmer, J.J., III. 1999. *Enterobacteriaceae*: introduction and identification p. 442-458. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
13. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods. 4th ed. American Public Health Association, Washington, D.C.
14. U.S. Pharmacopeial Convention, Inc. 2001. The U.S. pharmacopeia 25/The national formulary 20-2002. U.S. Pharmacopeial Convention, Inc. Rockville, Md.
15. Cunniff, P. (ed.). 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, Va.
16. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
17. Bopp, C.A., F.W. Brenner, J.G. Wells, and N.A. Strockbine. 1999. *Escherichia*, *Shigella*, and *Salmonella*, p. 459-474. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

 Becton, Dickinson and Company
7 Loveton Circle
Sparks, MD 21152 USA
800-638-8663
www.bd.com/ds

 Benex Limited
Pottery Road, Dun Laoghaire
Co. Dublin, Ireland

ATCC is a trademark of the American Type Culture Collection.

BD, BD Logo, BBL and Trypticase are trademarks of Becton, Dickinson and Company. ©2013 BD.