PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC52500 – Double Disk Diffusion Test	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director of Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
The double disk diffusion test is used to determine inducible clindamycin resistance in *Staphylococcus* spp., beta-hemolytic *Streptococcus* spp. and *Streptococcus pneumoniae* that are erythromycin resistant and clindamycin susceptible.

PURPOSE/RATIONALE:
This standard operating procedure describes how to perform the double disk diffusion test.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) performing the double disk diffusion test.

SAMPLE INFORMATION:

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

REAGENTS and/or MEDIA:

Type	Oxoid 2 µg Clindamycin disk and 15 µg Erythromycin disk
Stability and Storage Requirements	• Unopened cartridges must be stored at 2°C to 8°C • 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
- Sterile saline
- Sterile swabs
- Mueller Hinton agar and Mueller Hinton agar with 5% sheep blood
- Forceps
- Small, metric ruler

EQUIPMENT

- DensiCHEK Plus
- 35° ambient air and 37° CO_2 incubators

SPECIAL SAFETY PRECAUTIONS:

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

- Ensure that appropriate hang hygiene practices be used.
- 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 of 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. Routine Practices 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:
  - Positive: *Staphylococcus aureus ATCC* BAA-977, presence of D-zone
  - Negative: *Streptococcus pneumoniae* ATCC 49619, absence of D-zone
- A TQC order is automatically generated on Wednesdays to record the QC results

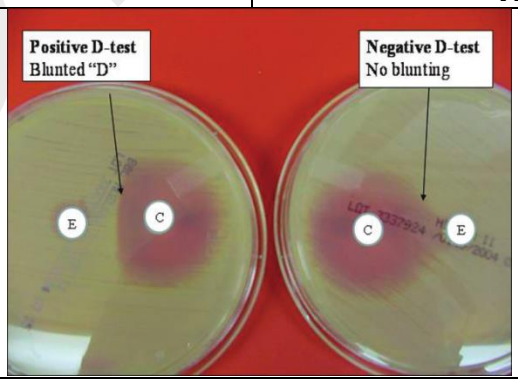
PROCEDURE INSTRUCTIONS:

Step	Action
Performing the double disk diffusion test	
1	Remove the antibiotic disks from the refrigerator for 1 hour and bring to room temperature.
2	Remove Mueller Hinton or Mueller Hinton with blood agar from the refrigerator and bring to room temperature.

3	Remove testing agar from the refrigerator and bring to room temperature: • For *Staphylococcus* spp. use Mueller Hinton agar • For *Streptococcus* spp. use Mueller Hinton agar with 5% sheep blood
4	Dispense 3 mL of 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.
5	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.
6	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.
7	Apply an erythromycin disk and clindamycin disk to the agar surface with forceps. Refer to MIC52510-DD Test Template for Disk Placement • Leave a 15 mm space between disks for *Staphylococcus* spp. • Leave a 12 mm space between disks for *Streptococcus* spp. • Apply gentle pressure to ensure complete contact of disk with agar
8	Invert the plate and incubate within 15 minutes of the disk application: • *Staphylococcus* spp.in the O_2 incubator for 20 to 24 hours • *Streptococcus* spp. in the CO_2 incubator for 20 to 24 hours
9	After incubation, read plates only if lawn of growth is confluent.
10	Use a ruler held on the back of the plate to measure the diameter of inhibition zone to the nearest millimeter, including the disk and observe for the presence or absence of D-zone.

INTERPRETATION OF RESULTS:

IF	THEN
Flattening of the zone of inhibition adjacent to the erythromycin disk	**Inducible clindamycin resistance = Positive**
Completely round zone of inhibition around clindamycin disk	**Inducible clindamycin resistance = Negative**



LIMITATIONS:

1. The inducible clindamycin resistance test is only standardized to detect inducible clindamycin resistance for *Staphylococcus* spp., *S.pneumoniae* and beta-hemolytic *Streptococcus.*
2. Despite a positive result for inducible clindamycin resistance, clindamycin may still be effective in some patients.
3. Numerous factors can affect results, including inoculum size, rate of growth, disk content and drug diffusion rate. Therefore, strict adherence to protocol is required to ensure reliable results.

CROSS-REFERENCES:

- MIC52510-DD Test Template for Disk Placement

REFERENCES:

1. Oxoid. (2018-07). *Antimicrobial Susceptibility Test Disks* product insert
2. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 31th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2021

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	23 Mar 19	Initial Release	L. Steven
2.0	30 Jun 21	Procedure reviewed and added to NTHSSA policy template	L. Steven