

D-zone Test for Beta-Hemolytic Streptococci

I. Principle

Macrolide (erythromycin)-resistant isolates of beta-hemolytic strep may have constitutive or inducible resistance to clindamycin or may be resistant only to the macrolides. When testing clindamycin susceptibility by disk diffusion, the inducible resistance found in some isolates will not be apparent unless the clindamycin disk is placed in close enough proximity to the erythromycin disk so that the zones of diffusion overlap. Organisms that show a flattening of the clindamycin zone adjacent to the erythromycin disk (referred to as a “D” zone) indicate inducible resistance and should be reported as clindamycin resistant.

II. Specimen

When antimicrobial susceptibility testing is requested on beta-hemolytic streptococci, the clindamycin and erythromycin disks should be placed in close enough proximity to reveal D-zones.

III. Reagents and Equipment

- A. Isolated colonies of beta-hemolytic streptococci
- B. BBL Prompt system
- C. Mueller-Hinton agar with 5% sheep blood
- D. Sterile swabs
- E. Antibiotic disks: clindamycin (2- μ g) and erythromycin (15- μ g)
- F. CO₂ incubator at 35 \pm 2°C

IV. Procedure

- A. Prepare and inoculate Blood Mueller-Hinton agar as outlined in the “Disk Diffusion Susceptibility Testing” procedure.
- B. CLSI recommends placing the clindamycin and erythromycin disks on the agar surface **12 mm apart** (as measured from the adjacent edges of the disks).
- C. Invert and incubate the plate in CO₂ incubator at 35 \pm 2°C for 24 h.

V. Interpretation

After 24 h incubation, examine for zones of inhibition. If the isolate is resistant to erythromycin and yields a zone of inhibition around the clindamycin, examine the shape of the clindamycin zone.

Positive D-zone:

A flattening of the zone on the side adjacent to the erythromycin disk indicates a positive D-zone test. Report these isolates as resistant to clindamycin, regardless of the zone size.

Negative D-zone:

If no flattening of the clindamycin zone is observed, measure the zone and report based on CLSI breakpoints.

VI. Quality Control

Weekly QC testing of D-zone is not necessary. CLSI lists strains of *Staphylococcus aureus* that may be used for QC testing. However, the intent of performing QC for D-zone is for training, competency testing, and test evaluation. Clinical isolates that demonstrate inducible clindamycin resistance are used for this purpose.

VII. Limitations

If disks are placed more than 12 mm apart, inducible resistance resulting in a D-zone will not be observed.

VIII. References

- A. Clinical and Laboratory Standards Institute M100-S17. Volume 27, Number 1. January 2007. Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement.

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Updates and Revisions: