Technical Data

Wilson Blair HiVeg™ Agar Base MV331

Intended Use:

With the addition of selective reagent used for the isolation of *Salmonella* Typhi

Composition**

Ingredients	g / L
HiVeg™ special peptone	10.000
HiVeg™ extract	5.000
Dextrose (Glucose)	10.000
Sodium chloride	5.000
Agar	30.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 60.0 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. Cool to 45-50°C. To sterile molten base, add 4 ml of 1% Brilliant green solution and 70 ml of selective reagent.

Selective Reagent

Solution 1 : 40 gm sodium sulphite in 100 ml distilled water.

Solution 2 : 21 gm dibasic sodium phosphate in 100 ml distilled water.

Solution 3 : 12.5 gm bismuth ammonium citrate in 100 ml distilled water.

Solution 4 : 0.96 gm ferrous sulphate in 20 ml distilled water with 2 drops of hydrochloric acid.

Prepare each solution separately and then combine. Boil the combined solution until a slate grey colour develops. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Salmonella is a genus of gram-negative *Enterobacteriaceae*- commonly implicated in foodborne illness and the causative agent of typhoid and paratyphoid fever. *Salmonella* species have been isolated from humans and animals. More than 2000 serovars of *Salmonella* exists with each showing different host specificities. For example, humans are the only known natural reservoir for serotype *Salmonella* Typhi and serotypes *Salmonella* Paratyphi A, B and C (1).The organism can be transmitted by the faecal-oral route. It is excreted by humans in faeces and may be transmitted by contaminated water, food, or by person-to-person contact (with inadequate attention to personal hygiene). Wilson and Blair Agar, formulated by Wilson and Blair (2) is recommended for isolating *Salmonella* species especially *Salmonella* Typhi from clinical specimens. The selective reagent formulation is a modification of the bismuth sulphite reagent described by Hajna and Perry (3). This medium is particularly valuable for the isolation of *S.* Typhi. The medium is highly selective for *Salmonella*, being inhibitory to coliforms, *Proteus* and *Shigella*; occasional strains of coliforms grow to form dull green or brown colonies, but without a surrounding metallic sheen. The medium is also suitable for the isolation of lactose-fermenting strains of *Salmonella* (which can not be differentiated on lactose containing differential media) since lactose is not the fermentable substrate used in this medium. Wilson Blair HiVeg™ Agar Base is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. HiVeg™ special peptone and HiVeg™ extract provide nitrogenous, carbonaceous compounds and other growth nutrients. Brilliant green dye inhibits all gram-positive bacteria. Dextrose is the fermentable carbohydrate. Ferrous sulphate aids in H_2S production. Bismuth is a heavy metal, which is inhibitory to most gram-negative enteric bacilli other than *Salmonella*. Ferrous sulphate is reduced by *Salmonella* species in presence of bismuth sulphite and dextrose to form iron sulphide, indicated by black coloured colonies. Disodium hydrogen phosphate buffers the medium well. Sodium chloride balances the osmotic equilibrium.

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 (4,5,6). 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. Do not store the medium in refrigerator (4°C) for longer than 2 days, as the medium changes to green colour and reduces its selectivity.
2. Further biochemical and serological tests need to be carried out for confirmation.

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

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 3.0% Agar gel.

Colour and Clarity of prepared medium

Basal Medium : Light yellow coloured clear to slightly opalescent gel. After addition of the selective reagent and 1%Brilliant green, greenish yellow coloured, opaque gel forms in Petri plates.

Reaction

Reaction of 6.0% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed with added 1% Brilliant green and selective reagents after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=50%	green
Salmonella Typhi ATCC 6539	50-100	luxuriant	>=50%	black with sheen
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	>=50%	black with sheen

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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

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4. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
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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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