**BD™ Tetrathionate Broth Base • BD Iodine Solution (for Tetrathionate Broth Base)**

**INTENDED USE**

BD Tetrathionate Broth Base, after supplementation with BD Iodine Solution, is a selective enrichment medium for *Salmonella* species from human stools and from a variety of foods. The completed medium is also known as TT Broth.

**PRINCIPLES AND EXPLANATION OF THE PROCEDURE**

Microbiological method. *Salmonella* species cause many types of infections, from mild self-limiting gastroenteritis to life-threatening typhoid fever.\(^1\) The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than two days and diarrhea lasting less than 7 days.\(^1\) Mueller demonstrated the effectiveness of Tetrathionate Broth for enriching typhoid and paratyphoid bacilli while inhibiting coliform organisms.\(^2\) Using modified Mueller’s broth, Kauffmann increased the number of positive isolates.\(^3,4\) Tetrathionate Broth was used in studies for the poultry industry and in a collaborative study for rapid screening of *Salmonella* in food.\(^5-7\) Tetrathionate Broth is specified in standard methods for *Salmonella* testing and is used in processing fecal cultures for bacteria.\(^8-15\) Tetrathionate Broth Base, when supplemented with iodine/iodide solution, is used as a selective enrichment for the cultivation of *Salmonella* species that may be present in small numbers and compete with intestinal flora. As a result, they may not be detected by primary plating of the specimen on selective differential solid media.

In BD Tetrathionate Broth Base, Proteose Peptone provides nitrogen, carbon, vitamins and amino acids. Selectivity is accomplished by the combination of sodium thiosulfate and tetrathionate, which suppresses commensal intestinal organisms.\(^16\) (Tetrathionate is formed in the medium upon addition of the iodine and potassium iodide contained in BD Iodine Solution.) Organisms containing the enzyme tetrathionate reductase will proliferate in the medium.\(^17\) Bile salts, a selective agent, suppress coliform bacteria and inhibit gram-positive organisms. Calcium carbonate neutralizes and absorbs toxic metabolites and provides a stable pH value.

**REAGENTS**

Formulas* Per Liter Purified Water

<table>
<thead>
<tr>
<th>BD Tetrathionate Broth Base</th>
<th>BD Iodine Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto™ Proteose Peptone 5.0 g</td>
<td>Iodine 300.0 g</td>
</tr>
<tr>
<td>Bacto Bile Salts 1.0</td>
<td>Potassium iodide 250.0</td>
</tr>
<tr>
<td>Sodium Thiosulfate 30.0</td>
<td>Appearance: reddish-brown</td>
</tr>
<tr>
<td>Calcium Carbonate 10.0</td>
<td></td>
</tr>
<tr>
<td>pH 8.4 +/- 0.2</td>
<td></td>
</tr>
<tr>
<td>Appearance: Nearly colorless to slightly yellowish, with heavy white precipitate</td>
<td></td>
</tr>
<tr>
<td>Appearance after addition of iodine solution: Brownish, heavy precipitate</td>
<td></td>
</tr>
</tbody>
</table>

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

**PRECAUTIONS**

IVD. For professional use only.

Do not use vials or bottles if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration. For completion of BD Tetrathionate Broth Base, follow the procedures described under Reagent Preparation.
Consult GENERAL INSTRUCTIONS FOR USE document for aseptic handling procedures, biohazards, and disposal of used product.

**Warning:** BD Iodine Solution (for Tetrathionate Broth Base): Hazard-determining components of labeling: iodine

**STORAGE AND SHELF LIFE**

On receipt, store vials with BD Tetrathionate Broth Base in the dark at 5 to 25°C until just prior to use. Avoid freezing and overheating. The vials may be inoculated up to the expiration date and incubated for the recommended incubation times. Vials from opened packages can be used up to the expiration date. Opened vials must be used immediately. Store BD Iodine Solution at 15 to 22°C in the dark; do not refrigerate. Close the bottle tightly! Bottles from opened packages can be used up to the expiration date. Opened bottles can be used repeatedly up to the expiry date if closed and stored properly after each use.

**USER QUALITY CONTROL**

Before use, complete BD Tetrathionate Broth Base by adding BD Iodine Solution (for details, see PROCEDURE - Reagent Preparation). Inoculate the vials with 10 to 100 CFU of the Salmonella strains per vial. Use $10^4$ to $10^5$ CFU for the remaining strains. Incubate for 18 to 24 h at 35 ± 2°C. After incubation, subculture 10 to 20 µl onto BD MacConkey II Agar plates; incubate the plates for 18 to 24 hours at 35 ± 2°C and record growth.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Growth</th>
<th>Growth upon subculture on BD MacConkey II Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella Typhimurium ATCC™ 14028</em></td>
<td>Good to excellent</td>
<td>Good to excellent</td>
</tr>
<tr>
<td><em>Salmonella Abony DSM 4224</em></td>
<td>Good to excellent</td>
<td>Good to excellent</td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 25922</em></td>
<td>Weak to good</td>
<td>Inhibition partial</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 19433</td>
<td>Weak or none</td>
<td>Inhibition partial to complete</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>Weak or none</td>
<td>Inhibition partial to complete</td>
</tr>
</tbody>
</table>

**PROCEDURE**

**Materials Provided**

- **BD Tetrathionate Broth Base**, 12 ml, provided in 30 ml screw-cap vials. Microbiologically controlled.
- **BD Iodine Solution (for Tetrathionate Broth Base)**, 40 ml, provided in 50 ml bottles with screw cap.

**Materials Not Provided**

Ancillary culture media, reagents and laboratory equipment as required.
Reagent Preparation
To complete BD Tetrathionate Broth Base, add 2% (or 0.24 ml) of BD Iodine Solution (for Tetrathionate Broth Base) per vial (=12 ml). After completion, close the tube and mix gently. Do not heat! Note that the white sediment (calcium carbonate) does not dissolve after completion. Inoculate the completed medium within 2 hours after addition of the iodine solution!

Specimen Types
The completed Tetrathionate Broth is used as an enrichment broth for Salmonella from a variety of foods and from human stool specimens (see also PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE).

Test Procedure
Add 1.0 to 3.0 g of solid specimens or 1.0 to 3.0 ml of liquid specimens per tube of completed Tetrathionate Broth. Smaller amounts of stool may be used during the acute phase of an infection. Mix carefully and incubate for 12 to 24 hours at 35 ± 2° C in an aerobic atmosphere. Food materials with high numbers of contaminants are incubated at 43 ± 0.2° C in a waterbath. For a complete discussion of the appropriate procedures for foods, refer to the references. Note that stool specimens should also be streaked directly (without prior enrichment) onto appropriate plated media.1,14,15

Results
After the incubation, subculture Tetrathionate medium onto appropriate selective differential media, e.g. BD XLD Agar or BD Hektoen Enteric Agar and on BD MacConkey II Agar. Consult the references.1,11-15

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE
BD Tetrathionate Broth Base, after completion with BD Iodine Solution, is a standard medium which is used for the enrichment of Salmonella species from foods and human stool specimens.1,8-15 Tetrathionate broth and Rappaport-Vassiliadis medium were shown to be the most sensitive media for food materials with low Salmonella counts.16 Since the nutritional requirements of organisms vary, some strains of Salmonella may be encountered that fail to grow or grow poorly in this medium. Isolation techniques should always include a variety of enrichment broths and isolation media. Consult the references.1,14,15 Isolates obtained from this medium must be subjected to further biochemical and serological tests to obtain a complete identification.1,14,15

REFERENCES


PACKAGING/AVAILABILITY
BD Tetrathionate Broth Base (partially completed bottled medium)
Cat. No. 257103 cpu 50 12 ml fill volume; in 30 ml screw-cap vials

BD Iodine Solution (for Tetrathionate Broth Base) (partially completed bottled medium)
Cat. No. 257199 cpu 1: 40 ml fill volume; in a 50 ml screw cap bottle

FURTHER INFORMATION
For further information please contact your local BD representative.

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