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Instructions for Use

MUELLER HINTON AGAR WITH BLOOD

Cat. no. A59	Mueller Hinton with Blood, 15x100mm Plate, 25ml	10 plates/bag
Cat. no. H21	Mueller Hinton with Blood, 15x150mm Plate, 72ml	10 plates/bag

INTENDED USE

Hardy Diagnostics' Mueller Hinton with Blood is recommended for use in disk diffusion sensitivity testing of *Streptococcus* spp., including *Streptococcus pneumoniae* .

SUMMARY

The basal medium for Mueller Hinton with Blood is Mueller Hinton Agar supplemented with 5% defibrinated sheep blood. Mueller and Hinton developed Mueller Hinton Agar in 1941 to be a protein free medium for isolating pathogenic strains of *Neisseria*.[10] It was found that Mueller Hinton Agar was useful in identifying sulfonimide-resistant and responsive strains of gonococci.[13] This medium is now used in standardized antimicrobial disk susceptibility testing as described by Bauer and Kirby et al.[5] Barry and Fay investigated the effects of altering the depth of plated Mueller Hinton Agar on disk diffusion testing, and determined a standardized depth of approximately four millimeters to be sufficient.[4] In 1970 Dewees et al., studied the effect of storage on Mueller Hinton Agar plates used for antimicrobial disk diffusion zone sizes. Their findings indicated commercially manufactured Mueller Hinton Agar plates were suitable for use in routine susceptibility testing.[6] In addition to the above criteria, Hardy Diagnostics' Mueller Hinton with Blood Agar meets the standards of performance established by the Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS).[11]

Mueller Hinton with Blood contains beef infusion, casamino acids and starch. Starch acts as a colloid that protects against toxic material in the medium. Beef infusion and casamino acids provide a source of energy and nutrients. Agar is added as a solidifying agent. The levels of tetracycline and sulfonamide inhibitors, thymidine, thymine, magnesium and calcium ions are controlled so as not to interfere with susceptibility testing and to yield good growth. Fresh, defibrinated sheep blood is added to support the growth of the fastidious microorganism, *Streptococcus pneumoniae*.

The Kirby-Bauer antimicrobial disk diffusion and sensitivity procedure is used with Mueller Hinton with Blood Agar plates and disks impregnated with specific antibiotics. [9,11,14] The medium is inoculated and a known concentration of organism is grown in the presence of disks containing relevant antibiotics. Antibiotics in the disks diffuse into the agar during incubation. If the organism is susceptible to a particular antibiotic, an area of clearing around the disk will result in which the organism does not grow. The diameter of this clearing is called a zone of inhibition. In general, larger zones indicate that an organism is more sensitive to an antimicrobial agent than smaller zones. However, zone sizes have been standardized in order to interpret the most effective antimicrobial agent against a particular organism. Established zone diameters can then be used to interpret resistant, intermediate, and sensitive results for pathogenic microorganisms and this data used to effectively manage patient care. Current zone diameters are updated annually and published in the Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), document M100-S, *Performance Standards for Antimicrobial Susceptibility Testing*.[9]

FORMULA

Ingredients per 950ml of deionized water:*

Acid Hydrolysate of Casein	17.5gm
Beef Extract	2.0gm
Starch	1.5gm
Sheep Blood	50.0ml
Agar	17.0gm

Final pH 7.3 +/- 0.1 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.[1-3,7,8] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

This product is not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organism. This product is used in conjunction with other biochemical tests to identify pure cultures of isolated organism.

Method of Use: Information pertaining to the preparation and inoculation of dilutions of organism for disk diffusion susceptibility testing can be obtained by consulting the CLSI publications M2-A; and M7-A.[9,11]

When inoculating, the surface of the medium should be moist but free of droplets of moisture. If excess surface moisture is present on the medium or on the petri dish covers, incubate uninoculated plates at 35 degrees C. until the moisture is lost by evaporation.

INTERPRETATION OF RESULTS

Consult the most current publications of the CLSI documents M100-S and M02-A.[9,11]

LIMITATIONS

In vitro susceptibility does not necessarily imply *in vivo* effectiveness.

Failure to follow CLSI recommended procedures as described in the standard documents may result in inaccurate results.[9, 11]

Bacterial suspensions must be prepared according to the CLSI recommended procedure and compared with the appropriate turbidity standard to ensure reliable results.[9,11]

The plates are to be incubated at 35ºC. in 5-10% CO_2 for 20-24 hours. A longer incubation time may lead to erroneous results: i.e. zones that are too small.

Refer to the "Disk Diffusion Trouble Shooting Guide", in the Hardy Diagnostics software program HUGO™, for additional information.

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, antimicrobial disks, Mueller Hinton Broth, calipers, forceps, incinerator, 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	
Streptococcus pneumoniae ATCC® 49619	F	20-24hr	35°C	CO_2**	Growth

Refer to Table 3A in the current CLSI publication of the M100-S.[9,11]

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

Mueller Hinton Agar with Blood should appear opaque, and cherry red in color.

Note: The fills may be adjusted to provide the proper quality control zone diameters.

Streptococcus pneumoniae (ATCC® 49619) growing on Mueller Hinton Agar with Blood (Cat. no. H21) for antimicrobial disk susceptibility test. Incubated in CO_2 for 24 hours at 35ºC.

REFERENCES

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4. Barry, and Fay. 1973. *Am. J. Clin. Pathol.*; 50:196.

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

9. *Performance Standards for Antimicrobial Susceptibility Testing; Informational Supplement (Aerobic Dilution)* , Supplemental Tables M100-S (M02 and M07). Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS),

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10. Mueller, J.H., and J. Hinton. 1941. A Protein-Free Medium for Primary Isolation of the *Gonococcus* and *Meningococcus*. *Proc. Soc. Exp. Diol. and Med.*; 48:330-333.

11. *Performance Standards for Antimicrobial Disk Susceptibility Tests* . M02-A. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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

13. Ryan, K.J., et al. 1970. Disk Sensitivity Testing. *Hosp. Prac.*; 5:91-100.

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