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| DTEST –Inducible Clindamycin Resistance Confirmation Test by Disk Diffusion |
| **Purpose** | This procedure provides instruction for confirming Inducible Clindamycin Resistance by DTEST. |
| **Principal** | Inducible clindamycin resistance (ICR) in staphylococci and streptococci can be detected by agar disk diffusion. CLSI recommends testing for inducible clindamycin resistance in all staphylococci,*Streptococcus pneumoniae*, and beta-hemolytic streptococci that are erythromycin resistant and clindamycin susceptible or intermediate prior to reporting clindamycin results. |
| **Policy Statements** | This procedure applies to Microbiologists who perform antimicrobial susceptibility testing |
| **Test Code** | DTEST |
| **Materials** |  |  |  |  |
|  | **QC Strains** | **Supplies** | **Equipment** | **Media** |
|  | • MSSA- *Staph aureus* ATCC® 25923 | * --Sterile cotton tip swabs
* --12 x 75 polystyrene tubes
 | * DTEST disk dispenser with CC (2 mcg), E (15 mcg), FOX (30 mcg)
* DensiCHEK Plus® (Vitek)
 | * Agar plates: store at 2-8ºC.
* Mueller-Hinton agar (MH) Mini
* Mueller-Hinton Agar with Sheep Blood (MHSB)
* Saline-0.45-0.9%
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| **Specimen** | 1. Prepare inoculum from 4 or 5 isolated colonies of similar colony morphology
2. Direct colony inoculums: use colonies grown overnight on nonselective medium (e.g. SB or CHOC).
3. Subculture QC stock, frozen, or lyophilized isolates 2 times prior to testing.
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| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual*and the *Virology Procedure Manual***:**1. [*Biohazard Containment*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.1%20Biohazard%20Containment.docx)
2. [*Safety in the Microbiology/Virology Laboratory*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx)
* [*Biohazardous Spills*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.4%20Biohazardous%20Spills.docx)
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| **Quality Control** | 1. DTEST QC is performed weekly.
2. If there is a QC failure, document observation, notify supervisor and proceed with corrective action. Do not report patient results until the problem is resolved.
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| **Out of Control Results due to obvious error** | 1. Document the reason and retest the strain on the day
2. If the repeated result is within range, no further corrective action is necessary.
3. Examples of obvious error include: Use of wrong disk, Use of wrong control strain, Contamination, Wrong incubation temperature or conditions.
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| **Out of Control Results not due to obvious error** | 1. Investigate possible procedural problems: Correct zone measurements, Standardization of the inoculum, Storage and expiration dates of the disks, Incubation conditions, Control strain was not contaminated, Control organism was more than 24 h old.
2. Perform alternate test method until the problem is resolved.
3. Suppress the results for the individual antimicrobial agent.
4. Test the antimicrobial agent for 5 consecutive days. Record all results.
5. If all 5 zone diameters are within range, no additional corrective action is necessary.
6. If the problem is not resolved (1 or more diameters out of range), daily QC testing must be done until the problem is resolved.
7. It may be necessary to obtain a new QC organism either from the frozen stock or from BD.
8. Call BD technical service at 1-800-638-8663 if it may be a manufacturer problem.
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| **Procedure** | 1. Bring plates and dispensers to RT before use. It is essential for the dispensers to be at room temperature to prevent moisture condensation, and loss of antibiotic potency. Dispensers need at least 30 minutes to warm up.
2. Pick isolated colonies from 18-24 h growth on non-selective media (SB or CHOC)
3. Make a direct suspension in 3ml saline and using the Vitek DensiCHEK Plus®, obtain a reading of 0.5 - 0.55, (**not** up to 0.62 as for Vitek methods).
4. Use the adjusted inoculum suspension to inoculate AST test plate within 15 minutes.
5. Dip sterile swab into the suspension. Rotate swab against the wall of the tube above the liquid to remove excess inoculum.
6. Inoculate the dried surface of the MH plate. First streak of swab should go down the middle of the plate. Swab entire surface of agar plate three times, rotating plate approximately 60º between streaking to ensure even distribution.
7. Run the swab around the rim of the agar to remove excess moisture.
8. Allow plate to stand 3-5 minutes, (no more than 15) before applying the disks.
9. Apply the disks using the self-tamping dispenser.
10. To ensure purity of the organism, prepare a purity plate by streaking the inoculum to a blood agar plate and incubating overnight.
11. Because some of the drug diffuses almost instantaneously, do not relocate disks once they have made contact with the plate.
12. The CC (clinda) and E (erythro) disks must be dispensed by hand, spaced 12 mm apart for *S. pneumoniae* and β- hemolytic streptococci.
13. Invert plates and incubate within 15 minutes after the disks are applied.
14. Incubate Staphylococci in ambient air incubator for 16-18 hours.
15. Incubate *S. pneumoniae* and β- hemolytic streptococci (MHSB) in CO2 incubator for 20-24 h.
16. Read the plates after incubation only if the lawn of growth is confluent or nearly confluent.
17. Examine purity plate for any possible contamination.
18. For translucent media, invert plate, use reflected light and hold the Petri plate a few inches above a black surface
19. For opaque media, remove lid and use reflected light.
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| **Interpretation/ Results/** | 1. Organisms that show flattening of the clindamycin zone are positive for inducible clindamycin resistance=Positive ICR

1. Organisms that do not show flattening of the Clindamycin zone are negative for inducible clindamycin resistance=Negative ICR
2. Hazy growth within the zone of inhibition around the clindamycin disk indicates clindamycin resistance, even if no D-zone is apparent.
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| **Method Performance Specifications** | 1. The CC (clinda) and E (erythro) disks must be dispensed by hand, spaced 12 mm apart for

 *S. pneumoniae* and β- hemolytic streptococci.1. For MHSB, measure the zone of growth inhibition, not the zone of hemolysis.
2. Do not hold plates up to the light to read, using transmitted light.
3. Despite positive result for inducible clindamycin resistance, clindamycin may still be effective in some patients.
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| **Result Reporting** |

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| **Positive DTEST** | **Negative DTEST** |
| Report ICR as POS | Report ICR as NEG |
| Report CD MIC as resistant (>=8, R). | Release the CD MIC that is online, if hidden, SS or R |
| Unhide the CD. | Online Vitek® result does not require modification. |

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| **References** | 1. Hindler, J.F., Section editor, Antimicrobial Susceptibility Testing, 5.1.6, “Disk Diffusion Test” in *Clinical Microbiology Procedures Handbook,* Amy L Leber, editor, 2016, ASM Press, Washington, D.C.
2. CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Thirteenth Edition*, CLSI document M02-A13, Wayne, PA: Clinical and Laboratory Standards Institute; 2018,
3. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*, *Twenty-Eight* *Informational Supplement*, CLSI document M100-S28, Wayne PA: Clinical and Laboratory Standards Institute, 2018.
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| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| -Employee must read the procedure-Employee will observe trainer performing the procedure.-Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer. | -Direct observation. |
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Susan DeMeyere | 6/1/2018 | Initial Version-Separated from MC 6.31 Dtest-ESBL Confirmatory tests. |
| 2 | Susan DeMeyere | 8/28/2019 | Added inoculate a purity plate and examine following day.  |
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| **Archived by:** |  | **Archived Date:** |  |