D-zone Test for Staphylococci

I. Principle

Macrolide (erythromycin)-resistant isolates of *S. aureus* and coagulase-negative *Staphylococcus* species 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

All significant isolates of *Staphylococcus* species should be tested for D-zone.

III. Reagents and Equipment

A. Isolated colonies of significant *Staphylococcus* species
B. BBL Prompt system
C. Mueller-Hinton agar
D. Sterile swabs
E. Antibiotic disks: clindamycin (2-μg) and erythromycin (15-μg)
F. Ambient air incubator at 35 ± 2°C

IV. Procedure

A. Prepare and inoculate 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 approximately 15 – 26 mm apart (as measured from the adjacent edges of the disks). However, D-zones are more readily apparent when the space between disks is closer to 15 mm.
C. Invert and incubate the plate in ambient air at 35 ± 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 (see figure 1 below). 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 (see figure 2 below).

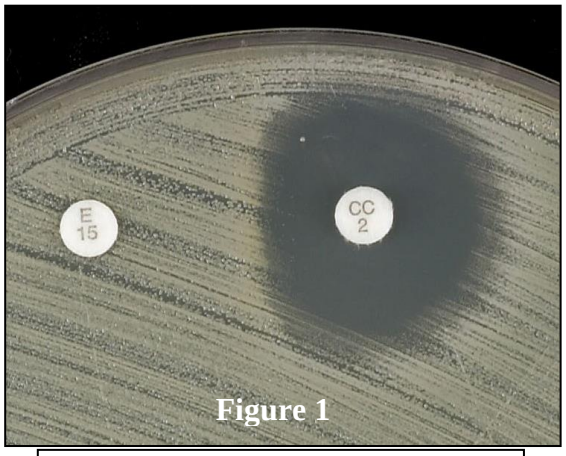

Figure 1

D-zone reaction indicating inducible clindamycin resistance. Report clindamycin as resistant.

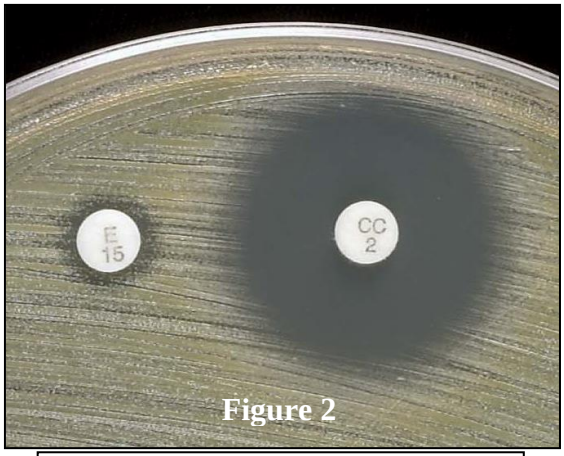

Figure 2

No D-zone. Measure clindamycin zone a 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

While most isolates with inducible clindamycin resistance will yield D-zones that are apparent when the disks are spaced 26 mm apart, D-zones are more apparent when the disks are spaced 15 mm apart.

VIII. Verification of Test Method

Surveillance testing was performed on 50 MRSA isolates that previously tested resistant to erythromycin. Isolates were subcultured to BAP and incubated overnight to prepare fresh inoculum for disk diffusion testing. D-zone testing was performed in duplicate on each isolate by placing a pair of clindamycin and erythromycin disks at 15 mm apart and a pair at 26 mm apart. After overnight incubation, the plates were examined for D-zones around the clindamycin disks. 7 (14%) of the isolates yielded D-zones. 6 (12%) isolates yielded D-zones that were readily apparent at both disk separation distances. 1 isolate yielded a D-zone that was more obvious at 15 mm disk spacing than at 26 mm.

IX. References

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

Effective 01/24/2003
Reviewed by Microbiology Director, Dr. Ann Robinson: 05/01/2007
Reviewed by Microbiology Supervisor, Jerry Claridge: 05/01/2007, 02/06/2008, 11/2008, 03/2010, 03/2011, 03/2013, Jason Ammons 05/2015
Updates and Revisions: Update 5/1/2007 Verification data for disk spacing.