Blood Agar Base (Infusion Agar)

Intended Use

Blood Agar Base (Infusion Agar), with the addition of sterile blood, is used for the isolation, cultivation and detection of hemolytic activity of streptococci and other fastidious micro-organisms.

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

User Quality Control

Identity Specifications

BBL™ Blood Agar Base (Infusion Agar)

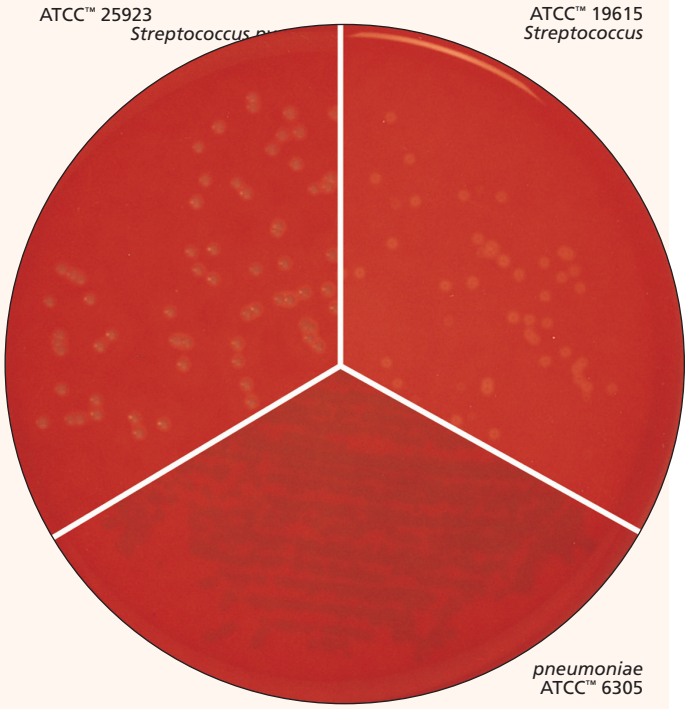
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Plain – Medium, yellow to tan, clear to slightly hazy.
	With 5% sheep blood – Cherry 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_2).

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



Staphylococcus aureus

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.[1] 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 media are specified in standard methods for food testing.[2-4] Infusion Agar has been largely replaced as a blood agar base 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. Medium contains 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.[5]

Formula

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

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

Directions for Preparation from Dehydrated Product

1. Suspend 40 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. For preparation of blood agar, cool the base to 45-50°C and aseptically add 5% sterile, defibrinated blood. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

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.[5]

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO_2. 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.[6] 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*.[5]

References

1. Snavely and Brahier. 1960. Am. J. Clin. Pathol. *33*:511.
2. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. 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 Yolken (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)