

- DO NOT AUTOCLAVE. Heating this medium for a period longer than necessary to just dissolve the ingredients destroys its selectivity.

References

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the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

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- Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
- Cintron. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
- Grasmick. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
- MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Bismuth Sulfite Agar

AOAC BAM CCAM COMPF SMWW USP

Cat. No. 273300 Dehydrated – 500 g

# Blood Agar Base (Infusion Agar)

## Blood Agar Base No. 2

Intended Use

Blood Agar Base (Infusion Agar) and Blood Agar Base No. 2, with the addition of sterile blood, is used for the isolation, cultivation and detection of hemolytic activity of streptococci and other fastidious microorganisms.

Summary and Explanation

Infusion Agar is an all-purpose medium which has been used for many years as a base for the preparation of blood agars. In a study of viability of streptococci, Snavely and Brahier performed comparative studies of horse, rabbit and sheep blood with Blood Agar Base, and found that sheep blood gave the clearest and most reliable colony and hemolysis characteristics at both 24 and 48 hours.<sup>1</sup> In the course of the investigation, about 1,300 isolations of streptococci were made with Blood Agar Base containing 5% sheep blood.

Blood Agar Base No. 2 is a nutritionally rich medium for maximum recovery of fastidious microorganisms.

Blood Agar Base media are specified in standard methods for food testing.<sup>2-4</sup> Infusion Agar and Blood Agar Base No. 2 have been largely replaced as blood agar bases by the Tryptic/Trypticase™ Soy Agar formulations, which contain milk and plant peptones in place of the variable infusion component.

Principles of the Procedure

Infusion from heart muscle, casein peptone and yeast extract provide nitrogen, carbon, amino acids and vitamins in Blood Agar Base. Proteose peptone is the nitrogen source for Blood Agar Base No. 2 while liver digest and yeast extract provide essential carbon, vitamin, nitrogen and amino acid sources. Both media contain sodium chloride to maintain osmotic equilibrium and agar is the solidifying agent.

Supplementation with blood (5-10%) provides additional growth factors for fastidious microorganisms, and is the basis

for determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood or type of base medium used.<sup>5</sup> Chocolate Agar for isolating *Haemophilus* and *Neisseria* species can be prepared from Blood Agar Base No. 2 by supplementing the medium with 10% sterile defibrinated blood (chocolatized).

Formulae

BBL™ Blood Agar Base (Infusion Agar)

Approximate Formula* Per Liter	
Heart Muscle, Infusion from (solids) .....	2.0 g
Pancreatic Digest of Casein .....	13.0 g
Yeast Extract .....	5.0 g
Sodium Chloride .....	5.0 g
Agar .....	15.0 g

Difco™ Blood Agar Base No. 2

Approximate Formula* Per Liter	
Proteose Peptone .....	15.0 g
Liver Digest .....	2.5 g
Yeast Extract .....	5.0 g
Sodium Chloride .....	5.0 g
Agar .....	12.0 g

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

Directions for Preparation from Dehydrated Product

- Suspend the powder in 1 L of purified water:
  - BBL™ Blood Agar Base (Infusion Agar) – 40 g;
  - Difco™ Blood Agar Base No. 2 – 39.5 g.
 Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Autoclave at 121°C for 15 minutes.
- For preparation of blood agar, cool the base to 45-50°C and aseptically add 5% sterile, defibrinated blood. Mix well.
- To prepare chocolate agar, add 10% sterile defibrinated blood to Blood Agar Base No. 2 at 80°C. Mix well.
- Test samples of the finished product for performance using stable, typical control cultures.



## User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

### Identity Specifications

#### Difco™ Blood Agar Base No. 2

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.95% solution, soluble in purified water upon boiling. Solution is medium to dark amber, very slightly to slightly opalescent.
Prepared Appearance:	Plain – Medium to dark amber, slightly opalescent. With 5% sheep blood – Cherry red, opaque.
Reaction of 3.95% Solution at 25°C:	pH 7.4 ± 0.2

### Cultural Response

#### Difco™ Blood Agar Base No. 2

Prepare the medium per label directions with 5% defibrinated sheep blood (SB) and 10% chocolated sheep blood (chocolate agar). Inoculate and incubate at 35 ± 2°C for 18-48 hours with added CO<sub>2</sub>.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY WITH SB	RECOVERY CHOCOLATE AGAR
<i>Haemophilus influenzae</i>	19418	10 <sup>2</sup> -3 × 10 <sup>2</sup>	N/A	Good
<i>Neisseria meningitidis</i>	13090	10 <sup>2</sup> -3 × 10 <sup>2</sup>	Good, gamma hemolysis	N/A
<i>Streptococcus pneumoniae</i>	6305	10 <sup>2</sup> -3 × 10 <sup>2</sup>	Good, alpha hemolysis	N/A
<i>Streptococcus pyogenes</i>	19615	10 <sup>2</sup> -3 × 10 <sup>2</sup>	Good, beta hemolysis	N/A

### Identity Specifications

#### BBL™ Blood Agar Base (Infusion Agar)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Plain – Medium, yellow to tan, clear to slightly hazy. With 5% sheep blood – Cherry red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 7.3 ± 0.2

### Cultural Response

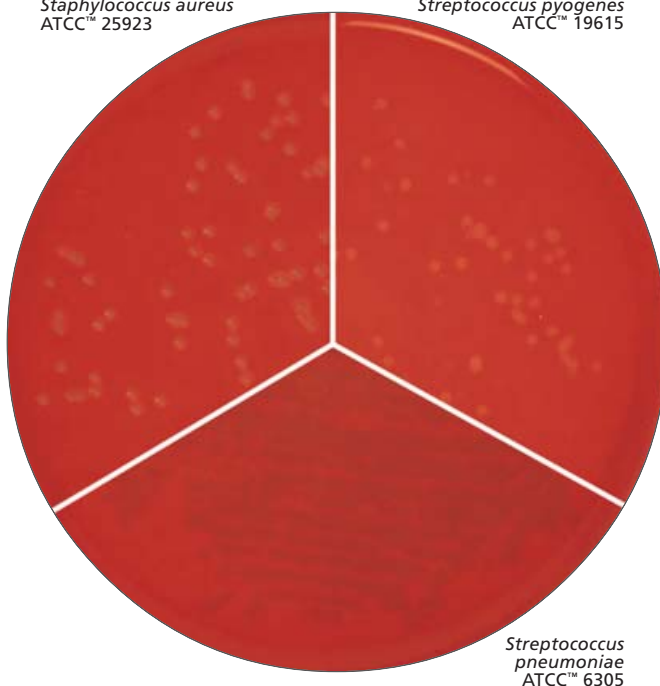
#### BBL™ Blood Agar Base (Infusion Agar)

Prepare the medium per label directions without (plain) and with 5% defibrinated sheep blood (SB). Inoculate and incubate at 35 ± 2°C for 18-24 hours (incubate streptococci with 3-5% CO<sub>2</sub>).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
<i>Candida albicans</i>	10231	30-300	N/A	Good, no hemolysis
<i>Listeria monocytogenes</i>	19115	30-300	N/A	Good, beta hemolysis
<i>Pseudomonas aeruginosa</i>	10145	30-300	Good	N/A
<i>Shigella flexneri</i>	12022	30-300	Good	N/A
<i>Staphylococcus aureus</i>	25923	30-300	Good	Good, beta hemolysis
<i>Streptococcus pneumoniae</i>	6305	30-300	Good	Good, alpha hemolysis
<i>Streptococcus pyogenes</i>	19615	30-300	Good	Good, beta hemolysis

*Staphylococcus aureus*  
ATCC™ 25923

*Streptococcus pyogenes*  
ATCC™ 19615



*Streptococcus pneumoniae*  
ATCC™ 6305

## Procedure

Use standard procedures to obtain isolated colonies from specimens. After streaking, stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the activity of both oxygen-stable and oxygen-labile streptolysins.<sup>5</sup>

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO<sub>2</sub>. Incubate plates at 35 ± 2°C for 18-24 hours.

## Expected Results

Colonial morphology on blood agar containing 5% sheep blood is as follows:

1. Hemolytic streptococci may appear as translucent or opaque, grayish, small (1 mm), or large matte or mucoid (2-4 mm) colonies, encircled by a zone of hemolysis. Gram stains should be made and examined to check the macroscopic findings. (Other organisms which may cause hemolysis include *Listeria*, various corynebacteria, hemolytic staphylococci,

*Escherichia coli* and *Pseudomonas*.) Approximate quantitation of the number of colonies of hemolytic streptococci may be helpful to the clinician.

2. Pneumococci usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of “green” (alpha) hemolysis.
3. Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.
4. *Listeria* may be distinguished by their rod shape in stains, and by motility at room temperature. Small zones of beta hemolysis are produced.
5. Other organisms representing minimal flora and clinically significant isolates can also be expected to grow on this nonselective formulation.

### Limitation of the Procedure

Colonies of *Haemophilus haemolyticus* are beta-hemolytic on horse and rabbit blood agar and must be distinguished from colonies of beta-hemolytic streptococci using other criteria.<sup>6</sup>

The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus*.<sup>5</sup>

### References

1. Snively and Brahier. 1960. *Am. J. Clin. Pathol.* 33:511.
2. U.S. Food and Drug Administration. 1995. *Bacteriological analytical manual*, 8th ed. AOAC International, Gaithersburg, Md.
3. Downes and Ito (ed.). 2001. *Compendium of methods for the microbiological examination of foods*, 4th ed. American Public Health Association, Washington, D.C.
4. Atlas. 1993. *Handbook of microbiological media*. CRC Press, Boca Raton, Fla.
5. Ruoff, Whiley and Beighton. 1999. *In Murray, Baron, Pfaller, Tenover and Tenover (ed.)*, *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.
6. Forbes, Sahm and Weissfeld (ed.). 1998. *Bailey & Scott's diagnostic microbiology*, 10th ed. Mosby, Inc., St. Louis, Mo.

### Availability

#### BBL™ Blood Agar Base (Infusion Agar)

BAM COMPF

Cat. No. 211037 Dehydrated – 500 g  
211038 Dehydrated – 5 lb (2.3 kg)

#### Difco™ Blood Agar Base No. 2

BAM ISO

Cat. No. 269620 Dehydrated – 500 g

## Bordet Gengou Agar Base • Bordet Gengou Blood Agar

### Intended Use

Bordet Gengou Agar Base, with the addition of glycerol and sterile blood, is used in qualitative procedures for the detection and isolation of *Bordetella pertussis* from clinical specimens.

### Summary and Explanation

Bordet Gengou Blood Agar is used in clinical laboratories as a method of diagnosing whooping cough. *Bordetella pertussis*, the etiologic agent of this disease, may be isolated from

aspirated bronchial or nasopharyngeal secretions, perinasal swabs or, perhaps with greater difficulty due to the diversity of flora, from throat swabs.<sup>1</sup>

Bordet and Gengou introduced the medium in 1906 as a method of maintaining stock cultures.<sup>2</sup> In 1934, Kendrick and Eldering replaced the 50% human or rabbit blood recommended in the original formulation with 15% sheep blood to make the medium more practical for laboratories to produce for routine clinical procedures.<sup>3</sup>

### User Quality Control

#### Identity Specifications

##### Difco™ Bordet Gengou Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble upon boiling in purified water containing 1% glycerol. Solution is light to medium amber, opalescent, may have a slight precipitate.
Prepared Appearance:	Plain – Light to medium amber, opalescent, may have a precipitate. With 15% blood – Cherry red, opaque.
Reaction of 3.0% Solution at 25°C:	pH 6.7 ± 0.2

#### Cultural Response

##### Difco™ Bordet Gengou Agar Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 48-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY WITH 15% RABBIT BLOOD
<i>Bordetella bronchiseptica</i>	4617	30-300	Good
<i>Bordetella parapertussis</i>	15311	30-300	Good
<i>Bordetella pertussis</i>	8467	30-300	Good

Uninoculated Plate

*Bordetella pertussis*  
ATCC™ 8467

