



Brucella Agar Base with Hemin and Vitamin K

M1039

Brucella Agar Base with Hemin and Vitamin K is recommended for the isolation, cultivation and subculture of *Brucella* species and other anaerobes.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Peptic digest of animal tissue	10.000
Yeast extract	2.000
Dextrose	1.000
Sodium chloride	5.000
Sodium bisulphite	0.100
Hemin	0.010
Vitamin K1	0.010
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 43.12 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 5% v/v sterile defibrinated sheep blood. Mix well before pouring into sterile Petri plates.

Principle And Interpretation

The agents of brucellosis, *Brucella* species are normal flora of the genital and urinary tracts of many animals including goats, pigs, cows and dogs. Most humans acquire the disease through ingestion of contaminating milk or through occupational exposure; the disease is particularly common among abattoir workers (1).

Brucella Agar Base w/ Hemin and Vitamin K1 is a modified (4, 5, 6) and highly enriched medium, which can be used for the isolation of *Brucella* and other anaerobic bacteria (2, 3).

The medium contain casein enzymic hydrolysate, peptic digest of animal tissue and yeast extract as sources of carbon, nitrogen and essential growth nutrients including B-complex vitamins. Dextrose serves as a source of energy. Addition of blood provides nutrients and helps to differentiate hemolytic organisms (2, 3). Presence of hemin and Vitamin K1 supports growth of other fastidious bacteria like *Bacteroides* species and gram-positive spore bearers like *Clostridium* species (7). The specimen should be inoculated onto the plate (reduced earlier by placing under anaerobic conditions for 18- 24 hrs) as early as possible. Swab cultures are directly streaked. Non-swab cultures are inoculated using an inoculating loop.

Incubation is carried out anaerobically at 35°C for at least 48 hours; however, negative results should be reported only after incubation for 7 days.

Quality Control

Appearance

Light yellow to tan homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Basal medium :Light amber coloured clear to slightly opalescent gel. After addition of 5% v/v sterile defibrinated blood: Cherry red coloured opaque 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

M1039: Cultural characteristics observed in presence of 10% CO₂ with added 5% v/v sterile defibrinated sheep blood, after an incubation at 35-37°C for 48 hours.

Organism**Growth****Cultural Response**

Bacteroides fragilis ATCC 25285 good-luxuriant

Clostridium perfringens ATCC 13124 good-luxuriant

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

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3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore
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5. Onderdonk A. B., Weinstein W. M., Sullivan N. M. and Bartlett J. G., 1974, Infect. Immun., 10:1256.
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