**MICRO.AST.9.0 Clindamycin Disk Induction Test**

**STATEMENT OF PURPOSE**

The clindamycin induction test (D-zone) is performed on *Staphylococci* and β-hemolytic *Streptococci* that test resistant to erythromycin and susceptible to clindamycin using Kirby-Bauer disk diffusion.

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

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

*msrA* = macrolide streptogramin (type B) resistance

*erm* = erythromycin ribosome methylase

\*requires induction to demonstrate resistance

The *erm* gene encodes enzymes that confer inducible or constitutive resistance via methylation of the 23S rRNA. For induced 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” or “D zone test”. For this test, an erythromycin disk is placed a specified distance 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.

**RESPONSIBILITIES**

Regional Microbiology Supervisor

**SPECIMEN**

Isolated colonies of *Staphylococci* or β-hemolytic *Streptococci* grown for 18-24 hours on non-inhibitory agar medium.

**MATERIALS**

**REAGENTS**

1. Mueller Hinton Agar (MH), 100 mm, stored at 2-8C -or-
2. Mueller Hinton Agar with 5% Sheep Blood (MHB), 100 mm, stored at 2-8C -or-
3. Blood Agar Plate (BAP), stored at 2-8C
4. Sterile saline, 0.85%, tubes, Vitek
5. Antimicrobic disks for the following agents at the concentrations specified:
   1. Erythromycin 15µg/ml
   2. Clindamycin 2µg/ml

**EQUIPMENT**

1. McFarland 0.5 standard, Remel #20-410
2. Sterile cotton-tipped swabs -or-
3. Sterile loop
4. Forceps
5. Calipers
6. Light source
7. Vortex mixer
8. Non CO2 incubator at 35C (+/- 2oC)
9. CO2 incubator at 35C (+/- 2oC)

**CALIBRATION**

Not applicable

**QUALITY CONTROL**

* + 1. For use with β-hemolytic *Streptococci*, quality control of the erythromycin and clindamycin disks is performed on each day patient testing is performed using *Streptococcus pneumoniae* ATCC 49619 to ensure acceptable potency of the disks before patient results are reported.
    2. For use with *Staphylococci*, quality control of the erythromycin and clindamycin disks is performed on each day patient testing is performed using *Staphylococcus aureus* ATCC 25923.

**PROCEDURE**

1. Standard disk diffusion test

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| **Step** | **Action** |
| 1. | Bring agar plates and disks to room temperature. Avoid prolonged exposure to elevated temperatures. |
| 2. | Using a sterile wooden applicator stick or sterile loop, pick 3 to 5 isolated colonies (18-24 hour culture) with the same morphology by touching just the top of each colony, and inoculate into 0.85% saline. Vortex suspension. |
| 3. | Adjust turbidity to equal a McFarland 0.5 standard |
| 4. | Within 15 minutes of adjusting the inoculum to a McFarland 0.5 standard, dip a sterile cotton swab into the suspension. Excess inoculum is removed by rotating the swab firmly against the wall of the tube above fluid level. |
| 5. | Inoculate the dried surface of a Mueller Hinton (MH) or Mueller Hinton with Blood (MHB) agar plate by streaking evenly in three directions over the entire surface. Vary the angle of streak by 60 for each direction to ensure even distribution. A final sweep is made of the agar rim.   |  |  | | --- | --- | | **If** | **Then** | | *Staphylococcus* spp. | Use Mueller Hinton (MH) agar | | *Streptococcus* spp. | Use Mueller Hinton with Blood (MHB) agar | |
| 6. | Allow inoculated plates to dry a minimum of 3 minutes, but no longer than 15 minutes to allow for any excess moisture to be absorbed, before applying antibiotic-impregnated disks. |
| 7. | |  |  | | --- | --- | | **If** | **Then** | | *Staphylococcus* spp. | Position erythromycin disk 15-26mm (edge to edge) from clindamycin disk on the first one-third quadrant of the plate. | | *Streptococcus* spp. | Position erythromycin disk 12mm (edge to edge) from clindamycin disk on the first on-third quadrant of the plate. | |
| 8. | Incubate the plate at 35oC (+/- 2oC)   |  |  | | --- | --- | | **If** | **Then** | | *Staphylococcus* spp. | 16-18 hours in ambient air | | *Streptococcus* spp. | 20-24 hours in CO2 | |
| 9. | Following incubation note the appearance of the clindamycin zone closest to the erythromycin disk. |

1. Purity plate variation

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| **Step** | **Action** |
| 1. | Following inoculation of MIC test, use 10µl loop or the Vitek straw to transfer an aliquot of the final inoculum suspension to a BAP. |
| 2. | Inoculate the first one-third of the agar surface in order to obtain confluent growth. |
| 3. | Streak the remaining quadrants to obtain isolated colonies. |
| 4. | |  |  | | --- | --- | | **If** | **Then** | | *Staphylococcus* spp. | Position erythromycin disk 15mm (edge to edge) from clindamycin disk on the first one-third quadrant of the plate. | | *Streptococcus* spp. | Position erythromycin disk 12mm (edge to edge) from clindamycin disk on the first on-third quadrant of the plate. | |
| 5. | Incubate the plate at 35-37°C for 18-24 hours. |
| 6. | Following incubation note the appearance of the clindamycin zone closest to the erythromycin disk. |

**INTERPRETATION**

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| **If** | **Then** |
| Demonstration of flattened clindamycin zone between the erythromycin and clindamycin disks is noted | The isolate is positive for inducible clindamycin resistance. Report the clindamycin as resistant and attach the comment “This isolate has inducible clindamycin resistance.” |
| No flattening of clindamycin zone is noted | The isolate is negative for inducible clindamycin resistance. Report the clindamycin as susceptible. |

**REPORTING RESULTS**

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| **Result** | **QLS** | **Misys** |
| Positive for inducible clindamycin resistance | * 1. Override the clindamycin result to resistant.   2. In the appropriate isolate field, append the comment **#JICCIN**. | 1. Click on **Micro Result Entry**.  2. Type Accession number in the **Value** field.  3. Click on the desired test and then click **Select**.  4. Click on the **Susceptibility** tab.  5. Use the drop down box to select the correct susceptibility method (Vitek, Kirby Bauer, etc)  6. Click on the correct organism.  7. Click **OK** on the displayed summary screen.  8. Arrow down to the **Clindamycin** field.  9. Override the result to **R**.  10. Click on **File** to save.  11. Click on the **Culture** tab.  12. Append **CCIN** to the correct organism by clicking on the organism identification and pressing **Tab** until a blue box appears after the organism name. Enter CCIN at this time.  13. Press the **down arrow** key until the blue box appears in an empty observation field.  14. Click **SAVE**. |
| Negative for inducible clindamycin resistance | Release result as susceptible with MIC. | Release result as susceptible with MIC. |

**PROCEDURE NOTES**

The D zone test is setup concurrently with KB testing of all Group A and B β-hemolytic *Streptococcus* spp. The D zone is only interpreted if the results of the KB test are erythromycin resistant and clindamycin susceptible. The D zone test for *Staphylococci* and *Streptococcus* is performed as needed by this method, routine testing is included in the Vitek MIC Card. Refer to the Vitek procedure for further information.

**REFERENCES**

1. CLSI, *Performance Standards for Antimicrobial Susceptibility Tests* – Twenty-second Edition, Approved Standard, CLSI document M100-S22, 2012.
2. Hindler, Janet. Clindamycin Disk Induction Test for Staphylococcus spp. CLSI procedure. 01/2004.

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