Brucella Media
Brucella Agar • Brucella Agar with 5% Horse Blood
Brucella Broth

Intended Use

Brucella Agar is a culture medium for the cultivation of *Brucella* organisms. With the addition of 5% horse blood, the medium is used in qualitative procedures for the isolation and cultivation of nonfastidious and fastidious microorganisms from a variety of clinical and nonclinical specimens.

Brucella Broth is used for the cultivation of *Brucella* species and for the isolation and cultivation of a wide variety of fastidious and nonfastidious microorganisms.

Summary and Explanation

Brucella Agar was developed for the cultivation of *Brucella* species from diagnostic specimens, such as blood, and from foods and other potentially contaminated material. Brucella Agar with 5% Horse Blood plates are particularly useful for the cultivation of the more fastidious aerobic and anaerobic microorganisms, including streptococci, pneumococci, *Listeria*, *Neisseria meningitidis* and *Haemophilus influenzae*.

Brucella Broth may be used for the isolation and cultivation of a wide variety of microorganisms including nutritionally fastidious specimens.[1] This medium is recommended for the cultivation of *Brucella* species and was recommended as one of several media suitable for use as the liquid medium component of biphasic blood culture bottles.[1,2] It is also used to cultivate *Campylobacter* spp.[3]

Principles of the Procedure

Brucella Agar and Brucella Broth support the growth of fastidious microorganisms due to their content of peptones, dextrose and yeast extract. The peptones supply organic nitrogen. The yeast extract is a potent source of the B-complex vitamins. Dextrose is utilized as an energy source. Sodium bisulfite is a reducing agent, and sodium chloride maintains the osmotic equilibrium. Agar is the solidifying agent in Brucella Agar.

In BBL™ Brucella Agar with 5% Horse Blood plates, the horse blood supplies both the X and V factors which are growth requirements for certain organisms; e.g., *Haemophilus influenzae*.[3] Sheep and human blood are not suitable for this purpose because they contain enzymes that inactivate the nicotinamide adenine dinucleotide (NAD) which is the V factor.[4]

Defibrinated horse blood may give hemolytic reactions different than sheep blood.[5] Some streptococci (e.g., group D) give hemolytic reactions on horse blood but not on sheep blood

User Quality Control

Identity Specifications

BBL™ Brucella Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.3% solution, soluble in purified water upon boiling. Solution is light to medium, tan to yellow, clear to slightly hazy, may contain small amount of sediment.
Prepared Appearance:	Light to medium, tan to yellow, clear to slightly hazy.
Reaction of 4.3% Solution at 25°C:	pH 7.0 ± 0.2

BBL™ Brucella Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.8% solution, soluble in purified water upon heating. Solution is pale to medium, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Pale to medium, tan to yellow, clear to slightly hazy.
Reaction of 2.8% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

BBL™ Brucella Agar

Prepare the medium per label directions without (plain) and with 5% defibrinated horse blood (HB). Inoculate and incubate at 35 ± 2°C for 3 days with 3-5% CO_2 (incubate *S. aureus* without CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH HB
Brucella abortus	11192*	10^3-10^4	Good	Good
Brucella melitensis	4309*	10^3-10^4	Good	N/A
Brucella suis	4314*	10^3-10^4	Good	N/A
Staphylococcus aureus	25923	10^3-10^4	Good	N/A
Streptococcus pneumoniae	6305	10^3-10^4	N/A	Good
Streptococcus pyogenes	19615	10^3-10^4	N/A	Good

**Minimally one strain of* Brucella *should be used for performance testing. If these strains are not available, verify performance with a known isolate.*

BBL™ Brucella Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 7 days with 3-5% CO_2 (incubate *S. pyogenes* for 66-72 hours without CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Brucella abortus	11192*	< 10^3	Growth
Brucella melitensis	4309*	< 10^3	Growth
Brucella suis	4314*	< 10^3	Growth
Streptococcus pyogenes	19615	< 10^3	Growth

**Minimally one strain of* Brucella *should be used for performance testing. If these strains are not available, verify performance with a known isolate.*

and may be mistakenly reported as group A. If a hemolytic reaction is obtained, the organism should be tested with a **Taxo**™ A bacitracin (0.04 unit) disc and it also should be grouped serologically or tested by the fluorescent antibody method.[6] Beta-hemolytic streptococci and *Haemophilus haemolyticus* may be differentiated by performing a Gram stain on a smear prepared from the colony.

Formulae

BBL™ Brucella Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Peptic Digest of Animal Tissue	10.0	g
Dextrose	1.0	g
Yeast Extract	2.0	g
Sodium Chloride	5.0	g
Sodium Bisulfite	0.1	g
Agar	15.0	g

BBL™ Brucella Broth

Consists of the same ingredients without the agar.

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

Precautions[7]

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   BBL™ Brucella Agar – 43 g;
   BBL™ Brucella Broth – 28 g.
   Mix thoroughly.
2. For the agar, heat with frequent agitation and boil for 1 minute to completely dissolve the powder. For the broth, heat slightly, if necessary, to obtain solution.
3. Autoclave at 121°C for 15 minutes.
4. For preparation of blood plates, add 5 to 10% sterile defibrinated blood to sterile agar which has been cooled to 45-50°C.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Agar (without or with added blood)

Use standard procedures to obtain isolated colonies from specimens.

Since many pathogens require carbon dioxide on primary isolation, incubate plates at 35 ± 2°C for 24-72 hours in anaerobic atmosphere supplemented with carbon dioxide.

Broth

For liquid specimens, use a sterile inoculating loop to transfer a loopful to the broth medium. Swab specimens may be inserted into the broth after the inoculation of plated media.

Incubate tubes for up to 7 days at 35 ± 2°C in an aerobic atmosphere with or without supplementation with carbon dioxide.

For the preparation of biphasic blood culture bottles, aseptically add sterile Brucella Broth to a blood culture bottle containing solidified sterile Brucella Agar, with increased agar at a final concentration of 2.5%. The bottles should contain 5-10% CO_2 and be vented. Blood cultures should be incubated at 35°C for up to 30 days with subcultures prepared every 4 to 5 days.[1,2]

Expected Results

Agar (without or with added blood)

After incubation, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more 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

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Moyer, Holcomb and Hausler. 1991. *In* Balows, Hausler, Herrmann, Isenberg, and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
3. Chapin and Murray. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.). Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
4. Krumweide and Kuttner. 1938. J. Exp. Med. 67:429.
5. Vera and Power. 1980. *In* Lennette, Balows, Hausler and Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
6. Vera. 1971. Health Lab. Sci. 8:176.
7. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

BBL™ Brucella Agar

CCAM 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 Broth

CCAM ISO USDA

Cat. No.	211088	Dehydrated – 500 g
	296185	Prepared Tubes (K Tubes), 5 mL – Ctn. of 100

**Store at 2-8°C.*