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| E TEST Method for Susceptibility Testing |
| **Purpose** | This procedure provides instruction for the performance of E TEST SUSCEPTIBILITY TESTING. |
| **Principal**  | Epsilometer test (E- Test) is an ‘exponential gradient’ method of determination of antimicrobial resistance. A standardized inoculum of bacteria is swabbed onto the surface of a Mueller-Hinton agar plate. Rectangular plastic test strips consisting of a predefined, continuous, and exponential gradient of antibiotic concentrations are placed on the surface of the agar. After overnight incubation, a drop-shaped inhibition zone intersects the graded test strip at the minimum inhibitory concentration (MIC) of the antibiotic. Using the CLSI MIC standard breakpoints, a quantitative MIC report is obtained. |
| **Test Code** | MICS |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
| **Materials** | • E test strip of antibiotic to be tested* Penicillin
* Vancomycin
* Oxacillin
 | • Sterile cotton tip swabs* 12 x 75 polystyrene tubes
 | * Vitek DensiCHEK Plus®
* Incubator (CO2) or ambient air
 | • Agar plates: store at 2-8ºC. --Mueller-Hinton agar (MH) 90mm and 150mm --MH with 5% sheep blood (MHB) 90mm and 150mm--Saline-0.45-0.9% |
| Specimen | Prepare inoculum from 4 or 5 isolated colonies of similar colony morphology. |
|  | 1. | Direct colony inoculum (stationary-phase): use 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 Manualand 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)

**E test strips for Benzylpenicillin**--*Streptococcus pneumonia* ATCC 49619**E test strips for Oxacillin****--***Staphylococcus aureus* ATCC 29213**E test strips for Vancomycin***--Enterococcus faecalis* ATCC 29212**Benzylpenicillin, Oxacillin, Vancomycin E test strips**1. Perform QC each day of use.
2. Perform QC with each new lot or shipment of the antimicrobial strip before put into service. Record results in QC manual.
3. 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.**Out- of -control results due to obvious error**. Possible errors include:Use of wrong stripUse of wrong control strainContaminationWrong incubation temperature or conditions1. 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.**Out-of-control results not due to an obvious reason.** Investigate possible procedural problems:Correct interpretation of intersection of ellipses with test stripStandardization of the inoculumStorage and expiration dates of the Etest stripsIncubation conditionsControl strain was not contaminated* Control organism was more than 24 h old
1. Retest the strain on the same day.
2. If the repeated result is within range, no further corrective action is necessary.
3. Test the antimicrobial agent for 5 consecutive days. Record all results.
4. If all 5 MIC’s are within range, no additional corrective action is necessary.
5. If the problem is not resolved (1 or more MIC’s out of range), day of use QC testing must be

performed until the problem is resolved. Contact Lab Director.1. It may be necessary to obtain a new QC organism either from the frozen stock or from BD.
2. Call BD technical service at 1-800-638-8663 if it may be a manufacturer problem.

 **Reporting patient results**1. Perform alternate test method until the problem is resolved.
2. Investigate potentially affected patient results performed since the last successful QC event.
3. Suppress the results for the individual antimicrobial agent.
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| **Quality Control/ QC strains** **Day of use QC testing.****Out of Control****Results** |
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| **Procedure** | 1. Bring plates RT before use.
2. Invert plates to equilibrate so that the condensation does not fall onto the agar.
3. Agar plates can be put in the 35ºC ambient air incubator to warm. Caution: (no longer than 30 minutes, to prevent agar dehydration).

**Inoculum preparation**1. Pick isolated colonies from 18-24 h growth on non-selective media (SB or CHOC)
2. Using saline and the Vitek DensiCHEK Plus®, obtain a reading of 0.5 - 0.55, (**not** up to 0.62 as for Vitek methods).
3. Avoid extremes in inoculum density. Never use an undiluted overnight broth culture.
4. Use the adjusted inoculum suspension to inoculate AST test plate within 15 minutes.

 **Inoculation of Test Plates**1. Dip sterile swab into the suspension. Rotate swab against the wall of the tube above the liquid

 to remove excess inoculum.1. Inoculate the dried surface of the MH plate. First streak of swab should go down the middle of

 the plate. Swab across the entire agar surface at a 90º angle.1. 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.1. Run the swab around the rim of the agar to remove excess moisture.
2. To ensure purity of the organism, prepare a purity plate by streaking the inoculum to a blood agar plate and incubating overnight.
3. Allow excess moisture to be absorbed for no longer than 20 minutes so that the surface is completely dry before applying the Etest strips.

**Application of strips to Inoculated Agar Plates**1. Apply strips to agar surface using forceps or E-test applicator. Do not touch the strips at any point along the gradient.
2. Ensure that the strip touches the agar completely with no air bubbles. Use forceps and/or an applicator stick to force out any air that has become trapped below the strip (without moving the strip!), working from the lowest concentration upwards. Small bubbles will not affect results.
3. Use templates to position 4 to 6 strips onto a 150 mm plate or 1-2 strips onto a 90 mm plate. For organisms expected to be highly susceptible, use fewer strips per 150 mm plate and only one on a 90 mm plate.
4. Because some of the drug diffuses almost instantaneously, **do not remove or adjust a strip** **once it has touched the agar**. If the strip moves while being applied, a new agar plate must be set up.

**Incubation**1. When using regular Mueller Hinton media, invert plates and incubate at 35ºC in an ambient air incubator within 15 minutes after the strips are applied.
2. When using Mueller Hinton with 5% SB, invert plates and incubate at 35ºC in CO2 incubator within 15 minutes after the strips are applied.

 **Reading plates**1. Read the plates after incubation only if the lawn of growth is confluent. If individual colonies

 are apparent, the inoculum concentration was too light and the test must be repeated.1. Read the MIC value where the pointed end of the inhibition ellipse intersects the side of the strip.
2. Examine purity plate for any possible contamination.
3. When growth occurs along the entire strip i.e. no inhibition ellipse is seen, report the MIC as ≥ the highest value on the MIC scale. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale.
4. Vancomycin inhibition ellipses can be slim. Read the actual intersection at the strip and not growth ”hugging” the side of the strip which may be caused by organisms growing in a tunnel of water.
5. Ignore swarming and hemolysis, read the inhibition of growth.
6. For bactericidal drugs e.g. ß-lactams, always read the MIC at the point of complete inhibition of all growth, including hazes, microcolonies and isolated colonies. Tilt the plate and/ or use a magnifying glass to carefully examine endpoints, especially for pneumococci, streptococci, enterococci, fusobacteria, *Acinetobacter* and *Stenotrophomonas* spp.
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| **Interpreting Results****Sunquest****Result****Reporting** | 1. Etest generates MIC values from a continuous scale and can give results in-between conventional two-fold dilutions i.e. half dilutions. An MIC value which falls between standard two-fold dilutions must be rounded up to the next two-fold value, i.e. 1.5 is rounded up to 2.
2. Interpretations (SS, I, R) will be made based on the rounded up value.
3. Refer to procedure MC 6.00 Susceptibility Testing Guidelines for interpretations on *S*. *pneumonia* and Benzylpenicillin.
4. For all other drug/bug combinations refer to the CLSI guidelines.
5. Criteria specified by CLSI are used to interpret the MIC’s.
6. Record the MIC in Sunquest function MRE by clicking on the susceptibility tab.
7. Use the drop-down arrow to select the MIC keyboard. Highlight the organism #. Enter the

MIC number at the appropriate drug prompt.1. In many instances, the computer will automatically interpret the results. Refer to CLSI guidelines for those instances the computer does not do an interpretation.
2. Display results to make sure they are correct by clicking the summary button.
3. Click on the File button to file results.
4. This method applies to rapid growing aerobes
5. Some bacteria may become resistant during antimicrobial therapy. Repeat testing on

 subsequent isolates should be performed every 3 days.1. Do not report patient results when quality control results are outside the stated QC ranges.2. MIC results for a quality control (QC) strain that fall a half dilution below the lower QC limit should be rounded up to the next upper two-fold value before establishing QC compliance. Similarly, MIC results that are a half dilution above the upper limit show non-QC compliance.3. Do not read the plate if the culture appears mixed or if the lawn of growth is too light or too heavy; repeat the test.4. Excessively wet plates prior to inoculation, insufficient drying before applying strips and/or unevenly streaked surfaces may give non-confluent growth, jagged ellipse edges and uneven MIC intersections. Repeat the test if MIC endpoints are difficult to read.5. When macrocolonies are present within the ellipse for bactericidal agents, read all macrocolonies within 1-3 mm from the strip.6. If inhibition ellipses for clindamycin, erythromycin or chloramphenicol ”dip” at the endpoint, extrapolate the MIC at the initial indentation, i.e. 0.5-1 dilution above the intersection.7. Be sure the agar plate is incubated for the recommended period before reading, especially for delayed expression of resistance and slow growing and fastidious organisms. |
| **Procedure****Notes****Limitations** |
| **References** | 1. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*, *Twenty-Eight Edition*, CLSI document M100-S28, Wayne PA: Clinical and Laboratory Standards Institute,20182. BioMérieux SA Marcy-l’Etoile – France ETest® package insert |
| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure.2. Employee will observe trainer performing the procedure.3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer. | 1. Direct observation. |
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Pat Ackerman | 1995 | Adopted |
| 2 | Jennifer JohnsonBecky Carlson | 10/29/1512/2/2015 | Reformatted Reviewed |
| 3 | Susan DeMeyere | 4/20/2018 | Added hyperlinks. Removed Weekly QC Testing. Removed Cefotaxime, Ceftriaxone, Erythromycin |
| **4** | Susan DeMeyere | **8/29/2019** | Added inoculate a purity plate and examine following day. |