



## Detection of inducible clindamycin resistance in *Staphylococcus* spp.

### Purpose

To detect the inducible clindamycin resistance (ICR) by using D-zone test

### Materials

1. Overnight culture of bacterial isolate on non-selective media (Sheep blood agar (SBA))
2. Sterile cotton swab
3. Mueller-Hinton agar (MHA)
4. Muller Hinton Broth (MHB)
5. Antimicrobial agents
  - 15- $\mu$ g erythromycin disk
  - 2- $\mu$ g clindamycin disk
6. Sterile forceps
7. 0.5 McFarland standard
8. Nephelometer
9. Vortex mixer
10. 35°C ambient-air incubator
11. Quality control strains
  - *Staphylococcus aureus* ATCC 25923 (Acceptable range for erythromycin is 22-30 mm and clindamycin is 24-30 mm)
  - *S. aureus* BAA-976™ (D-zone test negative)
  - *S. aureus* BAA-977™ (D-zone test positive)

### Procedure

1. Make a direct MHB or suspension of isolated colonies selected from an 18- to 24-hrs non-selective agar plate.
2. Adjust the turbidity of culture with MHB to achieve a turbidity equivalent to a 0.5 McFarland standard. **Use the inoculum within 15 mins.**
3. Dip a sterile cotton swab into the 0.5 McFarland adjusted suspension and rotate the swab several times and press firmly on the inside wall of the tube above the fluid level.
4. Inoculate the dried surface of an MHA plate by streaking the swab over the entire sterile agar surface.
5. Repeat these procedures by streaking 2 more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum.
6. Swab the rim of the agar.
7. Leave the lid ajar for (ideally) 3–5 mins, but no more than 15 mins.
8. Take a 2- $\mu$ g clindamycin disk and a 15- $\mu$ g erythromycin disk with sterile forceps and **place disks 15 to 26 mm apart (edge to edge).**
9. Press each disk down to ensure complete contact with the agar surface.

10. Invert the plates and place them in an incubator set to  $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$  within 15 mins after the disks are applied.
11. Incubate plate for 16 to 18 hrs and interpret the results

### Interpretation of results

1. Flattening of the zone of inhibition adjacent to the erythromycin disk (referred to as a D-zone) = inducible clindamycin resistance (ICR) (Figure 1).
2. Hazy growth within the zone of inhibition around clindamycin = clindamycin resistance, even if no D-zone is apparent.



**Figure 1** Positive D-zone test for inducible clindamycin resistance.

### References

- CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2022.
- CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests, 13th ed. CLSI M02. Clinical and Laboratory Standards Institute; 2018.