HealthLink
800-638-2625

Product No. 1103/1106
Revision No. 05

PRODUCT INFORMATION AND QUALITY CONTROL SHEET

MUELLER HINTON AGAR

I. INTENDED USE

Mueller Hinton Agar is the recommended medium for use in the standardized disc diffusion procedure for determining the susceptibility of common rapidly growing bacteria to antimicrobial agents.

II. SUMMARY AND EXPLANATION

Mueller Hinton Agar was originally formulated for the isolation of pathogenic *Neisseria* species. These organisms are now commonly isolated using selective media such as Modified Thayer Martin and Martin Lewis.

In the 1960s, a wide range of media and procedures were used in clinical microbiology laboratories to determine susceptibility patterns of bacteria to various antimicrobial agents. Bauer, Kirby and others selected Mueller Hinton Agar as the medium of choice to develop a standardized procedure for the determination of susceptibility patterns by the disc diffusion method. An international study confirmed the value of Mueller Hinton medium for this purpose due to its reproducability, simplicity of formula and large experimental data base.

The National Committee for Clinical Laboratory Standards (NCCLS) has published a performance standard for the standardized disc diffusion procedure and this document should be consulted for details.[1]

III. PRINCIPLES OF THE PROCEDURE

The standardized disc diffusion procedure is based on the ability of an antimicrobial agent impregnated on a paper disc to diffuse through an agar gel. Susceptibility results are determined by measuring the zones of inhibition created when a paper disc containing a specified amount of an antimicrobial agent is incubated in the presence of a confluent inoculation of a standardized suspension of a single microorganism. These measured zones of inhibition are compared to interpretive standards, derived by correlating zone sizes to minimum inhibitory concentrations (MICs), which provide an interpretation of susceptible, moderately susceptible, intermediate or resistant.

IV. TYPICAL FORMULA AND APPEARANCE

Appearance = pale amber, slightly opalescent
(Approximate formula* per liter of processed water)

Beef Extract	2.0g
Acid Hydrolysate of Casein	17.5
Starch	1.5
Agar	14.0

*adjusted and/or supplemented to meet performance criteria.

V. PRECAUTIONS

This product is for IN VITRO diagnostic use only. Culture specimens may contain microorganisms which can be potentially infectious to the user. Strict adherence to aseptic techniques and established precautions against microbiological hazards should be followed throughout the procedure. Carefully dispose of all items which contact patient specimens or isolated bacteria. (See Material Safety Data Sheet for further information.)

VI. STORAGE/SHELF LIFE

Plated media should be stored at 2-8°C (36-46°F), media side up, in the unopened or resealed package protected from light. DO NOT FREEZE OR EXPOSE TO HIGH TEMPERATURES. Allow unopened plates to warm to room temperature prior to inoculation. Prior to and during inoculation procedures, plates should be handled in a manner that minimizes product exposure to the environment. Product which has exceeded the assigned expiration date noted on the label should not be used. Do not use plates that exhibit evidence of drying, cracking, discoloration, microbial contamination or any other signs of deterioration. The presence of excessive condensate may indicate plates which have been damaged by exposure to temperature extremes.

VII. SPECIMEN COLLECTION

The standardized disc susceptibility procedure is designed for use with pure cultures. Isolated colonies of each microorganism type that may be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility. Guidelines for antimicrobial selection may be found in the NCCLS document M2-A5, "*Performance Standards for Antimicrobial Disk Susceptibility Tests*".[1]

VIII. MATERIALS PROVIDED

Mueller Hinton Agar Plates and lot specific Quality Control Certificate.

IX. MATERIALS REQUIRED BUT NOT PROVIDED

Incubator maintaining 35-37°C.
Antimicrobial discs
0.5 McFarland Standard
Tubed broth media
Measuring device in millimeters (mm)
Ancillary culture media, reagents. and laboratory equipment as required.

X. PROCEDURE

Refer to the NCCLS document M2-A5, "*Performance Standards for Antimicrobial Disk Susceptibility Tests*" for detailed procedural instructions.[1]

Standard Method:

1. Select at least 4 - 5 well isolated colonies of the same morphological type from an agar plate culture. Touch the top of each colony with an inoculation loop or needle and transfer to a tube containing approximately 5 ml of broth medium (example: trypticase soy broth.)

2. Incubate the broth culture at 35 - 37° C until it meets or just exceeds the turbidity of a 0.5 McFarland barium sulfate standard (approx. 2 - 6 hours).

3. Adjust the turbidity of the growing broth culture with sterile saline or broth to obtain a turbidity visually comparable to that of the 0.5 McFarland Standard. Tubes should be visually compared under adequate light against a white background with contrasting black lines.

4. Within 15 minutes after turbidity adjustment, dip a sterile swab into the suspension and rotate it firmly against the tube wall to express excess fluid.

5. Inoculate the surface of the Mueller Hinton plate by streaking the swab over the entire agar surface. Repeat this procedure two more times, rotating the plate approximately 60° each time to ensure even inoculum distribution. Note: Mueller Hinton plates should be free of excessive moisture prior to inoculation.

6. Place the impregnated antimicrobial discs on the surface of the agar plate using forceps or a disc dispenser. Gently press down each disc to ensure complete contact with the agar surface. Discs must be distributed evenly so that they are no closer than 24mm from center to center. Note: Discs should not be moved once in contact with the agar surface due to the

instantaneous diffusion of some of the drug.

7. Invert the plates and place them in a 35-37°C incubator within 15 minutes after the discs are applied. Note: Plates should not be incubated in an atmosphere of increased carbon dioxide.

8. Examine plates after 16-18 hours of incubation by holding against a non-reflective black background illuminated from above. Measure the zones of complete inhibition, including the diameter of the disc, to the nearest whole millimeter. The endpoint should be taken as the area showing no obvious visible growth with the unaided eye. Do not include the area of faint growth of tiny colonies at the edge of the obvious zone of inhibition.

If the test organism is a *Staphylococcus* or *Enterococcus* sp., 24 hours of incubation is required and plates are examined by being held up to the light to interpret light growth of methicillin or vancomycin resistance. Any discernable growth within the zone of inhibition is indicative of methicillin or vancomycin resistance.

Note: A confluent "lawn" of growth should be achieved. If only isolated colonies are present, the inoculum was too light and the test should be repeated.

9. Refer to Table 2 "Zone Diameter Interpretive Standards Table" contained within NCCLS document M2-A5 for expected values for testing common, rapidly growing pathogens.

XI. EXPECTED RESULTS

Observed zone diameters, measured to the nearest whole millimeter, should be compared to those listed in NCCLS document M2A5, Table 2 "Zone Diameter Interpretive Standards and Equivalent Minimum Inhibitory Concentration (MIC) Breakpoints for Organisms other Than *Haemophilus* and *Neisseria gonorrhoeae*." Specific organism results may then be reported as resistant, intermediate, or susceptible for each antimicrobial agent tested.

Note: Information supplements to NCCLS Document M2-A5, containing revised tables of antimicrobial discs and interpretive standards are published periodically. The latest tables should be consulted for current recommendations. For information on current publications, call HealthLink at (800-638-2625). The complete NCCLS document standard and informational supplements can be ordered from the National Committee for Clinical Laboratory Standards, 771 E. Lancaster Ave., Villanova, PA 19085. Telephone: (215) 525-2435 Fax: (215) 527-8399

Control cultures should be included each time a susceptiblity test is performed or weekly if satisfactory performance can be documented according to the NCCLS Standard. Zone sizes should fall within the ranges specified in NCCLS document M2A5, Table 3 "Control Limits for Monitoring Antimicrobial Disk Susceptibility Tests – Zone Diameter (mm) Limits for Individual Tests on Mueller Hinton Medium without blood or Other Supplements". (See Section, "QUALITY CONTROL".)

XII. LIMITATIONS

The NCCLS disc diffusion method has been standardized for testing rapidly growing pathogenic microorganisms. Organisms which require an anaerobic atmosphere, have a poor or slow growth rate on Mueller Hinton Agar, or have a definite strain to strain variation in growth rate should not be tested by the disc diffusion method. Fastidious organisms, such as *Haemophilus* sp., *Neisseria* sp, and *Streptococcus pneumoniae,* require a modification of the procedure listed. (See product information and quality control sheet for catalog #1104/1107.)

Improper storage of antimicrobial susceptiblity discs may cause a loss of potency and a falsely resistant result.

In vitro susceptibility of an organism to a specific antimicrobial agent does not necessarily mean that the agent will be effective *in vivo*. Consult appropriate microbiology references for guidance in the interpretation of results.

Certain bacteria requireing thymine or thymidine may not grow satisfactorily on Mueller Hinton Agar that contains low levels of these compounds.

For details discussing detection of MRSA resistant staphylococci, and antimicrobial resistance of enterococci (penicillin/ampicillin, vancomycin and high-level aminoglycoside), refer to NCCLS Document M2-A5.[1]

XIV. REFERENCES

1. National Committee for Clinical Laboratory Standards. 1993. Approved Standard: M2-A5. Performance Standards for Antimicrobial Disk Susceptibility Tests, 5th ed. National Committee for Clinical Laboratory Standards, Villanova, PA.

2. Finegold, S.M. and W.S. Martin. 1982. Bailey and Scott's Diagnostic Microbiology, 6th ed. C.V. Mosby Company, St, Louis.

3. Koneman, E.S., S.D.Allen, V.R. Dowell, Jr. and H. M. Sommers. 1983. Color Atlas and Textbook of Microbiology, 2nd ed. J.B. Lippincot Company, Philadelphia.

4. Lennette, E.H., ed. 1991. Manual of Clinical Microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

USER QUALITY ASSURANCE/ QUALITY CONTROL PROCEDURES AND INFORMATION

HealthLink manufactures all microbiological media in conformance with NCCLS guidelines where applicable. Dehydrated culture media used in this product has been manufactured according to NCCLS guideline M6-T, *"Evaluating Production Lots of Dehydrated Mueller Hinton Agar"*; Tentative Standard, June, 1993.

HealthLink recommends that the following quality assurance and quality control procedures be performed on each batch of product.

I. QUALITY ASSURANCE

The following quality assurance procedures must be performed to assure the product will perform according to its intended use within the assigned expiry date:

1. Daily, document that product storage refrigerator maintains temperature within the recommended range: 2-8°C.

2. Daily, document that laboratory incubator maintains temperature within the recommended range: 33-37°C.

II. QUALITY CONTROL

The following incoming inspection procedures must be performed for each batch (batch = same lot, same shipment) of culture media received in the laboratory :

1. Inspect plates according to instructions contained on the "Quality Control Log Sheet." (See also Section VI "STORAGE/SHELF LIFE")

2. Peel off the lower portion of a product bag label (Quality Control Certificate) for the lot being accepted into the laboratory and affix it to the Quality Control Log Sheet.

3. Initial and date the Quality Control Log Sheet.

Note: Notify Technical Service immediately if media

does not meet the inspection criteria.

4. To control the accuracy and precision of the disc diffusion test procedure, Section 9.0 "Quality Control Procedures" of the NCCLS document M2-A5 should be adhered to.

Test Organisms

Staphylococcus aureus ATCC 25923
Escherichia coli ATCC 25922
Escherichia coli ATCC 35218
Pseudomonas aeruginosa ATCC 27853
Enterococcus faecalis ATCC 33186 or 29212

Zone sizes should fall within established acceptable zone diameter ranges for each antimicrobial agent as specified in Table 3 of the NCCLS document M2-A5. (See information box under "EXPECTED RESULTS")

TECHNICAL SERVICE

HealthLink provides a toll free technical service line (1-800-638-2625) to assist with product usage. To receive additional product information, QA/QC log sheets, or to have technical questions answered; please call between the hours of 9:00 am to 5:00 pm EST.

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