ARENA BioScien

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BLOOD AGAR BASE

Medical laboratories media with the addition of sterile blood is used for the isolation, cultivation and detection of hemolytic activity of streptococci and other fastidious microorganisms.

REF: BS.1/BH01.100.0100	100 Gram	REF: BS.1/BH01.250.0250	250 Gram
REF: BS.1/BH01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE [1-4]

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 Base Agar, and found that sheep blood gave the clearest and most reliable colony and hemolysis characteristics at both 24 and 48 hours. In the course of the investigation, about 1,300 isolations of streptococci were made with Blood Base Agar containing 5% sheep blood. Blood Base Agar media are specified in standard methods for food testing. Infusion Agar has been largely replaced as a blood base agar by the Tryptic/Trypticase Soy Agar formulations, which contain milk and plant peptones in place of the variable infusion component.

METHOD PRINCIPLE [5]

Peptone mixture and yeast beef provide nitrogen, amino acids, minerals and vitamins essential for growth. Sodium chloride supplies essential electrolytes for transport and to maintain osmotic equilibrium and agar is the solidifying agent. Supplementation with blood (5%) 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.

MEDIA COMPOSITION

Item	Concentration %
Beef Extract	25 grs.
Peptone Mixture	25 grs.
Sodium Chloride	12.5 grs.
Bacteriological Agar	37.5 grs.

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the Blood Agar Base material safety data sheet.

MEDIA PREPARATION, STORAGE AND STABILITY [2]

BioScien Blood Agar Base dehydrated media are stable until expiration date stated on label when properly stored at 10-30°C. Hydrated Blood Base Agar media is prepared by suspend 40 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C and aseptically add 5-10% (w/v) sterile defibrinated blood, mix well and dispense into plates. When the medium is solidified, invert the plates to avoid excess moisture. The prepared medium should be stored at 8-15°C.

Formula in g/L

Beef Extract	10.0
Peptone Mixture	10.0
Sodium Chloride	5.0
Bacteriological Agar	15.00

Final pH 7.2 ± 0.2 at 25°C

Media Appearance and Deterioration

BioScien Blood Agar Base dehydrated medium is cream to yellow homogeneous free flowing powder. If there are any physical changes, discard the medium.
The hydrated medium is light amber colored, clear to slightly opalescent gel. After addition of 5% v/v sterile defibrinated blood the medium changed to cherry red colored, opaque gel forms in petri plates. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), and contaminations.

SPECIMEN COLLECTION AND PRESERVATION [1-3]

A big variety of clinical specimens were bacteriologically analyzed by Blood Agar Base including sputum, ETT, throat swab, conjunctival swab, ear swab, CSF, wound and pus swab, urine, urine catheter, and any effusion fluid.

Note: for patient preparation follow medical laboratory instruction, however, it is recommended to stop any antibiotic 4 days before culture.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile cups and petri-dishes
- Autoclave and Incubator

MICROBIOLOGICAL PROCEDURE

Inoculate sample onto the surface of the medium, streak for isolation with an inoculating loop. Incubate plates aerobically, anaerobically or under CO^2 (5-10%).
Examine plates for growth and hemolytic reactions after 18-24 hours and again after 40-48 hours incubation at 35 ± 2^0 C.

CHARACTERISTICS OF THE COLONIES [1]

Growth Characteristics on Blood Agar Base Medium

Streptococci	Translucent or opaque, grayish, small (1 mm), or large matte or mucoid (2-4 mm) colonies, encircled by a zone of hemolysis
Staphylococci	appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis
Pneumococci	usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of alpha (green) hemolysis
Listeria	May be distinguished by their rod shape in stains, and by motility at room temperature. Small zones of beta hemolysis are produced.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact ***BioScien*** technical support.

Positive controls:	**Expected results**
Streptococcus Pyogenes ATCC ® 19615	Good growth; Beta hemolysis
Streptococcus Aureus ATCC ® 25923	Good *Growth;* Beta hemolysis
Streptococcus Pneumoniae ATCC ® 6303	Good *Growth;* Alpha hemolysis
Negative controls:	**Expected results**
Uninoculated medium	No change

PERFORMANCE CHARACTERISTICS [4]

Performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 24 and 48 hours

Test Organisms	Results
Enterococcus Faecalis ATCC ® 19433	Growth; Alpha/gamma hemolysis
Escherichia Coli ATCC ® 25922	Inhibited
Staphylococcus Epidermidis ATCC ® 12228	Growth; Gamma hemolysis
Streptococcus Pneumoniae ATCC ® 6303	Growth; Alpha hemolysis
Streptococcus Pyogenes ATCC ® 19615	Growth; Beta hemolysis

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.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

EC REP

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