

of these areas should exhibit isolated colonies of the organisms contained in the specimen. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

Broth

Growth in the tubes is indicated by the presence of turbidity compared with an uninoculated control.

If growth appears, cultures should be examined by Gram stain and subcultured onto appropriate media; e.g., **Trypticase™ Soy Agar with 5% Sheep Blood** and/or **Brucella Agar** and **Chocolate II Agar**, **Eosin Methylene Blue Agar**, **Levine or MacConkey II Agar**.

References

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Brucella Media for Anaerobes Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K₁ • Brucella Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV)

Intended Use

Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K₁ is used for the isolation and cultivation of fastidious, obligately anaerobic microorganisms.

Brucella Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV) is used for the selective isolation of fastidious and slow growing, obligately anaerobic bacteria from the same specimen.

Summary and Explanation

The isolation of obligately anaerobic bacteria from clinical and nonclinical materials requires the use of selective, nonselective and enrichment media.¹ Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K₁ is an enriched, nonselective medium for the isolation and cultivation of a wide variety of obligately anaerobic microorganisms. Nonselective media are used to isolate organisms present in low numbers and to provide an indication of the numbers and types of organisms present in the specimen or sample.

Kanamycin and vancomycin are included in Brucella Laked Blood KV Agar for use in selective isolation of gram-negative anaerobes, especially *Bacteroides*. The combination of kana-

Availability

BBL™ Brucella Agar

ISO USDA

Cat. No.	211086	Dehydrated – 500 g
	221547	Prepared Plates with 5% Horse Blood – Pkg. of 20*
	221548	Prepared Plates with 5% Horse Blood – Ctn. of 100*

BBL™ Brucella Agar with 5% Horse Blood// MacConkey II agar with MUG

Cat. No.	298303	Prepared I Plate™ Dishes – Ctn. of 100*
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BBL™ Brucella Broth

USDA

Cat. No.	211088	Dehydrated – 500 g
	296185	Dehydrated – 5 lb (2.3 kg)

*Store at 2-8°C.

mycin and vancomycin for this purpose was first described by Finegold et al.² Vancomycin, however, may inhibit *Porphyromonas asaccharolytica*.³

Principles of the Procedure

Brucella Agar supports the growth of fastidious microorganisms due to its content of peptones, dextrose and yeast extract. The sheep blood, hemin and vitamin K₁, provide essential nutrients for certain obligate anaerobes.⁴

The addition of the antimicrobial agents, kanamycin and vancomycin, renders Brucella Laked Blood KV Agar selective for gram-negative microorganisms. The kanamycin inhibits protein synthesis in susceptible organisms, whereas the vancomycin inhibits gram-positive bacteria by interfering with cell wall synthesis.⁵ The laked blood improves pigmentation of the *Prevotella melanigenica* - *P. asaccharolytica* group.

Procedure

These media should be reduced immediately prior to inoculation by placing under anaerobic conditions for 18-24 hours.⁶ An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BBL™ GasPak™ EZ** anaerobic systems or an alternative system.⁷

Streak the specimen as soon as possible after it is received in the laboratory. Minimize exposure to air. With liquid specimens, media should be inoculated with one drop of the specimen. Tissue specimens should be minced and then ground in sterile broth such as BBL Enriched Thioglycollate Medium before inoculation. Inoculation is then performed as for liquid specimens. Swab specimens may be rolled onto the first quadrant of plated media and then used to inoculate liquid media. Alternately, the swab may be “scrubbed” in a small volume of reduced broth and the broth used to inoculate media as performed with liquid specimens.

An enrichment broth such as BBL Enriched Thioglycollate Medium should be inoculated at the same time as the primary isolation plates.

Incubate immediately under anaerobic conditions or place in a holding jar flushed with oxygen-free gas(es) until sufficient plates are accumulated (but no longer than 3 hours).⁸ Incubation should be at $35 \pm 2^\circ\text{C}$ for at least 48 hours and up to 7 days. Regardless of anaerobic system used, it is important to include an indicator of anaerobiosis such as a GasPak anaerobic indicator.

Expected Results

Examine colonies using a dissecting microscope and with a long-wave UV lamp to detect fluorescence. Colonies of the pigmenting *Bacteroides* group should fluoresce orange to brick-red under long-wave UV light. Fluorescence is visible before pigmentation.

In order to determine the relationship to oxygen of each colony type present on anaerobic solid media, follow established procedures.⁹ Those colony types that prove to contain obli-

gate anaerobes can be further studied using appropriate identification methods. Consult appropriate texts for additional information.^{10,11}

References

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Availability

BBL™ Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K₁

BS10 CMPH MCM7

United States and Canada

Cat. No. 297848 Prepared Plates – Pkg. of 20*
297716 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 255509 Prepared Plates – Ctn. of 20*

BBL™ Brucella Laked Sheep Blood Agar with Kanamycin and Vancomycin

BS10 MCM7

Cat. No. 297840 Prepared Plates – Pkg. of 20*

BBL™ Brucella 5% Sheep Blood Agar with Hemin and Vitamin K₁//Brucella Laked Sheep Blood Agar with Kanamycin and Vancomycin

Cat. No. 297849 Prepared 1 Plate™ Dishes – Pkg. of 20*

*Store at 2-8°C.

Brucella Broth with 20% Glycerol

Intended Use

This medium is used in the long-term frozen maintenance of bacterial stock cultures.

Summary and Explanation

Brucella Broth is a nutritious medium that, when supplemented with glycerol, may be used as a maintenance medium for the preservation of bacterial cultures.^{1,2}

Principles of the Procedure

Enzymatic digest of protein substrates act as protective colloids.

Glycerol is a cryoprotective agent that provides intracellular and extracellular protection against freezing.²

Procedure

Using a sterile swab or inoculating loop, remove fresh growth from the plated or slanted medium and suspend in the broth maintenance medium to achieve the desired concentration of viable cells. Freeze suspension immediately at -20°C or

below. Consult texts for detailed information about preparing stock cultures of specific organisms.²⁻⁴

Expected Results

Bacterial stock cultures frozen and stored at -20°C or below will remain viable for several months, and some may remain viable for years.

Limitations of the Procedure

The appropriate procedure, storage temperature, length of storage, etc., may vary for specific organisms.

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Availability

BBL™ Brucella Broth with 20% Glycerol

Cat. No. 297466 Prepared Tubes – Pkg. of 10