

## Fluid Tetrathionate HiVeg<sup>®</sup> Medium w/o Iodine and BG (Tetrathionate HiVeg<sup>®</sup> Broth Base w/o Iodine & BG)

MV032

### Intended Use:

Recommended for the selective enrichment method for isolating *Salmonella* serotype Typhi and other Salmonellae from faeces, urine, food and other material of sanitary importance.

### Composition\*\*

Ingredients	g / L
HiVeg <sup>®</sup> hydrolysate	2.500
HiVeg <sup>®</sup> peptone	2.500
Synthetic detergent	1.000
Calcium carbonate	10.000
Sodium thiosulphate	30.000

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 46.0 grams in 1000 ml purified / distilled water and heat just to boiling. **DO NOT AUTOCLAVE**. Cool below 45°C and add 20 ml iodine solution (iodine- 6 grams and potassium iodide- 5 grams in 20 ml distilled water) and 10 ml of 0.1% brilliant green solution. Mix well and dispense in 10 ml quantities. This complete medium should be used on the day of preparation otherwise sterilized broth base may be stored at 2-8°C for some time. Do not heat after the addition of iodine solution. Use the medium immediately after addition of iodine. Iodine Solution: Iodine- 6 grams and Potassium Iodide- 5 grams in 20 ml distilled water.

**Note:** Due to the presence of calcium carbonate, the prepared medium forms opalescent solution with white precipitate.

### Principle And Interpretation

*Salmonella* are ubiquitous in the environment. These organisms are usually present in small numbers compared to coliforms; therefore it is necessary to examine a relatively large sample to isolate the organisms (1). *Salmonella* present in food samples may be sub lethally damaged during various stages of food processing where they may be exposed to low temperatures, heat drying, radiations, various chemicals (2). These damaged cells are able to cause spoilage, and if ingested cause diseases under favorable conditions. Therefore it is important to resuscitate these damaged bacteria before enumeration. Fluid Tetrathionate Medium (with added iodine and brilliant green) is recommended for the selective enrichment of *Salmonella* including *Salmonella* Typhi from faeces, urine, food and other material of sanitary importance. The medium, originally formulated by Mueller (3) is recommended by APHA (4,5,6) for enrichment of *Salmonella*. Fluid Tetrathionate HiVeg<sup>®</sup> Medium is same as Fluid Tetrathionate Medium except that the animal based peptones are completely replaced with vegetable peptones to avoid BSE/TSE risks associated with animal peptones. Due to the addition of iodine and potassium iodide, tetrathionate is formed in the medium. Organisms possessing the enzyme tetrathionate reductase grow in this medium.

HiVeg<sup>®</sup> hydrolysate and HiVeg<sup>®</sup> peptone are the sources of carbon, nitrogen, vitamins and minerals. Synthetic detergent inhibit accompanying gram-positive microorganisms. The selectivity depends on the ability of thiosulphate and tetrathionate in combination to suppress commensal coliform organism (7,8). Calcium carbonate neutralizes the acidic tetrathionate decomposition products. Brilliant green also helps to select *Salmonella* by inhibiting the accompanying bacteria. For further confirmation, streak the enriched cultures after incubation, on plates of Brilliant Green HiVeg<sup>®</sup> Agar (MV016), MacConkey HiVeg<sup>®</sup> Agar (MV081) and Bismuth Sulphite HiVeg<sup>®</sup> Agar (MV027).

Aseptically inoculate test specimen into Fluid Tetrathionate medium (with added iodine and brilliant green) and incubate at 35-37°C for 18-24 hours. Following the incubation, isolate onto selective media plates. Refer standard procedures for enrichment and isolation (4,5,6).

### Type of specimen

Food samples.

## Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,9). After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations

1. For further confirmation, streak the enriched cultures after incubation, on plates of Brilliant Green HiVeg<sup>®</sup> Agar (MV016), MacConkey HiVeg<sup>®</sup> Agar (MV081) and Bismuth Sulphite HiVeg<sup>®</sup> Agar (MV027).

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

### Appearance

White to cream homogeneous free flowing powder

### Colour and Clarity of prepared medium

Complete medium with added brilliant green and iodine solution - Light green coloured, opalescent solution with heavy white precipitate, which on standing the precipitate settles down.

### Cultural Response

Cultural characteristics observed with added brilliant green and iodine solution when sub cultured on MacConkey HiVeg<sup>®</sup> Agar (MV081) after enrichment in Tetrathionate HiVeg<sup>®</sup> medium, after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Recovery	Colour of colony
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	little or no increase in number	pink-red with bile precipitate
<i>Salmonella</i> Choleraesuis ATCC 12011	50-100	good-excellent	colourless
<i>Salmonella</i> Typhi ATCC 6539	50-100	good-excellent	colourless
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	good-excellent	colourless
<i>Escherichia coli</i> ATCC 8739 (00012*)	50-100	little or no increase in numbers	pink-red with bile precipitate

Key : \*Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

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## Reference

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