IFU

Instructions for Use

anaeroGRO™

Pre-Reduced Anaerobic Culture Media

EGG YOLK AGAR (EYA), MODIFIED

Cat. no. AG401	Egg Yolk Agar, Modified*, 15x100mm Plate, 18ml	1 plate/pouch

* AnaeroGRO™ plated media is generally 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™ Egg Yolk Agar, Modified is an enriched, non-selective, and differential medium recommended for use in the detection of lecithinase and lipase production and proteolytic activity of certain obligate anaerobes and in the presumptive identification of various *Clostridium*, *Fusobacterium*, and *Prevotella* spp.; it is also used in the Nagler Test for the presumptive identification of *Clostridium perfringens*.

SUMMARY

If clostridia are suspected clinically or from the Gram stain of clinical material, an Egg Yolk Agar plate should be inoculated to check for the production of lipase and lecithinase.

Egg Yolk Agar, originally formulated by McClung and Toabe, is a non-selective medium supplemented with a suspension of egg yolk and enriched with hemin and vitamin K.[7] Egg yolk supplies lecithin and free fats, substrates needed to detect lecithinase and lipase production and proteolytic activity. Hemin and vitamin K are incorporated into the medium to enhance the growth of obligate anaerobic microorganisms.

Microorganisms that possess the enzyme lecithinase break down lecithin to insoluble diglyceride and phosphorylcholine. The insoluble diglyceride produces a white opaque zone of precipitation that spreads beyond the edge of the colony. Microorganisms that possess lipase hydrolyze free fats present in the medium to form glycerol and free fatty acids. Insoluble free fatty acids result in the formation of an iridescent sheen (as with oil on water) that can be seen when the plate is held at an angle to a light source.[3,6] As compared to lecithinase, lipase is not diffusible and produces a reaction only on the surface of the agar in the immediate vicinity of the colony. Proteolysis is noted by the development of clear zones in the medium surrounding colonial growth.

AnaeroGRO™ Egg Yolk Agar, Modified is packaged in an oxygen-free, reduced state to prevent the formation of toxic oxidized by-products that may damage obligate anaerobes and inhibit the growth of more fastidious species. 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.

FORMULA

Ingredients per liter of deionized water:*

Peptone	20.0gm
Yeast Extract	5.0gm
Disodium Phosphate	5.0gm
Sodium Chloride	2.5gm
Glucose	2.0gm
Reducing Agents/Peroxide Inhibitors	1.5gm
Pyruvate	0.5gm
L-Cystine	0.4gm
L-Tryptophan	0.2gm
Hemin	5.0ml
Tween® 80	1.0ml
Vitamin K	1.0ml
Magnesium Sulfate Solution	0.2ml
Agar	20.0gm

Final pH 7.0 +/- 0.2 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), 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 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: Specimen collection is not applicable since this medium is not intended for primary isolation from clinical specimens. As a general rule, infectious material should be submitted directly to the laboratory without delay and protected from excessive heat, cold and oxygen exposure. If there is to be a delay in processing, the specimen should be inoculated into an appropriate anaerobic transport media (Cat. no. S120D) and refrigerated until inoculation. Some fastidious anaerobes may require additional periods of incubation for proper recovery. Regardless of atmospheric system used, it is important to confirm anaerobiosis by using an anaerobic indicator, such as resazurin (Cat. no. BR55). Consult listed references for information on specimen collection.[1-6,8]

Note: If clostridia are suspected clinically or upon Gram stain, a primary Egg Yolk Agar plate can be inoculated to check for lipase and lecithinase production.[5] If boxcar-shaped cells are observed after Gram staining, a direct Nagler test can be performed.[5]

Method of Use:

1. Open the AnaeroGRO™ pouch just prior to use. Minimize specimen exposure to ambient oxygen levels in air.

2. Inoculate using a pure 24-72 hour culture. Streak the medium to obtain isolated colonies.

3. Immediately following inoculation, place the medium in an anaerobic atmosphere and incubate at 35-37ºC. for up to one week.

4. Observe for the appearance of lecithinase and lipase activity.

Nagler Test:

1. Prior to inoculation, allow medium to equilibrate to room temperature.

2. Swab one half of the medium with *C. perfringens* type A antitoxin and allow it to dry.

3. Starting from the side of the plate that does not contain antitoxin, make a single streak of the test organism.

4. Incubate the inoculated medium for 24-48 hours at 35ºC. in an anaerobic atmosphere.

INTERPRETATION OF RESULTS

Lecithinase

A positive lecithinase test is noted by the appearance of a white, opaque, diffuse zone that extends into the medium surrounding the colonies.

A negative lecithinase test is indicated by the absence of a white, opaque zone extending from the edge of the colony.

Lipase

A positive lipase test is noted by the appearance of an iridescent sheen (oil on water) that can be seen when the plate is held at an angle to a light source.

A negative lipase test is indicated by the absence of an iridescent sheen.

Nagler Test

A positive lecithinase reaction that occurs on the half of the medium without antitoxin and inhibition of lecithinase reaction on the half containing the antitoxin is indicative of a positive Nagler test.

A negative Nagler test is noted by a positive lecithinase reaction on both sides of the plate or no reaction on the agar.

Proteolysis

A positive proteolytic reaction is indicated by the development of clear zones in the medium surrounding colonial growth.

Lack of clearing around colonial growth is indicative of a negative proteolytic reaction.

Refer to the *Wadsworth-KTL Anaerobic Bacteriology Manual* or other texts for more information on identification of anaerobes.[5]

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.

A negative lecithinase test should be compared to an uninoculated control plate, as lecithinase can diffuse throughout the entire agar plate and make interpretation difficult.

Some microorganisms may require up to one week to produce a positive lipase reaction.

C. perfringens type A antitoxin is not specific for *C. perfringens*; a positive Nagler reaction can also be produced by *C. bifermentans*, *C. sordelli*, and *C. baratti*.

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 perform quality control of the media and processing procedures.

8. 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.

9. 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, swabs, applicator sticks, other culture media, incubators, incinerators, anaerobic culture materials, such as gas generators (Cat. no. AN25US), chambers, transports (Cat. no. S120D), jars (Cat. no. 16000), and oxygen 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:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Clostridium perfringens ATCC® 13124**	A	24hr	35°C	Anaerobic	Growth; lecithinase positive; white, opaque zone extending from edge of colonies, lipase negative; no sheen
Clostridium sporogenes ATCC® 11437	A	24hr	35°C	Anaerobic	Growth; lecithinase negative; lipase positive; iridescent sheen on agar surface when plate is held at an angle to the light source
Bacteroides fragilis ATCC® 25285**	B	24hr	35°C	Anaerobic	Growth; lecithinase and lipase negative; no reaction on agar

* 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

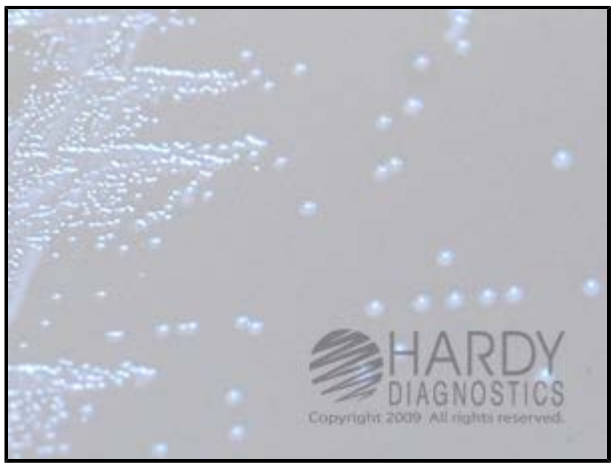
AnaeroGRO™ Egg Yolk Agar, Modified should appear slightly opaque, and light yellow to beige in color.

Clostridium perfringens (ATCC® 13124) colonies growing on AnaeroGRO™ Egg Yolk Agar, Modified (Cat. no. AG401). Incubated anaerobically for 24 hours at 35ºC.

Clostridium sporogenes (ATCC® 11437) colonies growing on AnaeroGRO™ Egg Yolk Agar, Modified (Cat. no. AG401). Photographed at an angle to show sheen (positive lipase). Incubated anaerobically for 24 hours at 35ºC.

Clostridium sordellii (ATCC® 9714) colonies growing on AnaeroGRO™ Egg Yolk Agar, Modified (Cat. no. AG401). Shown with backlight to emphasize white opaque zones around colonies (positive lecithinase). Incubated anaerobically for 24 hours at 35ºC.

Bacteroides fragilis (ATCC® 25285) colonies growing on AnaeroGRO™ Egg Yolk Agar, Modified (Cat. no. AG401). Incubated anaerobically for 24 hours at 35ºC.



Fusobacterium necrophorum (ATCC® 25286) colonies growing on AnaeroGRO™ Egg Yolk Agar, Modified (Cat. no. AG401). Incubated anaerobically for 24 hours at 35ºC.

Uninoculated plate of AnaeroGRO™ Egg Yolk Agar, Modified (Cat. no. AG401).

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. Dowell, V.R., Jr. and T.M. Hawkins. 1987. Laboratory Methods in Anaerobic Bacteriology. In: *CDC Laboratory Manual*. DHEW Publication No. (CDC) 87-8272. U.S. Department of Health, Education and Welfare, Public Health Service. Center for Disease Control, Atlanta, GA.

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

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

5. 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.

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

7. McClung, L.S. and R. Toabe. 1947. The Egg Yolk Plate Reaction for the Presumptive Diagnosis of *Clostridium sporogenes* and Certain Species of the Gangrene and Botulinum Groups. *J. Bacteriol.*; 53:139-147.

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

IFU-10030[A]

HARDY DIAGNOSTICS

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 2207A [D]