CHRISTUS SPOHN Health System

Policy / Procedure Title: E-test (MIC) Susceptibility

SOP Number:	M.6.130	**Effective Date:**	02/2007
Department:	Microbiology	**Revision Date:**	03/18/2015
Policy (P), Procedure (PR)or Both (P/P):	PR	**Version:**	02

Applicable Standards	
Standard	Organization
MIC.21460	CAP
MIC.21840	CAP
MIC.21910	CAP
MIC.21930	CAP
Related Documents	

Version History		
Version	Effective Date	Retired Date
01	6/2001	03/19/2015
02	03/19/2015	

Review History (Up to the Last 15 Occurrences)			
Date	Version	Revision Type	Review By
03/19/2015	02	System Director Approval	Joe A. Lewis, MD

Distribution
CSHCC Memorial Microbiology SOP

PRINCIPLE
E-Test is a quantitative technique for the determination of antimicrobial susceptibility of both non-fastidious Gram negative and Gram positive aerobic bacteria, and fastidious bacteria such as anaerobes, Pneumococci and Haemophilus. E-Test is based on a combination of the concepts of both dilution and diffusion tests. E-Test consists of a thin, inert and non-porous plastic strip 5mm wide and 50 mm long. One side of the strip is marked with a MIC reading scale in ug/ml. A predefined exponential gradient of antibiotic, dried and stabilized, is immobilized on the other side of the carrier. When an E-Test strip is applied onto an inoculated agar plate, there is an immediate effective release of the antibiotic from the carrier surface into the agar matrix. A continuous and exponential gradient of antibiotic concentrations is created directly underneath the carrier. After incubation a symmetrical inhibition ellipse centered along the carrier is seen. The zone edge intersects the strip at the MIC value given in ug/ml.

SPECIMEN
Growth should be taken from a 18-24 hour pure culture plate.

REAGENTS
E-Test strips are supplied in packages of 30 or 100 test strips of one antimicrobial agent.

STORAGE
Etest reagents should be stored according to the temperature specified on the packaging, until the given expiration date. Products can be stored lower than the maximum temperature specified.

Etest gradient strips left over from an opened package must be kept dry. The opened package should be either sealed with a sealing clamp of placed in an airtight storage container with active dessicant, and stored within the temperature range stated on the label. Protect etest strips from moisture, heat and direct exposure to strong light at all times.

HANDLING
- Before using the Etest gradient strips from an unopened package, visually inspect to ensure the package is intact. Do not use gradient strips if the package has been damaged.
- When removed from the refrigerator/freezer, allow the original package or storage container to reach room temperature before opening (+4C/approx 15 minutes, -20C approx 30 minutes) Ensure that the moisture condensing on the outer surface has evaporated completely before opeing the package. Packages stored at room temperature can be opened immediately.

Opening Instructions
<u>Single Pack</u>
1. Hold the packaging between the thumb and the index finger, placing the thumb tip on the indented area on the back.
2. Press forward with the thumb and back with the index finger to break open the aluminum film, ensuring that the dessicant remains in the top part of the packaging.
3. Bend the top part to open the packaging completely.
4. Remove the Etest strip from the packaging using forceps or other manual applicator.

<u>Blister</u>
1. Open one blister compartment by cutting the packaging along the dotted line using scissors.

<u>Foam</u>
1. Open the packaging by cutting off one end of the aluminum pouch using scissors.

- When handling Etest strips manually, grip only the handle of the strip i.e., the area labelled E. Do not touch the surface of the strip with the antibiotic gradient, i.e. the side

opposite the MIC scale. Strips can be placed in an applicator tray until ready to use. The Mini Grip-it or forceps can be used to efficiently pick up Etest strips.

QUALITY CONTROL:

Quality control testing is done along with each patient test unless QC has been validated for weekly testing.

To check the performance of Etest reagents, quality of media, inoculum and procedure used, test appropriate quality control strains as outlined under PROCEDURE. The reagents and test procedure are considered satisfactory if MIC values obtained fall within the quality control specifications provided on each Etest product supplement.

Do not report patient results when quality control results are outside the stated QC ranges. Frequency of quality control testing should be established by the individual laboratory. Guidelines are provided in CLSI® Antimicrobial Susceptibility Testing documents M7, M11 and M100 series.

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. However, MIC results that are a half dilution above the upper limit show non-QC compliance.

PROCEDURE:

Inoculation Preparation

Make a suspension equivalent to a 0.5 McFarland turbidity standard in broth or sterile 0.85%-0.9% saline by emulsifying several well-isolated colonies from an overnight agar plate. For fastidious organisms such as pneumococci, streptococci, gonococci, anaerobes and Haemophilus spp., use the suspension prepared in broth within 15 minutes.

Inoculaton

1. A suitable agar plate with an agar depth of 4.0 $\pm$ 0.4mm should be used. The medium and required supplements will depend on the bacterial species being tested. Following are examples:

Streptococcus spp.	Mueller-Hinton with 5% Sheep Blood
H. influenzae	HTM agar

2. Soak a sterile, cotton-tipped swab into the prepared inoculum suspension. Remove excess fluid by rotating and pressing the swab firmly against the inside wall of the test tube.
3. Swab the entire surface of the agar plate. Repeat the swabbing procedure three times, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum.
4. Allow excess moisture to be absorbed for approximately 15-20 minutes so that the surface is completely dry before applying the E-Test gradient strips.

Application

1. Check that the inoculated agar surface is completely dry before applying the gradient strips.
2. Open the package and handled as described under HANDLING.
3. With a pair of forceps, grip the handle of the strip (area labelled E) and place its bottom edge against the agar surface, holding it an angle to the surface with the MIC scale facing upwards and the concentration maximum nearest the periphery of the plate. Release it onto the agar surface.
4. Make sure that the strip is in complete contact with the agar surface. If necessary, remove air pockets underneath the strip by gently pressing the strip with the forceps, moving from the minimum concentration upwards. Small bubbles will not affect the results.
5. Once applied, the position of the E-test strip cannot be changed because of the immediate release of antibiotic from the strip into the agar.

Template

Six different E-Test strips can be applied onto a 140-150 mm diameter agar plate using the "wagon-wheel spokes" arrangement. The minimum concentration of each gradient should be oriented towards the center of the plate and the maximum concentration towards the periphery of the plate.

For single MIC determinations, two Etest strips can be used on a small agar plate, 90 mm in diameter, as shown above.

Incubation

Agar plates can be incubated in an inverted position (lid down) in stacks no higher than 5. The incubation temperature and atmosphere selected should be optimal for the bacterial species being tested and the particular bacterium/antibiotic combination being tested.

Organism Group	Agar Media	Inoculum Suspension	Turbidity (McFarland)	Incubation Temp	Atmosphere	Time
Non-fastidious Aerobes	Mueller Hinton	0.9%NaCl	0.5 (1 if mucoid)	35 ± 2°C	Ambient	16-20h
Anaerobes	Mueller Hinton	Mueller Hinton broth	1	35 ± 2°C	Anaerobic	24-48-72h, species dependent
Haemophilus influenzae	HTM	0.9% NaCl, Mueller Hinton or HTM broth	0.5 (1 if mucoid)	35 ± 2°C	5-10% CO2	20-24h
Streptococcus spp.	Mueller Hinton + 5% Blood	0.9% NaCl or Mueller Hinton broth	0.5 (1 if mucoid)	35 ± 2°C	5-10% CO2	20-24h
Neisseria meningitidis	Mueller Hinton + 5% Blood	0.9%NaCl broth	0.5	35 ± 2°C	5-10% CO2	20-24h

Note the following:

1. The type of bacterium/antibiotic combination may affect the nature of the zone edge at the MIC intersection. Bactericidal agents generally give distinct intersections while bacteriostatic antibiotics, in particular Sulfonamides and Trimethoprim, may give diffuse zone edges at the MIC intersection. In these cases, the principle of 80% inhibition should be used to read the intersection, i.e. the MIC is read as the lowest concentration showing a marked decrease in growth.
2. The inoculum density may affect the appearance of the MIC intersection of certain antibiotics. A heavy inoculum generally tends to give a less clear-cut intersection. If the zone edge is too diffuse or a double zone edge is seen spanning over a broad concentration range, the inoculum density may be unacceptably high and the test should be repeated.The inoculation technique may also affect the appearance of the MIC intersection. Excessively wet swabs and plates which are unevenly swabbed may cause zone edges to be jagged giving uneven intersections at the MIC.
3. Agar surfaces which are not dry prior to application of the E-Test strip may result in a thin line of growth from the MIC intersection and a few millimeters upwards along the edge of the strip. The line of growth should be ignored when reading the MIC.
4. The choice of medium may affect MICs in several aspects. Media as recommended by CLSI will support good growth and generally give distinct intersections. It is therefore essential to use a well defined and standarized susceptibility test medium to obtain accurate and reproducible MICs.
5. The mechanisms of antibiotic action and antibiotic resistance may result in different growth patterns. Enlarged, isolated mutant colonies may be present at the MIC intersections of b-lactam antibiotics. For Clindamycin, the otherwise symmetrical inhibition ellipse may occasionally be distorted at the MIC intersection giving a 'lightbulb' shape. When reading plates with Enterococcus and S. maltophilia species, the inhibition ellipse should be carefully examined for growth of micro colonies and the MIC read where complete inhibition of growth is seen.
6. When growth occurs along the entire strip, i.e. no inhibition zone is seen, the MIC should be reported as >than the highest value on the reading scale. When the inhibition ellipse is below the strip, i.e. the zone edge does not intersect the strip, the MIC should be reported as < than the lowest value on the reading scale.
7. An Etest MIC value which falls between two-fold dilutions must be rounded up to the next upper two-fold value before categorization.

INTERPRETATION OF RESULTS

Reading the MIC

After the required period of incubation and only when bacterial growth becomes distinctly visible, read the MIC value where the pointed end of the inhibition ellipse intersects the side of the strip. Do not read the plate if the culture appears mixed or if the lawn of growth is too light or heavy; repeat the test.

Etest MIC endpoints are usually clear-cut although different growth/inhibition patterns may be seen. Please consult the guidelines below and illustrations in the Etest Reading Guide.

IMPORTANT READING OBSERVATIONS

- Consult the Etest product supplement for information on the mode of action of each antibiotic (bactericidal or bacteriostatic)
- For bactericidal drugs, e.g. B-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.

- For bacteriostatic drugs e.g. trimethoprim/sulfamethoxazole, in case of trailing endpoints, read at 80% inhibition, i.e. the first point of significant inhibition as judged by the naked eye.
  • 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.
  • When macrocolonies are present within the ellipse for bactericidal agents, read all macrocolonies within 1-3 mm from the strip (consult ETEST READING GUIDE, Figure 21).
  • 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.
  • Organisms such as staphylococci, *Acinetobacter* spp., anaerobes and gonococci may be susceptible to sulbactam, tazobactam or clavulanic acid *per se*. For Etest PTc and TLc, this may result in an inhibition ellipse with an extended parallel band of inhibition alongside the strip. Extrapolate the upper elliptical curvature towards the strip to obtain the MIC (consult ETEST READING GUIDE, Figure 15).
  • 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.
  • For fosfomycin showing numerous (>5) macrocolonies in the inhibition ellipse, read the MIC at complete inhibition. A few (<5) colonies can be ignored.
  • For quinupristin/dalfopristin and linezolid, hazy and trailing growth for staphylococci and enterococci should be read 90% inhibition as judged by the naked eye. Read isolated macrocolonies in the inhibition ellipse at complete inhibition.
  • Vancomycin inhibition ellipses can be slim. Read the actual intersection at the strip and not growth ”hugging” the side of the strip.

Interpretation
MIC breakpoints for defining interpretive categories as published by the CLSI® and/or the FDA should be used for interpreting Etest MIC values.

Being a fully quantitative MIC method, Etest enables the laboratory to report the exact MIC value together with the interpretive category. Etest generates MIC values from a continuous scale and can give results in-between conventional two-fold dilutions i.e. half dilutions. An Etest MIC value which falls between standard two-fold dilutions must be rounded up to the next upper two-fold value before categorisation.

Example: Benzylpenicillin MIC (µg/mL) breakpoints for *Streptococcus pneumoniae* are:
S ≤ 0.06 I 0.12-1 R ≥ 2
An Etest MIC of 1 µg/mL is reported as intermediate (I) while 1.5 is rounded up to 2 µg/mL and the category reported as resistant (R).

See ORGANISM RELATED EFFECTS at end of procedure (Figures 7-26)

PROCEDURE NOTES:

1. When not in use, E-Test strips should be protected at all times from moisture, heat and direct exposure to strong light.
2. Because of the instantaneous release of antibiotic from the carrier to the agar, E-test strips once applied to the agar surface cannot be moved.
3. The inoculated surface of the agar must be completely dry before applying the E-test strip.
4. Do not place too may E-Test strips on each agar plate, six strips can be placed on a 150 mm plate and 2 strips on a 90 mm plate.
5. Use well-standardized media for accurate and reproducible results.

IMPORTANT OBSERVATIONS:

1. The results obtained with the E-test is in vitro values only and can only provide an indication of in vivo susceptibility.
2. For details of specific interpretive limitations and/or limitations on the clinical use of an antibiotic in various therapeutic situations, please refer to the tables and footnotes of MIC interpretive standards in the latest CLSI AST documents for dilution procedures (M7, M11 and M100 series) or the appropriate FDA drug labelling.

REFERENCES:

1. Package Insert. Etest Antimicrobial Susceptibility Testing. bioMerieux 15203D – en – 2012/11
2. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. Approved Standard, M7-A8.
3. CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*. Approved Standard, M45-A
4. CLSI Performance standards for Antimicrobial Susceptibility Testing. M100-S23.