PROCEDURE: DISK DIFFUSION

I. PRINCIPLE

Disks containing antibiotics are applied to the surface of Mueller Hinton Agar (MH) Plates or Mueller Hinton Agar plates containing 5% sheep blood (BMH). The type of plate used for testing will be dependent upon what organism is being tested. These MH agar plates have been swabbed with a standard inoculum of a pure culture of a clinical isolate prior to the placement of the antibiotic impregnated disk. These inoculated MH plates with the disks are incubated overnight. Following incubation, the plates are examined, and the diameter of zone size is measured around each disk. The diameters are compared to established standards in order to determine susceptibility of the organisms to the specific antibiotic tested.

II. SPECIMEN

Pure isolate of non-fastidious organisms that have standard susceptibility interpretations published in the CLSI M100 document.

III. MATERIALS

A. Mueller Hinton Agar
B. Mueller Hinton Agar with 5% sheep blood
C. Sterile Saline aliquots
D. Commercially prepared and standardized antimicrobial/chemotherapeutic impregnated disks
E. Sterile tipped swabs
F. Sterile plastic transfer pipettes
G. McFarland 0.5 turbidity standard
H. Densitometer
I. Forceps
J. Ruler
K. Vortex mixer
L. Incubators (35°C ambient-air and 5% CO_2)
M. Multi-disk dispensing apparatus
N. CLSI M100 guidelines for interpretation

IV. STORAGE AND HANDLING

A. MH and BMH - 2-8°C
B. Saline – 2-30°C
C. Antimicrobial disks – store with desiccant at 2-8°C or as otherwise indicated in the package insert.
    1. Meropenem is stored between -20°C to 8°C

V. QUALITY CONTROL

A. Quality control on the densitometer is performed and documented each day. Invert (do NOT vortex) 1.0 McFarland and 0.5 McFarland standards. Instrument should read as zero with no tubes inserted. Place the standards in the instrument and record the readings. Calibrate as needed.
    1. Calibrate 1.0 McF using the 1.0 CAL knob.
    2. Calibrate 0.5 McF using the 0.5 CAL knob.
B. Refer to *Quality Control Procedure for Antimicrobial Susceptibility Testing* for complete disk diffusion quality control instructions

VI. TEST PROCEDURE

A. Bring MH/BMH plates and disks to room temperature prior to use.
B. Prepare the bacterial inoculum by direct suspension method. Fresh (18-24hours old) growth from a non-selective media should be used.
    1. Select three to five similar colonies and transfer with sterile inoculation needle or loop into a sterile saline aliquot tube.
    2. Vortex the inoculated saline suspension to ensure even distribution of the bacteria.
    3. Check the turbidity of the inoculated saline. The turbidity equivalent to a 0.5 McFarland standard is required.
    4. Dilute by adding sterile saline with a sterile transfer pipet to the inoculated tube, if necessary, to achieve the desired 0.5 McFarland standard that is required.
C. Inoculation of MH/BMH
    1. Choose the appropriate Mueller Hinton Agar
        a. MH is used for most non-fastidious organisms such as *Staphylococcus* spp., *Enterococcus* spp., Enterobacteriaceae and *Pseudomonas aeruginosa*.
        b. BMH is used with most streptococci.
    2. Within 15 minutes of properly adjusting the inoculated saline, dip a sterile swab into the inoculum and rotate/press firmly several times against the upper inside wall above the fluid level to express extra fluid from the swab.
    3. Streak the entire MH/BMHA plate surface three times (in three planes – turning 60 degrees in between streaking) to obtain an even inoculation on the plate surface. Avoid hitting the side of the agar plate.
    4. Run the swab around the edge of the MHA/BMHA plate to remove excess moisture.
D. Application of Disks
    1. Select the appropriate panel of disks (refer to organism specific susceptibility panels in the *Antibiotic Battery* procedure).
    2. Apply disks with the loaded multi-disk dispenser – use aseptic precautions. The disks should be set so that their centers are at least 24mm apart.
    3. Gently press the disks down with sterile forceps to ensure they are making proper contact with the surface of the MH/BMH.
    4. There should be no more than 12 disks on the Mueller Hinton agar plate.
    5. Within 15 minutes of inoculation, place the plates (agar side up) in the appropriate incubator (5% CO2 is for the incubation of streptococci).
E. Incubation and reading zone sizes
    1. Examine the plates after 16-18 hours of incubation (20-24 hours for *Streptococcus* and a full 24 hours of incubation is recommended for *Staphylococcus/Enterococcus*. If the growth on the plates is not pure, the testing should be repeated. If isolated colonies are growing, the inoculum was too light, and the testing should be repeated.
    2. READ DISKS: Measure the diameter of the zone of inhibition in millimeters.
    3. For hemolytic colonies, measure the zone of inhibition of bacterial growth, not the zone of inhibition of hemolysis.
    4. If bacteria are growing up to the edge of the disk, the zone size is the diameter of the disk (6mm).
    5. For swarming *Proteus* spp., measure the obvious edge of inhibition. Ignore the swarming portion of growth.
    6. Measure the more obvious edge of growth around sulfonamides (example: SXT). Disregard light growth that is usually about 20% or less of the lawn growth.

VII. INTERPRETATION

A. Interpretive susceptibly criteria are obtained from CLSI M100 breakpoints/FDA STIC. Interpretations are auto populated based on zone sizes entered into the LIS system.

VIII. LIMITATIONS

A. For in vitro Diagnostic use only
B. Disk performance is dependent upon disk potency, proper inoculum and control cultures, functional pretested plates, proper storage and incubation factors.
C. Use only pure cultures.
D. Direct specimens should not be used for testing.
E. Overnight broth cultures should not be used for testing.
F. This method is standardized for rapidly growing aerobic organisms. Refer to CLSI M100 for complete reference.

IX. NOTES

A. No more than 12 disks per 150mm MH/BMH.
B. Do not relocate disks once they have made contact with the surface of the agar.

X. TECHNICAL SUPPORT

A. Problems, issues, or unusual results should be brought to the attention of the laboratory manager, a technical specialist, or laboratory director.

XI. REFERENCES

A. Clinical Microbiology Procedures Handbook, third edition. 2007. Antimicrobial Susceptibility Testing (section 5.1.1). Garcia, Lynne S.

XII. REVISIONS

A. 5/31/2024 – Added quality control for densitometer. Updated interpretation section.