

Brilliant Green HiVeg® Agar Base w/1.2% Agar

MV016A

Intended use

Recommended as enrichment medium for isolation of *Salmonellae* from food, dairy and water samples.

Composition**

Ingredients	g / L
HiVeg® peptone No. 3	10.000
Yeast extract	3.000
Lactose	10.000
Saccharose (Sucrose)	10.000
Sodium chloride	5.000
Phenol red	0.080
Brilliant green	0.0125
Agar	12.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. **AVOID OVERHEATING.** Cool to 45-50°C. For more selectivity, aseptically add rehydrated contents of two vials of S Selective Supplement (FD068). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Salmonella species cause many types of infections, from mild self-limiting gastroenteritis to life threatening typhoid fever. The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhoea lasting less than 7 days.

Brilliant Green Agar Base, Modified, as a primary plating medium for isolation of *Salmonella* species was first described by Kristensen et al (1) and further modified by Kauffmann (2) and recommended by APHA (3,4) FDA (5) and USP (6). Brilliant Green HiVeg® Agar Base w/1.2% Agar is same as Brilliant Green Agar Base w/1.2% Agar, except that the animal peptones are completely replaced by vegetable peptones to avoid BSE/TSE risks associated with animal peptones. These media contain brilliant green which inhibits growth of majority of gram-negative and gram-positive bacteria. *Salmonella* Typhi, *Shigella* species, *Escherichia coli*, *Proteus* species, *Pseudomonas* species *Staphylococcus aureus* are mostly inhibited. Clinical specimens can be directly plated on this medium. However, being highly selective, it is recommended that this medium should be used along with a less inhibitory medium to increase the chances of recovery. Often cultures enriched in Selenite (MV025) or Tetrathionate HiVeg® Broth (MV032) are plated on Brilliant Green HiVeg® Agar Base w/ 1.2% Agar as well as Bismuth Sulphite HiVeg® Agar (MV027), SS HiVeg® Agar (MV108) and MacConkey HiVeg® Agar (MV081).

HiVeg® peptone No. 3 and yeast extract provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Phenol red serves as an acid base indicator giving yellow colour to lactose and/or sucrose fermenting bacteria. Lactose non-fermenting bacteria develop white to pinkish red colonies within 18-24 hours of incubation.

Type of specimen

Food and dairy; Water samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(7). 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. *Salmonella* Typhi and *Shigella* species may not grow on this medium, moreover *Proteus*, *Pseudomonas* and *Citrobacter* species may mimic enteric pathogens by producing small red colonies.
2. However, being highly selective, it is recommended that this medium should be used along with a less inhibitory medium to increase the chances of recovery.

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

Beige to light pink coloured homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% agar gel.

Colour and Clarity of prepared medium

Greenish brown coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.0% w/v aqueous solution at 25°C. pH : 6.9±0.2

Cultural Response

Cultural response was carried out after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	good-luxuriant	≥50 %	pinkish white
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	good-luxuriant	≥50 %	pinkish white
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	≥50 %	pinkish white
<i>Salmonella</i> Typhi ATCC 6539	50 -100	fair-good	30 -40 %	reddish pink
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	none-poor	0 -10 %	yellowish green
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	none-poor	0 -10 %	yellowish green
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	≥10 ⁴	inhibited	0%	
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	≥10 ⁴	inhibited	0%	

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 (8,9).

Reference

1. Kristensen M., Lester V. and Jurgens A., 1925, Brit.J.Exp.Pathol.,6:291.
2. Kauffman F., 1935, Seit F. Hyg., 177:26.
3. Marshall R. (Ed.), 1992, Standard Methods for the Microbiological Examination of Dairy Products, 16th ed., APHA, Washington, D.C.
4. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Bacteriological Analytical Manual, 1988, AOAC, Washington D.C.
6. The United States Pharmacopoeia-National Formulary (USP-NF), 2022
7. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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Disclaimer :

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