

PHENYLETHANOL AGAR (7147)

Intended Use

Phenylethanol Agar is used with blood for the selective isolation of Gram-positive cocci.

Product Summary and Explanation

Brewer and Lilley^{1,2} reported the addition of phenylethanol to a nutritive medium permitted growth of Gram-positive organisms, but markedly to completely inhibited growth of Gram-negative organisms. Phenylethanol Agar inhibits swarming of *Proteus* spp., and can be used to selectively isolate anaerobic bacteria from clinical specimens with mixed flora. Phenylethanol Agar is specified for use in several reference methods.^{3,4}

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal. Sodium Chloride maintains the osmotic balance of the medium. Phenylethanol is bacteriostatic for Gram-negative bacteria and inhibits DNA synthesis. Agar is the solidifying agent. The addition of 5% defibrinated sheep blood to the basal medium can enhance microorganism recovery of the medium.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Soybean Meal	5 g
Sodium Chloride	5 g
Phenylethanol	2.5 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 42.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, beige with soft lumps.

Prepared Appearance: Prepared medium is trace to slightly hazy and pale yellow. Prepared medium with 5% sheep blood is red and opaque.

Expected Cultural Response: Cultural response on Phenylethanol Agar at 35 ± 2°C and examined for growth after 18 - 48 hours incubation in an aerobic atmosphere.

Microorganism	Approx. Inoculum (CFU)	Expected Growth Results
<i>Escherichia coli</i> ATCC® 25922	~1000	If recovered, pinpoint colonies
<i>Enterococcus faecalis</i> ATCC® 29212	10-300	fair to good growth
<i>Proteus mirabilis</i> ATCC® 12453	~1000	If recovered, pinpoint colonies
<i>Staphylococcus aureus</i> ATCC® 25923	10-300	fair to good growth
<i>Staphylococcus epidermidis</i> ATCC® 12228	10-300	fair to good growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	10-300	fair to good growth
<i>Streptococcus pyogenes</i> ATCC® 19615	10-300	fair to good growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, inoculate directly onto surface of the medium. Streak for isolation with inoculating loop.
2. Incubate plates at 35°C under conditions of increased CO₂ (5 - 10%) for 18 - 24 hour, and if necessary, 40 - 48 hours.

Results

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. Perform additional biochemical testing to identify the organism.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. *Pseudomonas aeruginosa* is not completely on this medium.⁶
2. Some Gram-positive cocci may be slightly inhibited and many require further incubation (up to 48 hours) for sufficient growth to be evident.⁷
3. Subculture Gram-positive colonies onto nonselective medium for biochemical testing.⁷

Packaging

Phenylethanol Agar	Code No.	7147A	500 g
		7147B	2 kg
		7147C	10 kg

References

1. **Brewer, J. H., and B. D. Lilley.** 1949. Paper presented at the December meeting of the Maryland Association of Medical and Public Health Laboratories.
2. **Lilley, B. D., and J. H. Brewer.** 1953. The selective antibacterial action of phenylethylalcohol. J. Pharm. Assoc. **42**:6.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
4. **Isenberg, H. D.** 1992. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.
5. **Washington, J. A., Jr.** 1981. Laboratory procedures in clinical microbiology. Springer-Verlag, New York.
6. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.
7. **MacFaddin, J. F.** 1985. Media for the isolation-cultivation-identification-maintenance of medical bacteria, vol. 1 Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.