BLOOD AGAR BASE #2

PRODUCT:

Plate Media:

Blood Agar Base #2 with 5% Sheep Blood, item no. P1146

PURPOSE:

Blood Agar Base (BAB) #2 is a highly nutritious base medium used for the cultivation of fastidious microorganisms. The addition of blood facilitates the recovery of more fastidious microorganisms and allows for the detection of hemolytic strains.

PRINCIPLE:

Blood Agar Base #2 supplies the preformed food supply necessary for fastidious microorganisms to replicate and grow. The pancreatic digestion of casein, a vegetable protein, and the peptic digestion of animal tissue provide a complementary source of peptones, carbohydrates, and vitamins which makes this base highly nutritious. In addition, the base contains yeast extract which has the largest percentage of vitamins of any supplement, as well as additional nitrogenous compounds and carbohydrates; liver digest provides additional nutrients.

FORMULAS:

Approximate, per liter of deionized filtered water.

Pancreatic Digest of Casein	7.5 g
Peptic Digest of Animal Tissue	7.5
Liver Digest	2.5
Yeast Extract	5.0
Sodium Chloride	5.0
Agar	12.0
Sheep Blood	50.0 ml

Final pH 7.4 ± 0.2 at 25°C

PRECAUTIONS:*

For in vitro diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light; store all horse blood-containing media in the dark as horse blood is extremely light-sensitive. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Limitations: Blood Agar Base #2 serves as a nonselective medium; biochemical and/or serological testing are necessary for definitive identification of microorganisms.

Sheep blood contains V factor-destroying enzyme (nucleotidase) which prevents the growth of *Haemophilus* species on sheep blood agar unless another microorganism, e.g., staphylococci, provides the V factor.

Small amounts of reducing sugars inhibit the expression of beta-hemolysis, and beta-hemolytic streptococci may develop a green zone or ring of hemolysis.

PROCEDURE:*

Specimen Collection: Information on specimen collection is found in standard reference material. In general, specimens should be protected from extremes of heat and cold and should be delivered to the laboratory without delay.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. Inoculate the plate according to standard microbiological procedures and streak the inoculum so as to obtain isolated colonies. Incubate under conditions that will permit the growth of microorganisms. In general, incubate at 35°C for 18-72 hours with adequate moisture in either aerobic, capnophilic, or anaerobic environments depending on the specific microorganisms being cultured.

Interpretation: The following growth characteristics are typical for organisms appearing on these media:

Organism	Colonial Morphology
Streptococcus pyogenes	Small, beta-hemolytic, transparent to opaque, domed, smooth, and entire edge.
Streptococcus viridans	Small, alpha-hemolytic, transparent to opaque, domed, smooth, and entire edge.
Streptococcus pneumoniae	Small, alpha-hemolytic, round, and mucoid with entire edge.
Staphylococcus aureus	Average, ± hemolysis, opaque, circular, smooth, raised, white to golden yellow pigment.
Staphylococcus epidermidis	Average, ± hemolysis, opaque, circular, smooth, raised, usually white to colorless.
Cornybacteria	Small, grayish colonies.
Listeria monocytogenes	Small, beta-hemolytic, transparent, gray to white.
Yeast	Small, white to gray in 48-72 hours.
Escherichia coli	Large, grayish colonies.

For other clinically significant organisms, a reference such as Lennette et al.[5] should be consulted.

Material Required but Not Provided: Standard microbiological supplies and equipment such as loops, needles, incubator, and incinerator are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC #):	Expected Results:
Streptococcus pneumoniae (6305)	Growth, alpha hemolysis
Streptococcus pyogenes (19615)	Growth, beta hemolysis
Staphylococcus aureus (25923)	Growth
Escherichia coli (25922)	Growth
	Key: See “Interpretation”

User Quality Control: Check for signs of contamination and deterioration.

BIBLIOGRAPHY:

1. Baker, F. J., and M. R. Breach, *Medical Microbiology Techniques*, Butterworth & Co., Boston, 1980.
2. Casman, E. P., *Am. J. Clin. Pathol*. 17:281-289, 1947.
3. *Diagnostic Procedures and Reagents*, 4th ed., American Public Health Association, New York, 1963.
4. Facklam, R. R., *Isolation and Identification of Streptococci,* USHEW, PHS, Centers for Disease Control, Atlanta, 1980.
5. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D. C., 1985.

* For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

PML MICROBIOLOGICALS, INC.
Data #175

Revision Date: January 2001