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

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

*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.

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.²⁻¹²⁻¹⁷

**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 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**