

## Brucella HiVeg<sup>®</sup> Agar Base

MV074

### Intended Use:

Recommended for enrichment, isolation and cultivation of *Brucella* or *Campylobacter* species from various samples.

### Composition\*\*

Ingredients	g / L
HiVeg <sup>®</sup> hydrolysate	10.000
HiVeg <sup>®</sup> peptone	10.000
Yeast extract	2.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
Sodium bisulphite	0.100
Agar	15.000
Final pH ( at 25°C)	7.0±0.2

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

### Directions

Suspend 21.55 grams in 500 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 and aseptically add sterile 5% (v/v) inactivated horse serum RM1239 (Inactivate RM1239 by heating at 56°C for 30 minutes) and rehydrated contents of one vial of Hexa Selective Supplement, Modified (FD161). For isolation of *Campylobacter* species add rehydrated content of one vial of Minerals Growth Supplement (FD009), 5-7% defibrinated sheep blood to 500 ml media. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

*Brucella* are intracellular parasites that cause epizootic abortions in animals and septicemic febrile illness or localized infections of bone, tissue or organ systems in humans (1,2). *Brucella* species are highly fastidious and therefore require a nutrient rich medium to be able to grow. Also, *Brucella* species are highly infective and so extreme care should be taken while handling.

Brucella HiVeg<sup>®</sup> Agar Base is used for the isolation and cultivation of *Brucella* species. The basal medium (with addition of Campylobacter Supplements) can be also used for the isolation of *Campylobacter* (3). Brucella Medium is a modified medium formulated to support luxuriant growth of fastidious bacteria like *Brucella*, Streptococci, Pneumococci, *Listeria*, *Neisseria meningitides* and *Haemophilus influenzae* (4).

Brucella Agar is also recommended by APHA for isolation of *Brucella* species from foods (5). Brucella HiVeg<sup>®</sup> Agar Base is same as Brucella Agar except that the animal based peptones are completely replaced by vegetable peptones to avoid the BSE/TSE risks associated with the animal peptones.

HiVeg<sup>®</sup> hydrolysate and HiVeg<sup>®</sup> peptone provide nitrogen and carbon source, long chain amino acids, vitamins and other essential nutrients. Yeast extract serves as a source of vitamin B complex, and additionally it also supplies some nitrogenous nutrients. Sodium bisulphite is a reducing agent and sodium chloride helps to maintain the osmotic equilibrium of the medium. Dextrose serves as an energy source. The medium can also be enriched with 5% v/v sterile defibrinated horse blood. For selective isolation of *Brucella* species antibiotic mixtures in the form of freeze dried supplements (FD) are incorporated into the base (6,7,8).

### Type of Specimen

Food samples

### Specimen Collection and Handling

For food samples follow appropriate techniques for handling specimens as per established guidelines (5).

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,9).

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. All presumptive anaerobic organisms must be identified by confirmatory test.

## 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 1.5% Agar gel

### Colour and Clarity of prepared medium

Yellow coloured, clear to slightly opalescent gel forms in Petri plates

### Reaction

Reaction of 4.31% w/v aqueous solution at 25°C. pH : 7.0±0.2

### pH

6.80-7.20

### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-72 hours in presence of 10% CO<sub>2</sub> with added sterile 5% v/v inactivated horse serum (RM1239) and NPBCVN Selective Supplement (FD005).

Organism	Inoculum (CFU)	Growth
<i>Brucella melitensis</i> ATCC 4309	50-100	luxuriant
<i>Brucella suis</i> ATCC 4314	50-100	luxuriant
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	≥10 <sup>4</sup>	inhibited
<i>Escherichia coli</i> ATCC 25922 (00013*)	≥10 <sup>4</sup>	inhibited

Key : \*Corresponding WDCM numbers.

## Storage and Shelf Life

Store below 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 (4,9).

## Reference

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Reference no:10,11,12 are extra

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