IFU

Instructions for Use

LKV (LAKED BLOOD WITH KANAMYCIN AND VANCOMYCIN)AGAR

Cat. no. A60	LKV Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. J87	Brucella with H & K / LKV, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J102	BBE / LKV, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Laked Blood with Kanamycin and Vancomycin Agar is recommended for use in the isolation and partial identification of anaerobic microorganisms.

SUMMARY

Brucella Agar is the basal medium for Laked Blood with Kanamycin and Vancomycin Agar. Dextrose, peptones, yeast extract, hemin, vitamin K and laked sheep blood are among the nutrients included in the 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 the added laked sheep blood, which also allows the demonstration of hemolytic reactions and enhances earlier pigmentation of *Prevotella* spp. Hemin and vitamin K are incorporated into the medium to enhance the growth of gram-positive spore-formers and *Bacteroides* species.[6] Vancomycin is employed to inhibit the growth of gram-positive organisms and kanamycin to inhibit gram-negative facultatively anaerobic bacilli.

FORMULA

Ingredients per liter of deionized water:*

Peptamin	20.0gm
Sodium Chloride	5.0gm
Yeast Extract	2.0gm
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 2-8ºC. 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 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.[1-5] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. 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 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.[1-5,9] Prior to inoculation, the medium should be brought to room temperature. A large amount of inoculum should be used. This will minimize the damaging

effects of toxic oxygen growth-limiting factors. Streak inoculum to obtain isolated colonies. Incubate plates anaerobically at 35-37ºC. for up 48 hours. Confirmation of anaerobic organisms should be performed by subculturing to an aerobic Blood Agar plate (Cat. no. A10). Examine anaerobic colonies for hemolytic reaction, colony morphology, gram stain, and further biochemical testing.

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.[2-5,9]

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.

It is suggested that LKV plates be reduced, prior to use, by placing them in an anaerobe jar at room temperature for a period of 6-24 hours.

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, other culture media, swabs, applicator sticks, 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	
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.

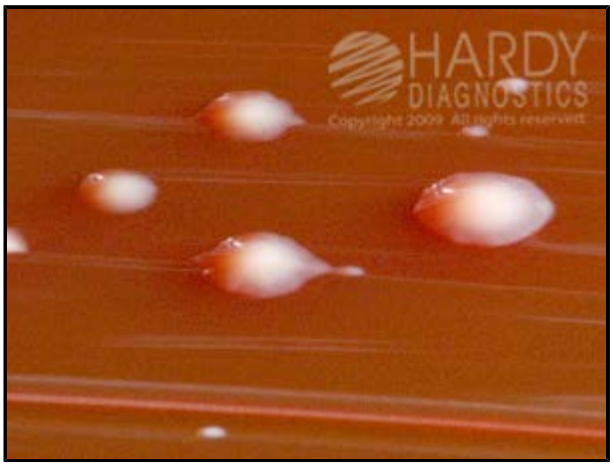
USER QUALITY CONTROL

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

LKV Agar should appear clear, and brown in color.



Bacteroides fragilis (ATCC® 25285) colonies growing on LKV Agar (Cat. no. A60). Incubated anaerobically for 48 hours at 35ºC.

Uninoculated plate of LKV Agar (Cat. no. A60).

REFERENCES

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

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

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

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9. 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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HDQA 2207F [D]