

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
<ul style="list-style-type: none"> MSSA- <i>Staph aureus</i> ATCC® 25923 	<ul style="list-style-type: none"> Sterile cotton tip swabs 12 x 75 polystyrene tubes 	<ul style="list-style-type: none"> DTEST disk dispenser with CC (2 mcg), E (15 mcg), FOX (30 mcg) DensiCHEK Plus® (Vitek) 	<ul style="list-style-type: none"> Mueller-Hinton agar (MH) Mini Mueller-Hinton Agar with Sheep Blood (MHSB) Saline-0.45-0.9%

Specimen

1. Direct colony inoculums: use colonies grown overnight on nonselective medium (e.g. SB or CHOC).
2. Prepare inoculum from 4 or 5 isolated colonies of similar colony morphology.
3. Subculture QC stock, frozen, or lyophilized isolates 2 times prior to testing.

Storage

- Store agar plates and disk dispenser at 2-8°C.

Special Safety Precautions Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the [Microbiology Procedure Manual](#).

- [Biohazard Containment](#)
- [Safety in the Microbiology Laboratory](#)
- [Biohazardous Spills](#)

Quality Control

1. DTEST QC is performed monthly on the first Thursday of each month.
2. If there is a QC failure, document observation, notify technical specialist and proceed with corrective action. Do not report patient results until the problem is resolved.

Out of Control Results due to obvious error

1. Document the reason and retest the strain on the same 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.

Out of Control Results not due to obvious error

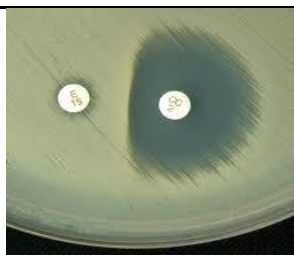
1. Investigate possible procedural problems: Correct zone measurements, standardization of the inoculum, storage and expiration dates of the disks, incubation conditions, control strain was not contaminated, control organism was more than 24 h old.
2. Perform alternate test method until the problem is resolved.
3. Suppress the results for the individual antimicrobial agent.
4. Investigate potentially affected patient results performed since the last successful QC event.
5. Retest the strain on the same day.
6. If the repeated result is within range, no further corrective action is necessary.
7. If repeated result is not within range, test the antimicrobial agent for 5 consecutive days. Record all results.
8. If all 5 zone diameters are within range, no additional corrective action is necessary.
9. If the problem is not resolved (1 or more diameters out of range), daily QC testing must be done until the problem is resolved.
10. It may be necessary to obtain a new QC organism either from the frozen stock or from BD.
11. Call BD technical service at 1-800-638-8663 as it may be a manufacturer problem.

Procedure

1. Use Mueller Hinton mini (MH) for *Staphylococcus* isolates and Mueller Hinton Sheep Blood (MHSB) for *S. pneumoniae* and beta-hemolytic *Strep*.
2. Allow plates and dispenser to come to room temperature 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.
3. Pick isolated colonies from 18-24 h growth on non-selective media (SB or CHOC).
4. Make a direct suspension in 3 ml saline and using the Vitek DensiCHEK Plus®.
5. Obtain a reading of 0.5 - 0.55, (**not** up to 0.62 as for Vitek methods).
6. Use the adjusted inoculum suspension to inoculate MH or MHSB plate within 15 minutes.
7. Dip sterile swab into the suspension. Rotate swab against the wall of the tube above the liquid to remove excess inoculum.
8. 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 60° between streaking to ensure even distribution.
9. Run the swab around the rim of the agar to remove excess moisture.
10. Allow plate to stand 3-5 minutes, (no more than 15) before applying the disks.
11. Apply the disks using the self-tamping dispenser.
12. Because some of the drug diffuses almost instantaneously, do not relocate disks once they have made contact with the plate.
13. The CC (clinda) and E (erythro) disks must be dispensed by hand, spaced 12 mm apart for *S. pneumoniae* and β - hemolytic streptococci.
14. Invert plates and incubate within 15 minutes after the disks are applied.
15. Incubate Staphylococci in ambient air incubator for 16-18 hours.
16. Incubate *S. pneumoniae* and β - hemolytic streptococci (MHSB) in CO₂ incubator for 20-24 h.
17. Read the plates after incubation only if the lawn of growth is confluent or nearly confluent.
18. Examine plate for any possible contamination.
19. For translucent media, invert plate, use reflected light and hold the Petri plate a few inches above a black surface
20. For opaque media, remove lid and use reflected light.

Interpretation/ Results/

1. Organisms that show flattening of the clindamycin zone are positive for inducible clindamycin resistance=Positive ICR



2. Organisms that do not show flattening of the Clindamycin zone are negative for inducible clindamycin resistance=Negative ICR
3. Hazy growth within the zone of inhibition around the clindamycin disk indicates clindamycin resistance, even if no D-zone is apparent.

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.
2. For MHSB, measure the zone of growth inhibition, not the zone of hemolysis.
3. Do not hold plates up to the light to read, using transmitted light.
4. Despite positive result for inducible clindamycin resistance, clindamycin may still be effective in some patients.

Result Reporting

Positive DTEST	Negative DTEST
Report ICR as POS	Report ICR as NEG
Report CD MIC as resistant (R).	

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. Clinical and Laboratory Standards Institute (CLSI) M100 Performance Standards for Antimicrobial Susceptibility Testing, 35th edition, 2025.

Training Plan/ Competency Assessment

Training Plan	Initial Competency Assessment
<ul style="list-style-type: none"> -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.

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 examine plate for any possible contamination.
3	Susan DeMeyere	6/2/2025	Changed to monthly QC from weekly