

Brucella Agar Base, Granulated[®]

GM074

Intended Use:

For selective isolation and cultivation of *Brucella* or *Campylobacter* species from clinical and non-clinical specimens.

Composition**

Ingredients	g/ L
Tryptone	10.000
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 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. If required, for additional selectivity of *Brucella* species: Aseptically add sterile 5% v/v inactivated Horse Serum (RM1239, inactivated by heating at 56°C for 30 minutes) and rehydrated contents of one vial of NPBCVN Selective Supplement (FD005).

For *Campylobacter*: Add rehydrated contents of 1 vial of Blaser-Wang Selective Supplement (FD006) or Butzler Selective Supplement (FD007) or Skirrow Selective Supplement (FD008) and 5-7% defibrinated sheep blood to 500 ml sterile medium. For growth enhancement add rehydrated contents of 1 vial of Minerals Growth Supplement (FD009). Mix well before pouring 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 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).

Tryptone and 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

Clinical : stool samples, rectal swabs, etc., Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,9). Swab specimens can be directly streaked on the plate. Liquid specimens can be inoculated by means of an inoculation loop. When non-selective medium is required, Brucella Broth Base may be employed with the addition of serum only (i.e. without antibiotics).

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

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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. 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 granular media

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% CO₂ 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>Escherichia coli</i> ATCC 25922 (00013*)	≥10 ⁴	inhibited
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	≥10 ⁴	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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,9).

Reference

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3. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Tenover F. C., Tenover F. C., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
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5. 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.
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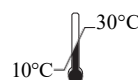
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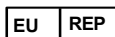
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**In vitro diagnostic
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