



PHENYLETHYL ALCOHOL AGAR (PEA)

- For in vitro use only -

Catalogue No. PP23 & PP24

Our PEA Agar is used for the selective isolation of gram-positive cocci from clinical specimens containing gram-negative organisms.

Lilley and Brewer whom reported the anti-bacterial effect of phenylethyl alcohol developed PEA Agar. They discovered that the medium allowed gram-positive organisms to grow while inhibiting the growth of gram-negative organisms. The medium also proved beneficial in inhibiting the swarming of *Proteus* species. Dowell Jr., Hill, and Altemeier later devised a supplemented version of PEA agar for the isolation of anaerobic bacteria. Anaerobic PEA is used for the selective isolation of anaerobic bacteria from mixed populations and prevents rapidly-growing, facultatively anaerobic gram-negative bacteria (i.e. *Proteus*) from overgrowing strict anaerobes. The medium allows both gram-positive and most gram-negative obligately anaerobic bacteria to grow.

The growth components include pancreatic digest of casein, papaic digest of soybean meal, and defibrinated sheep blood. The addition of sheep blood does not allow for the determination of hemolytic patterns since all organisms are somewhat inhibited on the medium and hemolysis is atypical in most cases. The addition of vitamin K₁ and hemin to the anaerobic formulation directly stimulates the growth of some anaerobes and is a direct requirement for some isolates.

Formula per Litre of Medium

PP24 PEA Agar

Pancreatic Digest of Casein..... 15.0 g
Papaic Digest of Soybean Meal..... 5.0 g
Sodium Chloride..... 5.0 g

Phenylethyl Alcohol 2.5 g
Agar 15.0 g
Defibrinated Sheep Blood 50.0 mL

Additional Ingredients per Liter:

PP23 PEA Anaerobic Agar

Vitamin K₁ 10.0 mg
Hemin 5.0 mg

pH 7.4 ± 0.2

Recommended Procedure

PP24 PEA Agar

1. Allow medium to reach room temperature.
2. Using a direct inoculum from the specimen, perform a four-quadrant streak to obtain well-isolated colonies. A non-selective blood agar plate should also be inoculated to fully characterize the different types of organisms present in the sample.
3. Incubate aerobically or in a CO₂-enriched environment at 35°C.
4. Examine after 24 hours. If growth is poor or if there is no growth, re-incubate plates for an additional 24 hours.

PP23 PEA Anaerobic Agar

1. Anaerobic plates should be prereduced before use by placing them in an anaerobic environment overnight at room temperature.
2. Using a direct inoculum from the specimen, perform a four-quadrant streak to obtain well-isolated colonies. A non-selective blood agar plate should also be inoculated to fully characterize the different types of organisms present in the sample.
3. Incubate anaerobically at 35°C.

- Examine after 48 hours; additional incubation may be required for some slow-growing and pigmented anaerobes.

- Pseudomonas aeruginosa* is not inhibited on this medium

Interpretation of Results

PEA plates should be examined closely after incubation. The growth characteristics of gram-positive cocci are similar to those seen on sheep blood agar (BAP) except that colonies are smaller. Although gram-negative bacteria such as *E. coli* and *Proteus* species are inhibited on PEA Agar, prolonged incubation (≥ 72 hours) reduces the selectivity of the medium and may allow these organisms to grow. Colonies of interest should be sub-cultured onto a non-selective medium so that further tests can be performed from pure culture.

Special precaution must be taken to avoid exposure of anaerobic culture plates to oxygen during examination. Some sensitive, obligate anaerobes may die even after a 10-minute exposure to oxygen. The colony morphology of isolates on PEA are similar to those observed on a BAP. Colonies on the PEA plate should be further processed if they are different than those growing on the BAP. In some instances, the PEA anaerobic plate may be used in place of the anaerobic blood agar plate if it is overgrown with swarming *Clostridium* or *Proteus* species.

Additional physiological or biochemical tests should be performed on isolated colonies from pure culture in order to complete identification of isolates.

- Many gram-negative bacilli may exhibit visible colonies on PEA but their size and numbers are reduced. Prolonged incubation may also allow some inhibited gram-negative organisms to grow
- Some gram-positive cocci may be slightly inhibited on initial incubation and may require further incubation (48-hrs) to produce visible colonies

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
PEA Agar	
<i>Streptococcus pyogenes</i> ATCC 19615	Growth
<i>Staphylococcus aureus</i> ATCC 25923	Growth
<i>Proteus mirabilis</i> ATCC 12453	Partial Inhibition
PEA Anaerobic Agar	
<i>Bacteroides fragilis</i> ATCC 25285	Growth
<i>Streptococcus pyogenes</i> ATCC 19615	Growth
<i>Proteus mirabilis</i> ATCC 12453	Partial Inhibition

Storage and Shelf Life

Our PEA Agar should be protected from direct light and stored at 4°C to 8°C with the medium side uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions both formulations have a shelf life of 5 weeks from the date of manufacture.

Ordering Information

Original: Feb 2003

Revised / Revisited: October 2014

Cat#	Description	Format
PP23	PEA Anaerobic Agar [Standard 15x100-mm plate]	10/pkg
PP24	PEA Agar [Standard 15x100-mm plate]	10/pkg

References

1. Lilley BD, Brewer JH. The selective antibacterial action of phenylethylalcohol. *J Pharm Assoc* 1953; 42:6-8.
2. Page LM, Rawlings BE. Phenylethyl-alcohol medium and antibiotic sensitivities of mixed bacterial flora. *Am J Med Technol* 1959; 25:389.
3. Dowell Jr. VR, Hill EO, Altemeier WA. Use of phenethyl alcohol in media for isolation of anaerobic bacteria. *J Bacteriol* 1964; 88:1811-3.
4. MacFaddin JF. *Media for isolation-cultivation-maintenance of medical bacteria*, Vol I. Baltimore: Williams & Wilkins, 1985.
5. Sutter VL, Citron DM, Edelstein MAC, Finegold SM. *Wadsworth anaerobic bacteriology manual*. 4th ed. Belmont, CA: Star Publishing Company, 1985.
6. Dowell Jr. VR, Lombard GL, Thompson FS, Armfield AY. *Media for isolation, characterization, and identification of obligately anaerobic bacteria*. Atlanta, Georgia: Center for Disease Control, 1987.
7. Isenberg HD, Ed. *Clinical microbiology procedures handbook*. Washington, DC: ASM, 1992.
8. NCCLS. *Quality assurance for commercially prepared microbiological culture media*. 2nd ed. NCCLS document M22-A2. Wayne, PA: NCCLS, 1996.
9. Murray, P.R., Tenover, R. Baron, M. Pfaller, F. Tenover, R. Tenover, R. Tenover. *Manual of Clinical Microbiology*. 7th ed. Washington: ASM, 1999.