HealthLink
800-638-2625

Product No. 1104/1107
Revision No. 05

PRODUCT INFORMATION AND QUALITY CONTROL SHEET

MUELLER HINTON AGAR WITH 5% SHEEP BLOOD

I. INTENDED USE

Mueller Hinton Agar with 5% sheep blood is the recommended medium for antimicrobial disc diffusion susceptibility testing of *Streptococcus pneumoniae.*

II. SUMMARY AND EXPLANATION

In 1977 an outbreak of multiple resistant *Streptotococcus pneumoniae* occurred in South Africa. In the United States the incidence of penicillin-relatively resistant pneumococci has increased and a few cases of penicillin-resistant pneumococci have been reported. The National Committee for Clinical Laboratory Standards (NCCLS) has published a performance standard for the standardized disc diffusion susceptibility procedure which includes testing the penicillin resistance of *Streptococcus pneumoniae.* This standardized procedure utilizes Mueller Hinton Agar supplemented with 5% sheep blood as the recommended test medium. This document should be consulted for further 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.

The addition of sheep blood to the standard Mueller Hinton Agar formula provides the enrichment necessary for good growth of *S. pneumoniae* and proper test interpretation.

IV. TYPICAL FORMULA AND APPEARANCE

Appearance = opaque, cherry red

(Approximate formula* per liter of processed water)

Beef Extract	2.0g
Acid Hydrolysate of Casein	17.5
Starch	1.5
Agar	14.0
Defibrinated sheep blood	5%

*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 diffusion susceptibility procedure is designed for use with pure cultures. Isolated colonies of *S. pneumoniae* should be selected from primary or overnight agar plates and tested for susceptibility to penicillin.

VIII. MATERIALS PROVIDED

Mueller Hinton Agar Plates with 5% sheep blood 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]

1. Remove growth of *S. pneumoniae* from an overnight agar plate with a sterile loop or swab and suspend in a suitable broth medium such as Mueller Hinton or 0.9% saline. Adjust the turbidity to equal that of a 0.5 McFarland barium sulfate standard.

2. Inoculate the adjusted broth culture onto Mueller Hinton Agar supplemented with 5% sheep blood as follows: a) dip a sterile swab into the suspension and rotate it firmly against the tube wall to express excess fluid, b) streak the swab over the entire agar surface, c) without redipping the swab, repeat this procedure two more times rotating the plate approximately 60° each time to ensure even inoculum distribution.

3. Aseptically place a 1 μg oxacillin disk onto the agar and gently press down to ensure adherence with the agar surface. Invert the plate and place it in a 35-37°C incubator in an atmosphere of 5-7% CO_2 for 20-24 hours.

Other antimicrobial agents that are suggested for routine testing of pneumococci are indicated in Table 1A of the NCCLS document M2-A5.

Note: A 10-U penicillin disc is not to be used to screen for penicillin resistance.

XI. EXPECTED RESULTS

Observed zone diameters, measured to the nearest whole millimeter, should be compared to those listed in NCCLS document M2A5, Table 2C.

Note: Isolates of *S. pneumoniae* with oxacillin zone sizes of $\geq$ 20mm are susceptible to penicillin. The disc test does not, however, distinguish intermediate strains from strains that are resistant. A penicillin MIC should be determined on isolates of *S. pneumoniae* with oxacillin zone sizes of $\leq$ 19mm.

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 BioClinical Systems, Inc. (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 susceptibility 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 3C. "Control Limits for Monitoring Antimicrobial Disk Susceptibility Tests – Zone Diameter (mm) Limits for Individual Tests on Mueller Hinton Medium with blood". (See Section, "QUALITY CONTROL".)

XII. LABORATORY RESULTS

Observed zone diameters, measured to the nearest whole millimeter, should be interpreted as follows; penicillin-susceptible strains of *S. pneumoniae* will have oxacillin zone sizes $\geq$ 20mm, penicillin-resistant or relatively resistant strains will have zone sizes $\leq$ 19mm. (Refer to document M2A5, Table 2C.)

XIII. LIMITATIONS

This NCCLS disc diffusion procedure method for *S. pneumoniae* has been standardized for determining relative resistance to penicillin and cannot be used to distinguish between penicillin-resistant and relatively resistant strains.

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. 1985. Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, D.C.

USER QUALITY ASSURANCE/ QUALITY CONTROL PROCEDURES AND INFORMATION

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: 22-35°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.

TECHNICAL SERVICE

HealthLink provides a toll free technical service line (1-800-638-2625) to assist with product usage. To receive additional product information, procedural instructions, QA/QC log sheets, Material Safety Data 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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