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Instructions for Use

NEOMYCIN ANAEROBIC BLOOD AGAR

Cat. no. A62	Neomycin Anaerobic Blood Agar, 15x100mm Plate, 17ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Neomycin Anaerobic Agar is recommended for use in the primary isolation of fastidious anaerobic microorganisms.

SUMMARY

The formulation for Neomycin Anaerobic Blood Agar was developed by Dowell and Hawkins at the Centers for Disease Control (CDC) in Atlanta, Georgia.[4]

The basal medium is composed of tryptic soy agar supplemented with yeast extract, vitamin K, hemin, cystine and 5% sheep blood. Digests of soybean meal and casein, components of tryptic soy agar, provide amino acids and other nitrogenous compounds. Sodium chloride is added to maintain osmotic equilibrium. Yeast extract is rich in vitamins and enhances the growth of fastidious microorganisms. Vitamin K produces enhanced growth of pigmented *Bacteroides* spp. Sheep blood, hemin and cystine provide additional growth factors required by certain anaerobic microorganisms. The medium is made selective by the incorporation of neomycin which inhibits gram-negative Enterobacteriaceae.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	15.0gm
Peptic Digest of Soybean Meal	5.0gm
Sodium Chloride	5.0gm
Yeast Extract	5.0gm
L-Cystine Dihydrochloride	0.4gm
Neomycin Sulfate	0.1gm
Vitamin K	10.0mg
Hemin	5.0mg
Sheep Blood	50.0ml
Agar	15.0gm

Final pH 7.2 +/- 0.3 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), 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 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.[1,2,5] 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 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: Allow the plates to warm to room temperature, and the agar surface to dry before inoculating. Inoculate and streak the specimen as soon as possible after collection. Streak for isolation with a sterile loop. Incubate plates in an anaerobic atmosphere at 35-37ºC. for at least 48 hours and up to seven days. Examine for typical colonial morphology and characteristics.

INTERPRETATION OF RESULTS

Consult listed references for the identification of colony morphology and further biochemical tests required for identification.[1-3,5]

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.

Recovery of some anaerobic microorganisms may necessitate prereduction of the medium by placing it in an oxygen-free holding jar just prior to inoculation.

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.

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.

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, anaerobic holding jars, anaerobic incubation systems, 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	24-48hr	35°C	Anaerobic	Growth
Bacteroides fragilis ATCC® 25285	A	24-48hr	35°C	Anaerobic	Growth
Escherichia coli ATCC® 25922	B	24hr	35°C	Aerobic	Inhibition

* 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

Neomycin Anaerobic Blood Agar should appear opaque, and red in color.

Bacteroides fragilis (ATCC® 25285) colonies growing on Neomycin Anaerobic Blood Agar (Cat. no. A62). Incubated anaerobically for 48 hours at 35ºC.

Escherichia coli (ATCC® 25922) inhibited on Neomycin Anaerobic Blood Agar (Cat. no. A62). Incubated aerobically for 24 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. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

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

4. Dowell, V.R. and Hawkins, T.M. CDC Laboratory Manual, Jan. 1974.

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

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

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

IFU-10606[A]

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Ordering Information

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

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