Brucella Vitamin K1 Blood Agar Base

M823

Intended Use:

Recommended for isolation and subculture of *Brucella* species and other anaerobes.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Dextrose (Glucose)	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 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 5% v/v sterile defibrinated sheep blood. Aseptically add sterile Vitamin K1 solution to give a final concentration of 10 mcg/ml. Mix well and pour 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 (2).

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 (7). Brucella Vitamin K1 Blood Agar Base is a highly enriched medium, which can be used for the isolation of anaerobic bacteria (6,9).

The medium contains tryptone 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 (6,9). Addition of hemin and Vitamin K1 supports growth of other fastidious bacteria like *Bacteroides* species and gram-positive spore bearers like *Clostridium* species (3). 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.

Type of specimen

Dairy samples

Specimen Collection and Handling

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8). 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. Some organisms may show poor growth due to nutritional variation.
2. Further biochemical and serological testing is required for complete identification.

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

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

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 (00007*)	good-luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 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 (4,5).

Reference

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8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
9. Zennette, Balows, Hausler and Shadomy, (Eds.), 1985, Manual of Clinical Microbiology, 4th Ed., ASM, Washington, D.C.

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