Brucella Vitamin K1 Blood Agar Base

M823

Brucella Vitamin K1 Blood Agar Base 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
Dextrose	1.000
Yeast extract	2.000
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 43.1 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 45-50°C and aseptically add 5% v/v sterile defibrinated sheep blood. Aseptically add sterile Vitamin K1 solution to give a final concentration of 10 mcg/ml. 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 was originally developed for isolation of *Brucella* species. But with the supplementation of blood and other nutritious substances, it can be used for growth and isolation of various fastidious organisms. Brucella Blood Agar Base was modified by the addition of Vitamin K1 by Sutter et al (4). Brucella Vitamin K1 Blood Agar Base is a highly enriched medium, which can be used for the isolation of anaerobic bacteria (2, 3).

The medium contains 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). Addition of hemin and Vitamin K1 supports growth of other fastidious bacteria like *Bacteroides* species and gram-positive spore bearers like *Clostridium* species (5). 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 an 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 K1 & 5% v/v sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates

Reaction
Reaction of 4.3% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
M823: Cultural characteristics observed in presence of 10% CO_2, under anaerobic condition with added 5%v/v defibrinated sheep blood and Vitamin K1, after an incubation at 35-37°C for 48 hours .

Organism	Growth
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
1. Baron E. J., Finegold S. M., (Eds.), 1990, Bailey and Scotts Diagnostic Microbiology, 8th Ed., The C.V. Mosby Co., St. Louis.
2. Zennette, Balows, Hausler and Shadomy, (Eds.), 1985, Manual of Clinical Microbiology, 4th Ed., ASM, Washington, D.C.
3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore
4. Sutter V. L., Citron D. M. and Finegold S. M., 1985, Wadsworth Anaerobic Bacteriology Manual, 4th Ed., Star Publishing Co., Belmont, Ca.
5. Gibbons and MacDonald, 1960, J. Bacteriol., 80:164.

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