IFU

Instructions for Use

anaeroGRO™

Pre-Reduced Anaerobic Culture Media

ANAEROBIC PEA (PHENYLETHYL ALCOHOL) AGAR

Cat. no. AG312	DuoPak B, BRU with Hemin and Vitamin K* Monoplate; BBE/PEA*, Biplate	2 plates/pouch
Cat. no. AG303	MultiPak A, BRU with Hemin and Vitamin K* Monoplate; PEA* Monoplate; BBE/LKV*, Biplate	3 plates/pouch
Cat. no. AG313	MultiPak B, BRU with Hemin and Vitamin K* Monoplate; PEA* Monoplate; LKV*, Monoplate	3 plates/pouch

* All AnaeroGRO™ plated media is provided in standard 15x100mm monoplates or biplates. Each plate or set of plates is packaged in an oxygen-free, gas-flushed foil pouch containing a desiccant and an oxygen scavenger sachet.

INTENDED USE

Hardy Diagnostics AnaeroGRO™ Anaerobic PEA Agar is recommended for use as an enriched and selective medium for the cultivation and selective isolation of gram-positive and negative obligate anaerobic bacteria. It is useful in isolating obligate anaerobes from mixed flora, by inhibiting gram-negative facultative anaerobes and the control of swarming organisms.

SUMMARY

PEA should be inoculated from purulent specimens and when mixed infections are suspected. PEA inhibits facultative Gram-negative rods, preventing *Enterobacteriaceae* from overgrowing the anaerobes, and inhibits swarming of *Proteus*. It also prevents certain clostridia, such as a *C. septicum*, from swarming, which will facilitate the isolation of other colonies. Most Gram-negative and Gram-positive anaerobes will grow on primary PEA medium, especially in mixed culture, and the morphology of the colonies is similar to that on blood agar plates; however, a longer incubation time may be necessary to detect the more slowly growing and pigmented anaerobes. However, pigment develops more rapidly than on non-selective Brucella Agar with H and K (Cat. no. AG301).

The basal medium of Anaerobic PEA consists of pancreatic digest of casein and enzymatic digest of soybean meal. Both provide amino acids, carbohydrates, and vitamins. The medium is supplemented with yeast extract, sheep blood, vitamin K, hemin, and L-cystine. Yeast extract enhances growth of fastidious microorganisms; sheep blood demonstrates hemolysis; vitamin K promotes growth and enhancement of pigmented *Prevotella* spp.; hemin and L-cystine improve the growth of *Clostridium haemolyticum*, *Fusobacterium necrophorum*, certain strains of *Actinomyces israelii*, *Bacteroides thetaiotaomicron*, and thiol-dependent streptococci.[6] The medium is made selective with the addition of phenylethyl alcohol which inhibits facultative anaerobic Gram-negative bacilli.

Culture media that is exposed to environmental oxygen leads to a build-up of reactive oxygen species (ROS) that

initiate damaging free radical reactions, which inhibit the growth of anaerobic bacteria. Therefore, ingredients have been added to the AnaeroGRO™ media to neutralize the growth inhibiting effects of peroxide and other reactive oxygen species (ROS) that may develop during the medium's brief exposure to oxygen after it is sterilized and before it is packaged in an oxygen-free environment.

AnaeroGRO™ Anaerobic PEA is packaged under oxygen-free conditions to prevent the formation of toxic oxidized products that may damage obligate anaerobes and inhibit the growth of more fastidious species. The pouches are flushed with nitrogen gas and a desiccant and oxygen scavenger sachet is inserted.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic and Enzymatic Digests of Casein	15.0gm
Papaic Digest of Soybean Meal	5.0gm
Sodium Chloride	5.0gm
Yeast Extract	5.0gm
Phenylethanol	2.5gm
Reducing Agents/Peroxide Inhibitors	1.5gm
Vitamin K	10.0mg
Hemin	5.0mg
L-Cystine	0.4mg
Sheep Blood	50.0ml
Agar	15.0gm

Final pH 7.3 +/- 0.3 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 15-30ºC. 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 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 quality control incubation times as stated below.

The plates must be inoculated **immediately** after opening the AnaeroGRO™ pouch. After inoculation, the plates must be placed **immediately** into an anaerobic atmosphere (pouch, jar, or chamber) to avoid exposure to oxygen and ensure optimal growth of anaerobic bacteria.

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.[1,2,4,6,9,10] It is not recommended that a swab be used for specimen collection. Swabs are prone to drying and may be easily exposed to ambient air. The preferred means of anaerobic specimen collection is aspiration with needle and syringe. The specimen should be transferred to an anaerobic transport system (Cat. no. S120D or AG25H) in order to protect it from oxygen exposure. Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold.

Method of Use: Open pouch and immediately inoculate and streak the specimen for isolated colonies. A large inoculum should be used in order to minimize the toxic effects of oxygen.[8] Incubate plates at 35-37ºC. for 18-48 hours in an anaerobic atmosphere. Examine for typical colonial morphology and characteristics.

A non-selective medium such as Brucella with H and K (Cat. no. A30) should be inoculated in parallel to the selective medium for enhanced recovery of anaerobic microorganisms.

Aerotolerance Testing: Confirmation of obligate anaerobic microorganisms should be performed. A Chocolate Agar plate (Cat. no. E14) incubated in 5-10% CO_2 is required for aerotolerance testing to detect isolates that require CO_2, especially slow-growing, fastidious, facultative or microaerophilic species that do not grow alone on media containing blood (such as *Haemophilus* and *Actinobacillus* spp.). Use of traditional blood agar media alone for CO_2 incubation may yield false-negative results. An additional Blood Agar plate (Cat. no. A10) incubated in air will further detail the atmospheric requirements and hemolytic properties of facultatively anaerobic microorganisms.

INTERPRETATION OF RESULTS

Refer to the *Wadsworth-KTL Anaerobic Bacteriology Manual* or other texts for more information on identification of anaerobic bacteria.[10]

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.

The plates must be inoculated **immediately** after opening the AnaeroGRO™ pouch. After inoculation, the plates must be placed **immediately** into an anaerobic atmosphere (pouch, jar, or chamber) to ensure optimal growth of anaerobic bacteria.

Many anaerobes are more sensitive to oxygen during the log phase of growth; therefore, it may be necessary to incubate inoculated media for a full 48 hours prior to examination and exposure of the culture to ambient air.

Some organisms for which the medium is designed to isolate may be inhibited by the selective nature of phenylethyl alcohol.

Large numbers of anaerobic bacteria are normally present in the following sites: throat, gingiva, sputum, gastric contents, small bowel, feces, rectal swabs, surfaces of decubitus ulcers, encrusted walls of abscesses, mucosal lining, eschar, voided urine, vagina or cervix, skin and adjacent mucous. Specimens for anaerobic culture, therefore, should not be collected from these sites.

Phenylethyl alcohol is volatile and will dissipate over time with air exporsure, thus reducing the selective nature of this medium.

Failure to cultivate and/or isolate obligate anaerobes may be due to the following:

1. Exposure of specimen to oxygen during transport or processing.

2. Overgrowth of aerobic, facultative organisms, or normal flora. Overgrowth can occur in transport media, Thioglycollate broth, or on non-selective plated media. This can be controlled by avoiding normal flora during specimen collection and by utilizing selective plated media.

3. Leaks in the anaerobic incubation system; e.g. faulty O-rings or vents.

4. Failure to use an anaerobic indicator (such as resazurin) to monitor for complete anaerobiosis.

5. Anaerobic gas mixture contains toxic gas, oxygen; or does not include CO_2, which is necessary for some anaerobes.

6. Failure to use a non-selective medium as part of the primary specimen set-up, since some fastidious anaerobes are inhibited by selective media.

7. Failure to incubate cultures for extended periods of time. Some fastidious anaerobes are slow growers (such as *Porphyromonas* and *Actinomyces* spp.), especially if present in small numbers, and may require 5 to 7 days of incubation in order to be visible on plated media.

8. Exposure of developing colonies on plated media to air, especially when opening jars to check for growth. Anaerobes are most sensitive to oxygen during the log phase of growth. Do not open jars or pouches at less than 48 hours of incubation (except when incubating BBE or EYA plates, since organisms that are selected for on these plates grow rapidly). Some fastidious anaerobes may lose viability with only 15 minutes of exposure to oxygen. Plates incubated in anaerobic chambers or unopened pouches can be inspected at 24 hours, since the plates are not exposed to oxygen during inspection.

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, slides, staining supplies, other culture media, microscopes, incinerators, anaerobic holding jars (Cat. no. 16000), gas generators (Cat. no. AN25US), anaerobic transports (Cat. no. S120D or AG25H), indicators (Cat. no. BR55), 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:

		Incubation	

Test Organisms	Inoculation Method*				Results
		Time	Temperature	Atmosphere	
Bacteroides fragilis ATCC® 25285**	A	18-24hr	35°C	Anaerobic	Growth
Proteus mirabilis ATCC® 12453**	B	18-24hr	35°C	Aerobic	Partial inhibition; slight growth without swarming

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

** 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.

PHYSICAL APPEARANCE

AnaeroGRO™ Anaerobic PEA should appear opaque, and dark red in color.

Bacteroides fragilis (ATCC® 25285) colonies growing on AnaeroGRO™ Anaerobic PEA Agar. Incubated anaerobically for 24 hours at 35°C.

Proteus mirabilis (ATCC® 12453) partial to complete inhibition on AnaeroGRO™ Anaerobic PEA Agar. Incubated aerobically for 24 hours at 35°C.

Clostridium perfringens (ATCC® 13124) colonies growing on AnaeroGRO™ Anaerobic PEA Agar. Incubated anaerobically for 48 hours at 35°C.

REFERENCES

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5. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*,, Lipincott Williams & Wilkins, Philadelphia, PA.

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9. Jousimies-Somer, H., et al. 2002. *Wadsworth Anaerobic Bacteriology Manual*, 6th ed. Star Publishing Company, Belmont, CA.

10. Jousimies-Somer, H.R., S.P. Citron, D. Baron, E.J. Wexler, and H.M. Finegold. 2002. *Wadsworth-KTL Anaerobic Bacteriology Manual*, 6th ed. Star Publishing Company, New York, N.Y.

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