

Bismuth Sulphite HiVeg™ Agar

MV027

Intended Use:

This medium is prepared by completely replacing animal based peptones with vegetable peptones. Recommended as a general culture medium for the selective isolation and preliminary identification of *Salmonella* Typhi and other *Salmonellae* species from various samples.

Composition**

Ingredients	g / L
HiVeg™ peptone	10.000
HiVeg™ extract	5.000
Dextrose (Glucose)	5.000
Disodium phosphate	4.000
Ferrous sulphate	0.300
Bismuth sulphite indicator	8.000
Brilliant green	0.025
Agar	20.000
Final pH (at 25°C)	7.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 52.33 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. 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 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). Of the various media employed for the isolation and preliminary identification of *Salmonellae*, particularly *Salmonella* Typhi; Bismuth Sulphite Agar is the most productive. Bismuth Sulphite Agar is a modification of original Wilson and Blair Medium (3-5). It is also recommended by various Associations (2,6-8) for the isolation and preliminary identification of *Salmonella* Typhi and other *Salmonellae* from pathological materials, sewage, water, food and other products.

Bismuth Sulphite HiVeg™ Agar is same as Bismuth Sulphite Agar except that the animal based peptones are completely replaced with vegetable peptones to avoid BSE/TSE risks associated with 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. Also this medium favors use of larger inoculum as compared to other selective media, as it has unique inhibitory action towards gram-positive organisms and coliforms. HiVeg™ peptone and HiVeg™ extract serve as sources of carbon, nitrogen, long chain amino acids, vitamins and essential growth factors. Dextrose is the carbon source. Disodium 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

Clinical samples, Foodstuff, water samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (2). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. 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 clinical 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* and *S.Arizonae* 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

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 2.0% agar gel.

Colour and Clarity of prepared medium

Yellow to Greenish yellow opalescent with flocculent precipitate forms in Petri plates.

Reaction

Reaction of 5.23% w/v aqueous solution at 25°C. pH : 7.7±0.2

pH

7.50-7.90

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
# <i>Klesiella aerogenes</i> ATCC 13048 (00175*)	50-100	none-poor	<=10%	brown-green (depends on the inoculum density)
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	>=10 ⁴	inhibited	0%	
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	none-poor	<=10%	brown-green (depends on the inoculum density)
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	>=50%	black with metallic sheen
<i>Salmonella</i> Typhi ATCC 6539	50-100	good-luxuriant	>=50%	black with metallic sheen

<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	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>Escherichia coli</i> ATCC 8739 (00012*)	50-100	none-poor	$\leq 10\%$	brown to green, depends on inoculum density
<i>Salmonella</i> Abony NCTC 6017 (00029*)	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 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

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