PURPOSE: The inducible clindamycin resistance 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.

SAMPLE INFORMATION:

Type	Few, well isolated colonies of *Staphylococcus* spp., beta-hemolytic *Streptococcus* spp. and *Streptococcus pneumoniae* 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. • 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 and Mueller Hinton agar with 5% sheep blood
- 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 where 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:

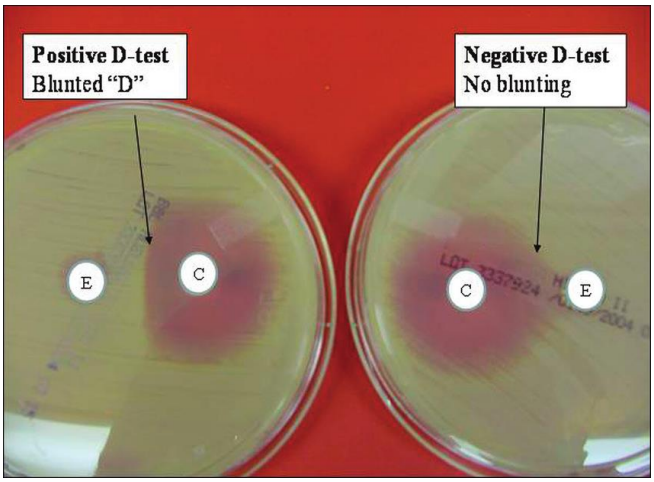
- Quality control is performed weekly:
  - Positive: *Staphylococcus aureus ATCC* BAA-977, presence of D-zone
  - Negative: *Streptococcus pneumoniae* ATCC 49619, absence of D-zone
- A TQC order is automatically generated on Wednesdays to record the QC results.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the inducible clindamycin resistance 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. use Mueller Hinton agar. • For *Streptococcus* spp. use Mueller Hinton agar with 5% sheep blood.
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 an erythromycin disk and clindamycin disk to the agar surface with forceps. Refer to MIC50611 – 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.
7	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.
8	After incubation, read plates only if lawn of growth is confluent.
9	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 (D-zone)	**Inducible clindamycin resistance = Positive**
Completely round zone of inhibition around clindamycin disk	**Inducible clindamycin resistance = Negative**



LIMITATIONS/PRECAUTIONS:

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.

REFERENCES:

- Oxoid Antimicrobial Susceptibility Test Disk package insert, 2018
- 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	23 MAR 19	Initial Release	L. Steven