IFU

Instructions for Use

anaeroGRO™

Pre-Reduced Anaerobic Culture Media

LKV (LAKED BLOOD WITH KANAMYCIN AND VANCOMYCIN) AGAR

Cat. no. AG061	BBE/LKV* Biplate	1 plate/pouch
Cat. no. AG302	DuoPak A, BRU with Hemin and Vitamin K*, Monoplate; BBE/LKV*, 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

* 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™ LKV (Laked Blood with Kanamycin and Vancomycin) Agar is recommended for use in the selective isolation and partial identification of obligately anaerobic gram-negative bacilli, such as *Prevotella* spp. and *Bacteroides* spp.

SUMMARY

LKV Agar is useful for the rapid isolation of *Prevotella* species. Yeast and other kanamycin-resistant organisms, such as *Capnocytophaga* species, sometimes grow on LKV; therefore, one should perform a Gram stain and determine the aerotolerance of all isolates.

Brucella Agar is the basal medium for LKV (Laked Blood with Kanamycin and Vancomycin) Agar. Dextrose, peptones, yeast extract, hemin, vitamin K and laked sheep blood are among the nutrients included in this medium. Dextrose serves as an energy source; peptones provide nitrogenous compounds, and yeast extract supplies B vitamins. Sodium chloride is incorporated to provide essential electrolytes. Sodium bisulfite, a reducing substance, is added to help maintain reduced conditions and a low pH.

Growth factors required by some anaerobic bacteria are provided by laked sheep blood. Hemin and vitamin K are incorporated to enhance the growth of *Bacteroides* species and to facilitate recovery and earlier pigment production of *Prevotella* spp.[7] Vancomycin inhibits the growth of gram-positive microorganisms and *Porphyromonas* spp. Kanamycin inhibits most gram-negative facultatively anaerobic bacilli, aerobes, and anaerobic gram-negative rods

except for *Prevotella* and *Bacteroides* spp.

AnaeroGRO™ LKV Agar 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:*

Peptamin	20.0gm
Sodium Chloride	5.0gm
Yeast Extract	2.0gm
Reducing Agents/Peroxide Inhibitors	1.5gm
Dextrose	1.0gm
Sodium Bisulfite	0.1gm
Hemin	5.0mg
Vitamin K	1.0mg
Vancomycin	40.0ml
Kanamycin	40.0ml
Laked Sheep Blood	50.0ml
Agar	17.0gm

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 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: 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. Immediate and proper transport to the laboratory is essential for successful recovery of significant anaerobic pathogens. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate anaerobic transport medium (Cat. no. S120D) and refrigerated until inoculation.

Recovery of anaerobes from clinical specimens requires reduced oxygen tension, low oxidation-reduction potential and the use of both selective and non-selective media.

Method of Use: Consult listed references for the correct inoculation procedure.[1-6,8] Minimize specimen exposure to ambient oxygen levels in air. Open the AnaeroGRO™ pouch just prior to use and immediately apply a liquid specimen directly to the agar surface. An enrichment broth, such as AnaeroGRO™ Thioglycollate Broth with H and K (Cat. no. AG22H), should be inoculated concurrently with primary isolation plates.

A large amount of inoculum should be used; streak inoculum to obtain isolated colonies. Incubate plates anaerobically at 35-37ºC. for up to 48 hours. 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

Examine for characteristic colonial growth and morphology. Use aerotolerance testing, biochemical testing, and/or gas-liquid chromatography for complete identification of anaerobes. Consult listed references for the interpretation of growth of anaerobic species.[2-6,8,9,11]

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.

Some organisms may be inhibited on LKV Agar. Therefore, it is recommended that a non-selective medium, such as AnaeroGRO™ Brucella Agar with H and K (Cat. no. AG301), be inoculated in parallel to ensure growth of all species present.

Some species of facultative organisms may grow on LKV medium, so a test for aerotolerance should be used to confirm colony type.

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, transport media (Cat. no. S120D), incubators, incinerators, anaerobic culture materials, such as gas generators (Cat. no. AN25US), compact systems (Cat. no. AN010C), sealing clips (Cat. no. AN005C), chambers, 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	
Bacteroides fragilis ATCC® 25285**	A	24-48hr	35°C	Anaerobic	Growth
Prevotella melaninogenica ATCC® 25845	A	24-48hr	35°C	Anaerobic	Growth
Escherichia coli ATCC® 25922**	B	24hr	35°C	Aerobic	Inhibited
Staphylococcus epidermidis ATCC® 12228	B	24hr	35°C	Aerobic	Inhibited

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

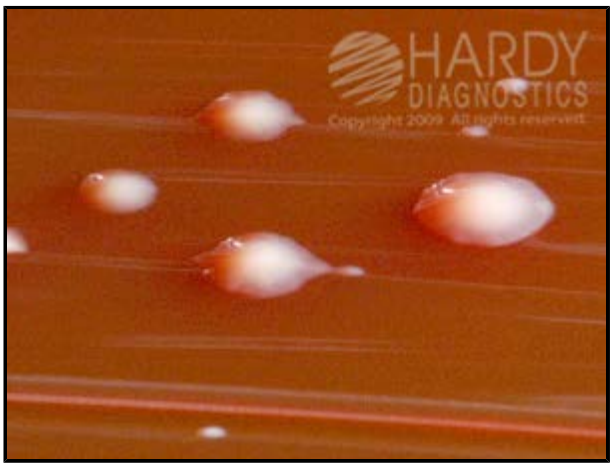
** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

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™ LKV Agar should appear clear, and reddish-brown in color.



Bacteroides fragilis (ATCC® 25285) colonies growing on AnaeroGRO™ LKV Agar. Incubated anaerobically for 48 hours at 35ºC.

Uninoculated plate of AnaeroGRO™ LKV Agar.

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

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