PROCEDURE: D TEST

I. PRINCIPLE

Staphylococcus spp. and Beta hemolytic Streptococci resistant to macrolide antibiotics (such as Erythromycin) may also have constitutive or inducible enzymes that cause resistance to lincosamide (clindamycin) and type B streptogramin (quinupristin-dalfopristin (Synercid)) antibiotics. The mechanism of resistance is methylation of the 23SrRNA encoded by the erm gene. This resistance mechanism is also referred to as MLS_B resistance. MLS_B stands for macrolide-lincosamide-streptogramin type B. For MLS_B inducible strains, erythromycin will induce production of the methylase enzyme, which allows clindamycin resistance to be expressed.

Inducible clindamycin resistance can be detected with a simple disk approximation test, commonly referred to as the "D test". For this test, an erythromycin disk is placed 15mm to 26mm (edge to edge) for Staphylococcus and 12 mm for Beta-hemolytic Streptococcus, from a clindamycin disk in a standard disk diffusion test. Following incubation, a flattening of the zone in the area between the disks where both drugs have diffused indicates that the organism has inducible clindamycin resistance.

II. AVAILABILITY

This test is only performed upon request in the situation where Erythromycin is intermediate or resistant and Clindamycin is susceptible.

The physician will be prompted with the comment: This isolate may have an inducible enzyme exhibiting resistance to clindamycin. However, clindamycin may still be effective in some patients. If clindamycin susceptibility is required for treatment, contact RIH Micro Lab at 444-5273 or TMH Micro Lab at 793-4236.

III. TEST CODE

DTEST

IV. SPECIMEN

A. Colonies of Staphylocccus spp./Beta hemolytic Streptococcus spp. as described for routine disk diffusion testing of rapidly growing nonfastidious bacteria.

V. MATERIALS

A. As stated for routine disk diffusion testing of rapidly growing non-fastidious bacteria
B. 15 ug erythromycin disk, 2 ug clindamycin disk

VI. TEST PROCEDURE

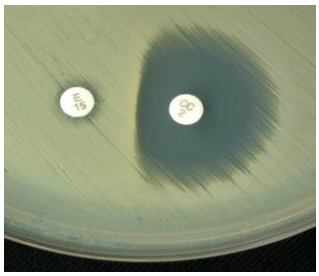
A. Follow standard disk diffusion testing for inoculum preparation, inoculation and incubation.
B. Order DTEST template in procedure worksheet and follow directions.
C. For Staphylococcus, position erythromycin disk 15-26 mm (edge to edge) from clindamycin disk.
D. For Beta hemolytic Streptococcus, position the erythromycin disk 12 mm (edge to edge) from the clindamycin disk.
E. Following incubation, note appearance of the clindamycin zone closest to the erythromycin disk

VII. QUALITY CONTROL

A. NCCLS has not recommended a QC strain at this time. In the interim, QC of the disks is performed with *S. aureus* ATCC 25923 according to standard disk diffusion QC procedure.

VIII. TEST INTREPRETATION

A. Positive for inducible clindamycin resistance (DTESTPOS)
   1. Demonstration of a flattening of the clindamycin zone between the erythromycin and clindamycin disks



B. Negative for inducible clindamycin resistance (DTESTNEG)
   1. No flattening of clindamycin zone

IX. REPORTING RESULTS

A. Positive:
   1. Report Code is **DTESTPOS** Comment will automatically be added stating the following: *This isolate is presumed to be resistant to clindamycin based on detection of inducible clindamycin resistance. Clindamycin may still be effective in some patients*. Add drug DTEST under the Kirby-Bauer tab and result as POS.

B. Negative:
   1. Report Code is **DTESTNEG**. Comment will automatically be added that states: *No inducible clindamycin resistance detected.* Add drug DTEST under the Kirby-Bauer tab and result as NEG.
   2. Unsuppress clindamycin results, changing the N/A to S. If MIC test, report clindamycin susceptible with MIC value.

X. PROCEDURE NOTE

A. Usually, sensitivity is increased by placing the disks closer to the lower end of the acceptable range.

XI. REFERENCES

A. NCCLS. Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. NCCLS document M100-S14 (ISBN 1-56238-516-X). NCCLS, 940 West Valley Road. Suite1400, Wayne, Pennsylvania 19087-1898 USA, 2006.
B. Fiebelkorn, K. R., S. A. Crawford, M. L. McElmeel, and J. H. Jorgensen. 2003. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. J Clin Microbiol. 41:4740-44
C. Frank, A. L., J. F. Marcinak, P. D. Mangat, J. T. Tjhio, S. Kelkar, P. C. Schreckenberger, and J. P. Quinn. 2002. Clindamycin treatment of methicillin-resistant *Staphylococcus aureus* infections in children. Pediatr Infect Dis J. 21:530-34.

XII. REVISIONS

A. 04/03/2023 Updated reporting guidelines for clarification