IFU

Instructions for Use

HARDY DIAGNOSTICS
A Culture of Service™

anaeroGRO™
Pre-Reduced Anaerobic Culture Media

BRUCELLA AGAR WITH HEMIN AND VITAMIN K (BRU)

Cat. no. AG301	Brucella Agar with Hemin and Vitamin K*, Monoplate	1 plate/pouch
Cat. no. AG304	Brucella Agar with Hemin and Vitamin K* 4Pak, Monoplate	4 plates/pouch
Cat. no. AG302	DuoPak A, BRU with Hemin and Vitamin K*, Monoplate; BBE/LKV*, Biplate	2 plates/pouch
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; LKV*, Monoplate; PEA*, Monoplate	3 plates/pouch

* These AnaeroGRO™ plated media are 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™ Brucella Agar with Hemin and Vitamin K is recommended for use in the primary isolation, quantitation, and partial identification of obligately anaerobic microorganisms from clinical specimens. This non-selective medium is also suitable for the growth of aerobic and microaerophilic bacteria when incubated under the appropriate conditions.

SUMMARY

Brucella Agar with Hemin and Vitamin K is a modification of the formulation given by the American Society for Microbiology (ASM).[4] According to Finegold, this medium is preferable to Heart Infusion Blood Agar Base for the cultivation of anaerobic bacteria.[8] Onderdonk et al. and Weinstein et al. both reported the addition of hemin.[9,11] Jousimies-Somer et al. further describe supplementing the medium with vitamin K.[5] It has been shown that Brucella Agar supports the growth of anaerobic gram-negative bacilli better than CDC (trypticase soy) or Schaedler's agar.[5]

AnaeroGRO™ Brucella Agar with Hemin and Vitamin K is an enriched medium designed to support and enhance the growth of fastidious microorganisms. The medium contains dextrose for energy, peptones to provide nitrogenous

compounds, and yeast extract as a source of B vitamins for cell maintenance and metabolism. Sheep blood provides additional growth factors required by some fastidious microorganisms and can also be used to assess hemolytic reactions as seen by the double zone beta-hemolysis of *Clostridium perfringens*. Hemin and vitamin K are nutritious supplements known to enhance the cultivation of some species of anaerobes, and to further promote the pigment production of *Prevotella melaninogenica*.[7] The medium is also suitable for use in susceptibility testing, differential disk, and spot biochemical testing.

AnaeroGRO™ Brucella Agar with Hemin and Vitamin K 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:*

Pancreatic Digest of Casein	10.0gm
Pancreatic Digest of Animal Tissue	10.0gm
Sodium Chloride	5.0gm
Yeast Extract	2.0gm
Reducing Agents/Peroxide Inhibitors	1.5gm
Dextrose	1.0gm
Sodium Bisulfite	0.1gm
Hemin	10.0mg
Vitamin K	10.0mg
Sheep Blood, Defibrinated	50.0ml
Agar	15.0gm

Final pH 7.0 +/- 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, moisture, and freezing.

The expiration dating 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.

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.

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 blood precautions. 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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

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

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[1-6,8] 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 transport medium (Cat. no. S120D) and refrigerated until inoculation.

When possible, clinical specimens should be inoculated directly onto the medium to prevent loss of organism viability. Minimize specimen exposure to ambient oxygen levels in air.

Method of Use: Consult listed references for the correct inoculation procedure.[1-6,8] Open the AnaeroGRO™ pouch just prior to use and immediately apply a liquid specimen directly to the agar surface; streak with a sterile inoculating loop to obtain isolated colonies. An enrichment broth, such as AnaeroGRO™ Thioglycollate Broth with Hemin and Vitamin K (Cat. no. AG22H), should be inoculated concurrently with primary isolation plates. For best results, specimens should be plated onto non-selective and selective media.

Immediately after culture, incubate plates anaerobically at 35-37ºC. for up to 72 hours. 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).

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

Consult listed references for the interpretation of growth of anaerobic species.[1-6,8]

Examine colonies using a dissecting microscope and a long-wave UV lamp to determine fluorescence. Certain strains of pigment producing *Bacteroides* spp. should fluoresce orange to brick-red under long-wave UV light. Fluorescence should be visible prior to colony pigmentation.

Refer to the *Wadsworth-KTL Anaerobic Bacteriology Manual* or other texts for more information on the 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.

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.

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, 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. Identification disks that are useful on Brucella with H and K would include Vancomycin (Cat. no. Z7501, Kanamycin (Cat. no. Z7191), SPS (Cat. no. Z7381), Colistin (Cat. no. Z8411) and Nitrate (Cat. no. Z7071).

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Bacteroides fragilis ATCC® 25285**	A	24-48hr	35°C	Anaerobic	Growth
Porphyromonas levii ATCC® 29147	A	24-48hr	35°C	Anaerobic	Growth
Clostridium perfringens ATCC® 13124**	A	24-48hr	35°C	Anaerobic	Growth; beta-hemolysis
Fusobacterium nucleatum ATCC® 25586	A	24-48hr	35°C	Anaerobic	Growth
Peptostreptococcus anaerobius ATCC® 27337	A	24-48hr	35°C	Anaerobic	Growth

* 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

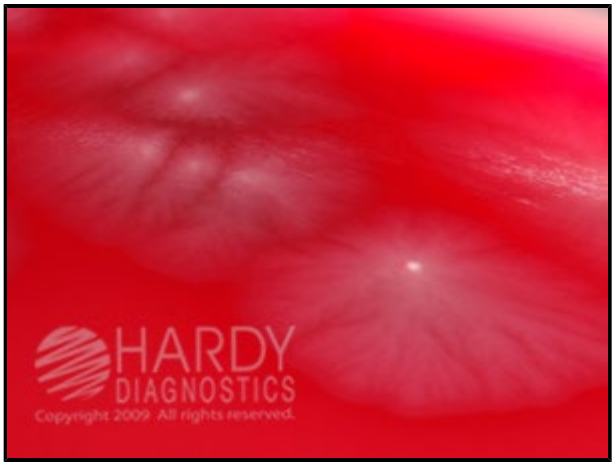
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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 certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

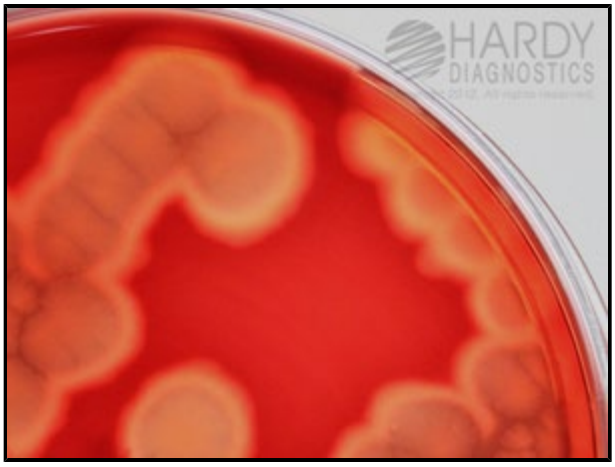
AnaeroGRO™ Brucella Agar with Hemin and Vitamin K should appear opaque, and medium to dark red in color.

Fusobacterium nucleatum (ATCC® 25586) colonies growing on AnaeroGRO™ Brucella Agar with H and K (Cat. no. AG301). Incubated anaerobically for 24 hours at 35ºC.

Bacteroides fragilis (ATCC® 25285) colonies growing on AnaeroGRO™ Brucella Agar with H and K (Cat. no. AG301). Incubated anaerobically for 24 hours at 35ºC.



Porphyromonas levii (ATCC® 29147) colonies growing on AnaeroGRO™ Brucella Agar with H and K (Cat. no. AG301). Incubated anaerobically for 24 hours at 35ºC.



Clostridium perfringens (ATCC® 13124) colonies growing on AnaeroGRO™ Brucella Agar with H and K (Cat. no. AG301). Incubated anaerobically for 48 hours at 35ºC.

Peptostreptococcus anaerobius (ATCC® 27377) colonies growing on AnaeroGRO™ Brucella Agar with

Uninoculated plate of AnaeroGRO™ Brucella Agar with H and K (Cat. no. AG301).

H and K (Cat. no. AG301). Incubated anaerobically for 48 hours at 35ºC.

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

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

9. Onderdonk, A.B. et al. 1974. *Infect. Immun.*; 10:1256.

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

11. Weinstein, W.M. et al. 1974. *Infect. Immun.*; 10:1250.

ATCC is a registered trademark of the American Type Culture Collection.

IFU-10026[B]

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: www.HardyDiagnostics.com
Email: TechService@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 [C]