

# BD BBL™ Prepared Tubed Media for Cultivation of *Salmonella* Species

8808871JAA  
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Tetrathionate Broth Base, Tetrathionate Broth Base with Brilliant Green

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## INTENDED USE

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

## 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.<sup>1</sup> Kauffmann modified Mueller's medium and achieved a higher percentage of isolates.<sup>2,3</sup> The medium now is formulated according to specifications of the American Public Health Association (APHA), the United States Pharmacopeial Convention (USP), AOAC International (AOAC) and the Food and Drug Administration (FDA). Tetrathionate Broth (with iodine-iodide solution and brilliant green) is specified as an enrichment medium for *Salmonella* in recent editions of compendia of microbiological methods.<sup>4-7</sup>

Tetrathionate Broth with Brilliant Green is more selective than Tetrathionate Broth.<sup>8</sup>

## 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.<sup>8</sup> The addition of brilliant green renders the medium more inhibitory to coliforms.

## REAGENTS

### Tetrathionate Broth Base

Approximate Formula\* Per Liter Purified Water

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

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

Tetrathionate Broth Base with Brilliant Green contains 10 mL of a 1:1000 solution of brilliant green.

### Warnings and Precautions

For *in vitro* Diagnostic Use or

For Laboratory Use as labeled.

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"<sup>9-12</sup> 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 to 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.

### 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.<sup>13-16</sup>

### PROCEDURE

**Material Provided:** Depending upon which product is ordered, one of the prepared tubed media listed in "Availability."

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

**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 iodine-iodide solution to each tube: 0.2 mL for each 10 mL of medium. 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 to 24 h at 35 ± 2°C in an aerobic atmosphere.

### User Quality Control:

1. Examine tubes for signs of deterioration as described under "Product Deterioration."
2. Check performance of the complete medium by inoculating a representative sample of tubes with pure cultures of stable control organisms that give known, desired reactions. The following test strains are recommended:

### EXPECTED RESULTS

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

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 NCCLS guidance and CLIA regulations for appropriate Quality Control practices.

### RESULTS

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

### 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.<sup>4-7,14,16,17</sup>

### 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 *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 24 h of incubation at 35-37°C in an aerobic atmosphere. Plates are incubated overnight at 35-37°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.

### AVAILABILITY

Cat No.	Description
298249	BBL™ Tetrathionate Broth Base, Pkg. of 10 size D tubes, 10 mL C€
297710	BBL™ Tetrathionate Broth Base with Brilliant Green, Ctn. of 100 size A tubes, 20 mL
292526	BBL™ Tetrathionate Broth Base, Ctn. of 100 size D tubes, 10 mL
293049	BBL™ Tetrathionate Broth Base, Ctn. of 100 size D tubes, 9 mL
292527	BBL™ Tetrathionate Broth Base with Brilliant Green, Pkg. of 10 size D tubes, 10 mL

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