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| ESBL Confirmation by Disk Diffusion |
| **Purpose** | This procedure provides instruction for the ESBL confirmation test by disk diffusion. |
| **Principal** | Extended-spectrum beta-lactamases (ESBLs) are enzymes that hydrolyze or inactivate extended-spectrum cephalosporins, aztreonam, and expanded-spectrum penicillins. ESBL activity is blocked by beta-lactamase inhibitors such as clavulanic acid, and laboratory tests for confirming ESBL-producingbacteria are based on this property.The confirmatory disk diffusion test for ESBLs requires four disk tests: ceftazidime alone, cefotaxime alone, ceftazidime plus clavulanic acid, and cefotaxime plus clavulanic acid. If the zone diameter of either ceftazidime or cefotaxime (or both) in the presence of clavulanic acid is ≥5 mm larger than the zone diameter of the respective agent alone, the test is considered positive for ESBL production. |
| **Policy Statements** | This procedure applies to Microbiologists who perform antimicrobial susceptibility testing |
| **Test Code** | ESBL |
| **Materials** |  |  |  |  |
|  | **QC Strains** | **Supplies** | **Equipment** | **Media** |
|  | * *--E. coli* ATCC® 25922
* *Klebsiella pneumoniae* ATCC® 700603
 | * Sterile cotton tip swabs
* 12 x 75 polystyrene tubes
 | ESBL Disk dispenser with ESBL Disks--CTX 30mcg --CTX/CLA 30/10 mcg --CAZ 30mcg --CAZ/CLA 30/10 mcgDensiCHEK Plus® (Vitek) | Agar plates: store at 2-8ºC.--Mueller-Hinton agar (MH)--Mini MH Saline-0.45-0.9% |
| **Specimen** | 1. Prepare inoculum from 4 or 5 isolated colonies of similar colony morphology using colonies grown overnight on nonselective medium (e.g. SB or CHOC).
2. 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***:*** [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)
* [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. ESBL QC is done on each day of use.
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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| **ESBL Procedure** | 1. Bring plates and dispensers to RT before use. ESBL dispenser (stored frozen) should equilibrate to RT for at least an hour.
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.
7. Repeat this procedure 3 times, rotating the plate approximately 60º between streaking to ensure even distribution. Avoid hitting the sides of the plate to prevent aerosols.
8. Run the swab around the rim of the agar to remove excess moisture.
9. Allow plate to stand 3-5 minutes, (no more than 15) before applying the disks.
10. Apply the disks using the self-tamping dispenser. Because some of the drug diffuses almost instantaneously, do not relocate disks once they have made contact with the plate.
11. Invert plates and incubate within 15 minutes after the disks are applied.
12. Incubate 35ºC, 16-18 hr, in ambient air.
13. Freeze positive isolates for further studies.
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| **Interpretation/ Results** | 1. **Positive for ESBL production**:a ≥ 5 mm increasein a zone diameter for either CTX/CLA or CAZ/CLA vs. CTX or CAZ alone.
2. **Negative for ESBl production:** a< 5 mm increase in a zone diameter for either CTX/CLA or CAZ/CLA vs. CTX or CAZ alone.
3. **Indeterminate for ESBL production**: a decrease in the zone diameter for

either CAZ or CTX tested in combination with clavulanicacid versus its zone diameter when tested alone; or, no change in the diskzones with the addition of clavulanic acid. |
| **Limitations** | 1. Indeterminate results: Indeterminate results on ESBL tests may indicate the

presence of a resistance mechanism other than or in addition to an ESBL, suchas an AmpC with or without a porin loss.1. Some organisms that produce ESBLs express other beta-lactamases or resistance

mechanisms (e.g., porin alterations) that can mask ESBL production in the confirmatorytest, resulting in a false-negative result. |
| **Susceptibility Reporting Guidelines for ESBL Positive isolates** | **Change the following to’ R’**

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| **Penicillin’s** | **Cephalosporin’s** | **Monobactams** |
| Ampicillin | Cefazolin | Aztreonam |
| Pipercillin | Cefepime |  |
| Ticarcillin | Cefotaxime |  |
|  | Ceftazidime |  |
|  | Ceftriaxone |  |
|  | Cefpodoxime |  |
|  | Cefuroxime |  |
|  | Ceftizoxime |  |

Beta-Lactamase inhibitor combinations and cephamycins are not affected by ESBL; do not edit the susceptibility results.

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| **β-lactamase inhibitor combinations** | **Cephamycins** |
| Amoxicillin-clavulanic acid (Augmentin) | Cefoxitin |
|  Ampicillin-sulbactam (Unasyn) | Cefotetan |
|  Piperacillin/tazobactam (Zosyn) |  |
|  Ticarcillin/clavulanic acid (Timentin) |  |

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| **Result Reporting** | 1. When reporting, append the Sunquest code **ESBL** onto the organism code, i.e., **KLPN-ESBL**.
2. Also include the code **DRO** on a separate line.
3. Call the patient’s caregiver with the result and document in the patient’s report
4. Report ESBL positive organisms resistant to all Penicillin’s, Cephalosporin’s and Aztreonam.
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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 | 5/23/2018 | Initial Version-Separated from MC 6.31 Dtest-ESBL confirmation tests. |
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| **Archived by:** |  | **Archived Date:** |  |