**Intended Use**

To detect inducible clindamycin resistance in erythromycin resistant Staphylococcus, beta hemolytic

Streptococcus, and Streptococcus pneumoniae.

**Principle**

 The clindamycin induction test is performed on Stapylococcus spp., beta hemolytic Streptococcus, and

 Streptococcus pneumoniae that test resistant to erythromycin and susceptible to clindamycin using

 routine antimicrobial susceptibility test methods.

Resistance to macrolides (e.g. erythromycin) can occur by two different mechanisms with the resulting phenotypes noted below:

|  |  |  |  |
| --- | --- | --- | --- |
|  Mechanism  |  Determinant  (gene) |  Erythromycin  |  Clindamycin |
| Efflux |  msrA |  R |  S |
| Ribosome alteration  |  erm |  R |  S\* |
|  erm |  R |  R(constitutive) |

 **mrsrA** = macrolide streptogramin (type B) resistance

 **erm** = erythromycin ribosomal methylase; encodes enzymes that confer inducible (MLS*B*i) or

 constitutive (MLS*B*c) resistance to MLS agents via methylation of the 23S rRNA

 \* requires induction to demonstrate resistance

 **MLS** = macrolide lincosamide (e.g. clindamycin) streptogramin (type B)

For MLS*B*i strains, erythromycin will induce production of the methylase, 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 disc is placed 12 mm to 26mm (edge to edge) 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.

**Reagents**

 Sheep blood agar plate or Mueller Hinton Agar

 Antibiotic Disks

 Erythromycin 15 mcg

 Clindamycin 2 mcg

**Precautions**

 Handle all specimens as if capable of transmitting disease and dispose of biohazards in marked

 containers.

**Specimen**

 Colonies of Staphylococcus, beta hemolytic Streptococcus, or Str. pneumoniae to be tested

**Procedure**

 **Staphylococcus spp**.

 1. **Standard disk diffusion test**

 a. Follow the standard disk diffusion testing recommendations for inoculum preparation,

 b. **Position erythromycin disk 15-26 mm (edge to edge) from clindamycin disk**.

 c. Following incubation in non-CO2 incubator, note the appearance of clindamycin zone

 closest to the erythromycin disk.

 2. **Purity plate variation**

 a. Following inoculation of MIC test, use a 10 *u*l loop to transfer an aliquot of the final inoculum suspension to a BAP. Inoculate the first one-third of the agar surface in

 order to obtain confluent growth. Streak the remaining quadrants to obtain isolated

 colonies.

 b. **Position erythromycin disk 15 mm (edge to edge) from clindamycin disk** on the first one-third quadrant of the plate.

 c. Following incubation, note the appearance of the clindamycin zone closest to the

 erythromycin disk.

 **Beta hemolytic Streptococcus spp. And Streptococcus pneumoniae**

 1. **Standard disk diffusion test** (Beta hemolytic Streptococcus from vaginal strep screens)

 a. Follow the standard disk diffusion testing recommendations for inoculum preparation,

 inoculation, and incubation. Use Mueller Hinton W/5% SB, 0.5 McFarland Std., and

 incubation in 5% CO2 for 20-24 hours. Place sensitivity discs on the plate as directed

 below. Measure zone diameter of CC and E at the widest point for standard Kirby

 Bauer results.

 b. **Position erythromycin disk 12 mm (edge to edge) from clindamycin disk**.

 c. Following incubation, note the appearance of clindamycin zone closest to the

 erythromycin disk.

 2. **Purity plate variation** (Beta hemolytic Streptococcus)

 a. Following inoculation of MIC test, use a 10ul loop to transfer an aliquot of the final

 inoculum suspension to a BAP. Inoculate the first one-third of the plate in order to

 obtain confluent growth. Streak the remaining quadrants to obtain isolated colonies.

 b. **Position erythromycin disk 12 mm (edge to edge) from clindamycin disk**

 on the first one-third quadrant of the plate.

 c. Following incubation, note the appearance of the clindamycin zone closest to the

 erythromycin disk.

**Interpretation and Reporting of Results**

 1. **Positive** for inducible clindamycin resistance

 a. Demonstration of flattened clindamycin zone between the erythromycin and clindamycin

 disks.

 b. Report clindamycin as Resistant. If MIC test delete clindamycin result.

 Add Comment: This isolate is presumed to be resistant based on detection of inducible

 Clindamycin resistance. Clindamycin may still be effective in some patients.

 2. **Negative** for inducible clindamycin resistance.

 a. No flattening of clindamycin zone

 b. Report clindamycin susceptible. If MIC test, report susceptible with MIC value.

**Quality Control**

 Staphylococcus aureus ATCC 25923 – For routine weekly QC of clindamycin and erythromycin

 discs. (Required)

 Positive control: Staphylococcus aureus BAA977 – To be run with each new lot # and/or shipment of

 antibiotic discs

 Negative control: Staphylococcus aureus BAA976 – To be run weekly

**Notes**

1. Reporting clindamycin as susceptible for staphylococcus or beta hemolytic Streptococcus that test erythromycin resistant and clindamycin susceptible without checking for inducible clindamycin resistance may result in inappropriate clindamycin therapy.
2. Many of the recently recognized MRSA that cause community-associated infections have the msrA gene and oral clindamycin may be a treatment option for these patients.
3. The test described here is acceptable for all Staphylococcus sp. including oxacillin susceptible or oxacillin resistant S. aureus or coagulase-negative staphylococci.
4. Erythomycin and clindamycin should not be reported on urine specimens.

**References**

1. Fiebelkorn,K.R., S.A. Crawford, M.L. McElmeel, and J.H. Jorgenson, 2003. Practical disk diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci. J.Clin Microbiol. 41:4740-44
2. Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement, M100-S23 Vol.33 No.1, January 2013