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
Escherichia coli ATCC® 25922	~1000	If recovered, pinpoint colonies
Enterococcus faecalis ATCC® 29212	10-300	fair to good growth
Proteus mirabilis ATCC® 12453	~1000	If recovered, pinpoint colonies
Staphylococcus aureus ATCC® 25923	10-300	fair to good growth
Staphylococcus epidermidis ATCC® 12228	10-300	fair to good growth
Streptococcus pneumoniae ATCC® 6305	10-300	fair to good growth
Streptococcus pyogenes 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 CO_2 (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.
2. Some Gram-positive cocci may be slightly inhibited and many require further incubation (up to 48 hours) for sufficient growth to be evident.[6]
3. Subculture Gram-positive colonies onto nonselective medium for biochemical testing.[6]
4. When supplemented with blood this medium may demonstrate atypical hemolytic reactions. The prepared medium should not be used for the classification or determination of hemolytic reactions.
5. *Pseudomonas aeruginosa* is **not** inhibited on this medium.[5]

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. Yolken (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. **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.

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