



## BD™ Brucella Blood Agar with Hemin and Vitamin K1

### INTENDED USE

**BD Brucella Blood Agar with Hemin and Vitamin K1** is a highly nutritious medium for the isolation and cultivation of strict anaerobes from clinical specimens.

### PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

**BD Brucella Blood Agar with Hemin and Vitamin K1** is a modification of Brucella Agar that has been supplemented with hemin and vitamin K1 to support the growth of fastidious anaerobes, especially *Bacteroides*, *Prevotella*, and *Porphyromonas* when incubated anaerobically.<sup>1-3</sup> It is used for the isolation of strict anaerobes from clinical specimens. It is also used for susceptibility testing of anaerobes with the E test® method.<sup>4-6</sup>

In **BD Brucella Blood Agar with Hemin and Vitamin K1**, peptones and yeast extract, together with glucose, supply nutrients. Sodium bisulfite lowers the redox potential to a range suitable for strict anaerobes. Hemin and vitamin K1 have been shown to be necessary for supporting the growth of certain strict anaerobes.<sup>7</sup> Sheep blood provides additional nutrients and is used to detect hemolytic reactions.

### REAGENTS

#### **BD Brucella Blood Agar with Hemin and Vitamin K1**

Formula\* Per Liter Purified Water

Pancreatic Digest of Casein	10.0 g
Peptic Digest of Animal Tissue	10.0
Yeast Extract	2.0
Glucose	1.0
Sodium Chloride	5.0
Sodium Bisulfite	0.1
Hemin	0.005
Vitamin K1	0.01
Agar	15.0
Sheep Blood, defibrinated	5%

pH 7.2 +/- 0.2

\*Adjusted and/or supplemented as required to meet performance criteria.

### PRECAUTIONS

**IVD** . For professional use only. Ⓢ

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

### STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

## USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate for 48 to 72 hours in an anaerobic atmosphere (e.g. **BD GasPak™** Anaerobic System) at 35 to 37° C.

Strains	Growth Results
<i>Bacteroides fragilis</i> ATCC™ 25285	Growth good to excellent; grey colonies
<i>Clostridium perfringens</i> ATCC 13124	Growth good to excellent; large, lobate, grey-white colonies; beta (double-zone) hemolysis
<i>Fusobacterium nucleatum</i> ATCC 25586	Growth good to excellent; grey-white colonies, surrounded by dark-grey zones
<i>Peptostreptococcus anaerobius</i> ATCC 27337	Growth good to excellent; whitish colonies
<i>Porphyromonas levii</i> ATCC 29147	Growth fair to good; dirty whitish to grey-brown colonies
Uninoculated	Red to dark red (blood color)

## PROCEDURE

### Materials Provided

**BD Brucella Blood Agar with Hemin and Vitamin K1** (90 mm **Stacker™** plates).  
Microbiologically controlled.

### Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

### Specimen Types

**BD Brucella Blood Agar with Hemin and Vitamin K1** is a universal medium for the isolation and cultivation of strict anaerobes from all types of specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). Observe approved techniques for collection and transport of anaerobic specimens.<sup>8-10</sup> Suitable transport media, e.g., **BD Port-A-Cul™**, must be used.

**BD Brucella Blood Agar with Hemin and Vitamin K1** is also used for susceptibility testing of strict anaerobes with the E test which requires the use of pure cultures.<sup>4-6</sup>

### Test Procedure

Streak the specimen directly after arrival, using an approved streaking technique onto **BD Brucella Blood Agar with Hemin and Vitamin K1**. Directly after streaking, place the plates into anaerobic jars supplied with an anaerobic atmosphere. It is recommended to use **BD GasPak** jars and **BD GasPak H<sub>2</sub>/CO<sub>2</sub>** envelopes together with a catalyst. Incubate for 2 to 3 days or longer, if necessary, at 35 to 37° C. Regardless of the anaerobic system used, it is important to include an indicator of anaerobiosis such as the **BD GasPak** disposable anaerobic indicator. If specimens containing mixed flora are streaked onto the medium, it is recommended to also include selective media such as **BD Schaedler Kanamycin-Vancomycin Agar with 5% Sheep Blood** and/or **BD Wilkins-Chalgren Agar with Amikacin and 7% Sheep Blood**. Also, facultative anaerobes might be present in the specimen. Therefore, it is recommended to always include an aerobic medium (such as **BD Columbia Agar with 5% Sheep Blood**) when the primary cultures are set up. This plate is incubated aerobically enriched with carbon dioxide together with the anaerobic cultures.<sup>8</sup> It allows the detection of facultative organisms in the specimen.

For use of **BD Brucella Blood Agar with Hemin and Vitamin K1** in anaerobic susceptibility testing with the E test, consult the references or the instructions of the manufacturer.<sup>4-6</sup>

### Results

After incubation, the plates are inspected for growth. Colonies which appear on this medium are suspected to be strict anaerobes if they do not grow on aerobically incubated blood agar plates. Also, growth on **BD Schaedler Kanamycin-Vancomycin Agar with 5% Sheep Blood** is compared to the growth on the other media. If mixed cultures of strict and facultative

anaerobes are present, appropriate subcultures on non-selective media, incubated aerobically and anaerobically, must be made from the anaerobic media to confirm that the isolate is a strict anaerobe.

Further microscopic and biochemical examination is necessary for the identification of the genera and species of the strict anaerobes. Consult appropriate texts for further information, including identification procedures.<sup>6, 8,9,11</sup>

For reading the E test results obtained on this medium, consult the references or the instructions of the manufacturer.<sup>4-6</sup>

## PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

On **BD Brucella Blood Agar with Hemin and Vitamin K1** which is one of the nonselective standard media for the isolation of strict anaerobes, *Bacteroides*, *Prevotella*, *Porphyromonas*, *Fusobacterium*, *Clostridium*, *Peptostreptococcus*, strictly anaerobic non-sporeforming rods (e.g., the former genus *Eubacterium*), *Mobiluncus*, *Actinomyces* and many others will grow.<sup>6,8-11</sup> Additionally, it is used for susceptibility testing with the E test method.<sup>4-6</sup>

### Performance Results<sup>12</sup>

**BD Brucella Blood Agar with Hemin and Vitamin K1** was evaluated internally with clinical isolates and collection strains of the following strictly anaerobic species and compared to **BD Schaedler Agar with Vitamin K1 and 5% Sheep Blood** (= reference medium): *Bacteroides fragilis*, *B. distasonis*, *Prevotella bivia*, *Fusobacterium nucleatum*, *Porphyromonas levii*, *Porphyromonas gingivalis*, *Campylobacter (Bacteroides) gracilis*, *Peptostreptococcus anaerobius*, *Clostridium perfringens*, *Mobiluncus mulieris*, *Eggerthella lenta (Eubacterium lentum)*. Both media were inoculated with 10<sup>3</sup> to 10<sup>4</sup> cfu per plate and were incubated anaerobically for 3 days. The number of colonies on the test medium was equal to or higher than that on test reference medium for all of the strains tested.

### Limitations of the Procedure

Note that the growth rates of strict anaerobes vary considerably: While *Bacteroides fragilis* will grow well after 24 hours, *Mobiluncus* or strains of *Porphyromonas* need 4 to 5 days, and *Actinomyces* may need 1 to 3 weeks to produce well visible colonies. If cultures are negative after 2 or 3 days of incubation, re-incubate anaerobically for an additional 2 to 3 days. If *Actinomyces* is suspected, dedicated culture plates of this and other media (e.g., **BD Columbia Agar with 5% Sheep Blood**) should be inoculated that are inspected after 1, 2 and eventually 3 weeks of incubation.

This medium is not specifically selective for strict anaerobes; facultative organisms will also grow. Therefore, it is important to compare the result of the anaerobic culture with that of an aerobically incubated plate if mixed cultures are obtained.

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## **PACKAGING/AVAILABILITY**

### **BD Brucella Blood Agar With Hemin And Vitamin K1**

Cat. No. 255509

Ready-to-use Plated Media, cpu 20

## **FURTHER INFORMATION**

For further information please contact your local BD representative.



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