IFU

Instructions for Use

PEA (PHENYLETHYL ALCOHOL) WITH 5% SHEEP BLOOD

Cat. no. A93	PEA with 5% Sheep Blood, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics PEA with 5% Sheep Blood is a selective medium recommended for the isolation of gram-positive microorganisms, particularly gram-positive cocci, from mixed specimens.

SUMMARY

Proteus spp. are frequently isolated from urinary tract specimens and many species are responsible for a variety of systemic and localized infections. However, the characteristic growth of *Proteus* , frequently described as "swarming," interferes with the enumeration of microorganisms from mixed cultures. Numerous media and techniques have been employed to counteract swarming. However, Brewer first reported the selective phenomenom of adding phenylethyl alcohol (PEA) to an absorbent pad in the lid of various petri dishes.[3] When used, organisms like *Pseudomonas* and *Proteus* do not grow well, while gram-positive organisms like *Staphylococcus* grow abundantly. As a result, Brewer and Lilley developed a selective medium containing phenylethanol that allowed for the growth of gram-positive cocci, while inhibiting gram-negative flora.[2] Brewer and Lilley later confirmed that microorganisms grown on PEA containing media do not change genetically and exhibit normal growth characteristics when subcultured to a non-selective medium without added PEA.

The basal medium of PEA agar consists of pancreatic digest of casein and enzymatic digest of soybean meal. Both ingredients provide amino acids, nitrogen, carbon, sulfur, vitamins and trace elements to promote bacterial growth. PEA with 5% Sheep Blood is further enriched by growth factors contained in sheep blood which help to facilitate the isolation of more fastidious microorganisms. Hemolytic reactions, however, should not be interpreted on this medium since they may be atypical. Sodium chloride is added to the medium to help maintain osmotic balance. PEA with 5% Sheep Blood is made selective by the addition of phenylethyl alcohol, which inhibits *Proteus* swarming and the growth of most facultative anaerobic gram-negative bacilli by suppressing DNA synthesis . PEA with 5% Sheep Blood supports the growth of most gram-positive and gram-negative obligately anaerobic microorganisms.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	15.0gm
Papaic Digest of Soybean Meal	5.0gm
Sodium Chloride	5.0gm
Phenylethanol	2.5gm

Sheep Blood	50.0ml
Agar	15.0gm

Final pH 7.3 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection and transfer.[4-8]

Method of Use: Allow plates to warm to room temperature and the agar surface to dry before inoculating. Inoculate and streak the specimen as soon as possible after collection. Streak for isolation using a sterile inoculating loop. Incubate plates at 35-37ºC. for 24 to 48 hours under appropriate atmospheric conditions. PEA with 5% Sheep Blood can be incubated under aerobic, anaerobic or 5% CO_2 conditions, depending upon the type of specimen and the organism of interest. However, incubation in CO_2 is most effective for detecting bacteria requiring increased levels and generally results in improved growth of most pathogens.[4-8] Examine plates for typical colonial morphology and growth characteristics.

INTERPRETATION OF RESULTS

Consult listed references for the identification of colony morphology and further biochemical tests required for identification.[4-8]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Phenyl ethyl alcohol is prone to evaporation. Prepared plates must be kept in a well-sealed bag during storage.

Detection of hemolytic reactions on PEA with 5% Sheep Blood is not recommended since atypical reactions may be observed.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Streptococcus pyogenes ATCC® 19615***	A	24hr	35°C	CO_2**	Growth
Streptococcus pneumoniae ATCC® 6305	A	24hr	35°C	CO_2**	Growth
Staphylococcus aureus ATCC® 25923	A	24hr	35°C	CO_2**	Growth
Proteus mirabilis ATCC® 12453***	B	24hr	35°C	CO_2**	Partial inhibition
Escherichia coli ATCC® 25922	B	24hr	35°C	Aerobic	Partial to complete inhibition

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Atmosphere of incubation is enriched with 5-10% CO_2.

*** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Brewer, J.H. and B.D. Lilley. 1949. Paper presented at the December meeting of the Maryland Association of Medical and Public Health Laboratories.

3. Brewer, J.H. 1950. The Utility of Metal Petri Dish Covers with Absorptive Disks. J. Bacteriol. 59:237-239.

4. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

6. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

7. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

8. Versalovic, J., et al. *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, D.C.

9. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

IFU-10642[A]

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]