

## Bismuth Sulphite HiVeg™ Agar Modified

MV1004

### Intended use

Recommended for the selective isolation and preliminary identification of *Salmonella* Typhi and other *Salmonellae* from sewage, water supplies, food etc.

### Composition\*\*

Ingredients	g / L
HiVeg™ peptone	5.000
HiVeg™ extract	5.000
Dextrose (Glucose)	5.000
Disodium hydrogen phosphate	4.000
Ferrous sulphate	0.300
Bismuth sulphite indicator	8.000
Brilliant green	0.016
Agar	12.700
Final pH ( at 25°C)	7.6±0.2

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

### Directions

Suspend 40.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT STERILIZE IN AUTOCLAVE** or by fractional sterilization since overheating may destroy the selectivity of the medium. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

*Note: The sensitivity of the medium depends largely upon uniform dispersion of precipitated bismuth sulphite in the final gel, which should be dispersed before pouring into the sterile Petri plates.*

### Principle And Interpretation

The *Salmonellae* constitute the most taxonomically complex group of bacteria among *Enterobacteriaceae* (1). Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta. Humans are the only reservoirs of *S. Typhi* (2). Four clinical types of *Salmonella* infections may be distinguished (3) namely gastroenteritis, bacteremia or septicemia, enteric fever and a carrier state. Of the various media employed for the isolation and preliminary identification of *Salmonellae*, particularly *Salmonella* Typhi; Bismuth Sulphite Agar is the most productive (4). Bismuth Sulphite Agar, Modified is a modification of the original formulation of Wilson and Blair Medium (5). It is also recommended for the isolation of *Salmonella* Typhi and other *Salmonella* (6,7). Bismuth Sulphite HiVeg™ Agar Modified is same as Bismuth Sulphite Agar Modified except that the animal based peptones are completely replaced by vegetable peptones to avoid the BSE/TSE risks associated with the animal peptones.

*S. Typhi*, *S. Enteritidis* and *S. Typhimurium* typically grow as black colonies with a surrounding metallic sheen resulting from hydrogen sulphide production and reduction of sulphite to black ferric sulphide. *Salmonella* Paratyphi A grows as light green colonies.

Bismuth Sulphite HiVeg™ Agar may be inhibitory to some strains of *Salmonella* species and therefore should not be used as the sole selective medium for these organisms. *Shigella* species are mostly inhibited on this medium; exceptions being *S. flexneri* and *S. sonnei* (8) and also some *Salmonella* like *S. Sendai*, *S. Berta*, *S. Gallinarum*, *S. Abortus-equi* are inhibited (8). Also this medium favors use of larger inoculum as compared to other selective media, as it has unique inhibitory action toward gram-positive organisms and coliforms.

HiVeg™ peptone and HiVeg™ extract serve as sources of carbon, nitrogen, vitamins and essential growth factors. Dextrose (Glucose) is the carbon source. Disodium hydrogen phosphate maintains the osmotic equilibrium. Bismuth sulphite indicator along with brilliant green inhibits the intestinal gram-positive and gram-negative bacteria. Ferrous sulphate aids in detection of hydrogen sulphide production. Clinical samples can be directly used to inoculate Bismuth Sulphite HiVeg™ Agar. In case of food samples, pre enrichment of the sample is done prior to inoculation.

## Type of specimen

Food and dairy samples ; Water samples.

## Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9,10,11).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (12). 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. **DO NOT AUTOCLAVE OR OVERHEAT THE MEDIUM**, as it destroys the selectivity of the medium.
2. *S. Typhi* exhibit typical brown colonies, with or without metallic sheen.
3. This medium is highly selective and must be used in parallel with less selective media for isolation.
4. With certain *Salmonella* species, typical black colonies with metallic sheen is observed near heavy inoculation and isolated colonies may show green colonies.
5. *Shigella* species are mostly inhibited on this medium; exceptions being *S. flexneri* and *S. sonnei* .
6. Some *Salmonella* like *S. Sendai*, *S. Berta*, *S. Gallinarum*, *S. Abortus-equi* are also inhibited .

## 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

Light yellow to greenish yellow homogeneous free flowing powder

### Gelling

Firm, comparable with 1.27% Agar gel.

### Colour and Clarity of prepared medium

Greenish yellow coloured opalescent with flocculent precipitate forms in Petri plates.

### Reaction

Reaction of 4.0% w/v aqueous solution at 25°C. pH : 7.6±0.2

### pH

7.40-7.80

### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<b>Preparation of test strain</b>				
# <i>Klesiella aerogenes</i> ATCC 13048 (00175*)	50-100	none-poor	<=10%	brown-green(depends on inoculum density)
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	>=10 <sup>4</sup>	inhibited	0%	
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	none-poor	<=10%	brown-green(depends on inoculum density)
<i>Salmonella Typhi</i> ATCC 19430	50-100	good-luxuriant	>=50%	black with metallic sheen

<i>Salmonella</i> Paratyphi B ATCC 8759	50-100	good-luxuriant	$\geq 50\%$	black with metallic sheen
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	$\geq 50\%$	black with metallic sheen
<i>Shigella flexneri</i> ATCC 12022 (00126*)	50-100	none-poor	$\leq 10\%$	brown
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	$\geq 50\%$	black with metallic sheen

Key : (\*) - Corresponding WDCM numbers.

(#)- Formerly known as *Enterobacter aerogenes*

### Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C, but not for more than 2 days as after which dye oxidizes to give green medium that could be inhibitory to some Salmonellae. 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 (13,14).

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