PURPOSE: The disk diffusion test is used to determine the *in vitro* susceptibility of bacteria that grow aerobically to certain antimicrobial agents.

SAMPLE INFORMATION:

Type	Few, well isolated colonies that are 18 to 24 hours old

REAGENTS and/or MEDIA:

Type	Oxoid Antimicrobial Susceptibility Test Disks
Stability and Storage Requirements	• Unopened cartridges must be stored at 2°C to 8°C. • Unopened cartridges should be allowed to come to room temperature before removing them from the packaging to minimize condensation. • Opened cartridges need to be stored at 2°C to 8°C, in an opaque, air tight container with a charged desiccant to protect the disks from moisture. • Once a cartridge is opened, it should be stored for no longer than a month.

SUPPLIES:

- Plastic Vitek tubes and caps
- 0.9% sterile saline
- Sterile swabs
- DensiCHEK Plus
- Mueller Hinton agar
- Mueller Hinton agar with 5% sheep blood
- Haemophilus Test Media
- Forceps
- 35° ambient air and 37° CO_2 incubators
- Small, metric ruler

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Quality control is performed weekly.
- Refer to MIC60020 - Antibiotic Quality Control and MIC60021 - Antibiotic Quality Control Job Aid.
- A TQC order is automatically generated on Wednesdays to record the QC results.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the disk diffusion test	
1	Remove the antibiotic disks from refrigerator for 1 hour and bring to room temperature.
2	Remove testing agar from the refrigerator and bring to room temperature: • For *Staphylococcus* spp., *Enterococcus* spp., Enterobacteriaceae and *Pseudomonas aeruginosa* use Mueller Hinton agar. • For *Streptococcus* spp. use Mueller Hinton agar with 5% sheep blood. • For *Haemophilus* spp. use Haemophilus Test Media.
3	Dispense 3 mL of 0.9% sterile saline into a labelled plastic test tube. Pick several colonies from a fresh agar plate and prepare a suspension equivalent to a 0.5 McFarland standard.
4	Within 15 minutes of adjusting turbidity, dip a sterile cotton swab into the inoculum and rotate against the wall of the tube above the liquid to remove excess inoculum.
5	Swab the entire surface of the agar three times, rotating plate approximately 60° between streaking to ensure even distribution. To minimize aerosols, avoid hitting the sides of the plate. Finally, run swab around the edge of the agar to remove any excess moisture. Allow inoculated plate to stand for 3 to 15 minutes before applying disks.
6	Apply the antibiotic disks to agar surface with forceps: • Apply gentle pressure to ensure complete contact of disk with agar. • Do not place disks closer than 24 mm from center to center. • Do not place more than 4 disks on a 100 mm plate for *Haemophilus influenzae* *Streptococcus pneumoniae* and beta-hemolytic *Streptococc*i. • Do not place more than 5 disks on a 100 mm plate for all other organisms. • Do not relocate disk once it has made contact with the agar surface. Instead, place a new disk in another location on the agar.
7	Invert the plate and incubate within 15 minutes of the disk application: • *Staphylococci* spp. and *Enterococci* spp. in O_2 incubator for 24 hours. • *Streptococci* spp. in CO_2 incubator for 20 to 24 hours. • *Haemophilus* spp. in CO_2 for 18 hours. • All other organisms in O_2 incubator for 18 hours.

INTERPRETATION OF RESULTS:

Step	Action
1	After incubation, read plates only if lawn of growth is confluent.
2	For Mueller Hinton agar and Haemophilus Test Medium: • Hold inverted plate a few inches above a black, non-reflecting surface. • Illuminate plate with reflected light. • Use a ruler held on the back of the plate to measure the diameter of inhibition zone to the nearest whole mm, including the disk. For Mueller Hinton agar with 5% sheep blood: • Remove the cover of the plate. • Illuminate the plate with reflected light. • Measure diameter of inhibition zone at agar surface to the nearest whole mm. • When testing hemolytic organisms, ensure that the diameter of the zone of inhibition of growth and not the zone of inhibition of hemolysis is measured.
3	The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth should be ignored.
4	Disregard swarming of *Proteus* spp. and measure the edge of the obvious inhibition under the veil of swarming.
5	For trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, slight growth (20% or less of the lawn of growth) should be disregarded and the more obvious margin measured to determine the zone diameter.
6	Discrete colonies growing within the inhibition zone may represent a mixed culture or resistant variants. Subculture a single colony from the primary culture plate, re-identify and retest for susceptibility. If the discrete colonies are still apparent, measure the colony-free inner zone.

REPORTING RESULTS:

Step	Action
1	In the LIS, report disk diffusion test results under the “Kirby - Bauer” tab in the sample screen. Only the interpretation will appear on the final report.
2	If the antibiotics have not already been generated, add them by selecting “Generate Drugs” if panel has been entered into the LIS or by selecting “Add Drug” to add drugs individually.
3	In the “Result” column add the zone that was read from the disk. The “Interpretation” column will automatically be filled out by the LIS. If the interpretation is not completed by the LIS, consult the CLSI guidelines and manually add the interpretation. Refer to the ASTM for the reporting of results.

LIMITATIONS/PRECAUTIONS:

1. This method is standardized only for rapidly growing aerobes.
2. Numerous factors can affect results, including inoculum size, rate of growth, formulation and pH of media, incubation environment and length of incubation, disk content and drug diffusion rate, and measurement of endpoints. Strict adherence to the procedure is required to ensure reliable results.
3. Some bacteria may become resistant during antimicrobial therapy.
4. *Haemophilus influenzae* and *Aggrigatibacter aphrophilus* (formerly *Haemophilus parainfluenzae)*: exercise care in preparing the 0.5McFarland suspension because higher inoculum concentrations may lead to false-resistant results with some β-lactam antimicrobial agents.

REFERENCES:

- Oxoid, Antimicrobial Susceptibility Test Disks product insert, 2018-07
- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	5 APR 19	Initial Release	L. Steven