PURPOSE: The Etest is a quantitative test used to determine the *in vitro* MIC (minimum inhibitory concentration) of antimicrobial agents against microorganisms.

SAMPLE INFORMATION:

Type	Few, well isolated colonies that are 18 to 24 hours old

REAGENTS and/or MEDIA:

Type	Oxoid M.I.C.Evaluator and bioMerieux Etest strips
Stability and Storage Requirements	• Unopened strips should be stored at 2°C to 8°C. • Allow the strips to come to room temperature before opening. • Once opened, strips should be used within 15 minutes.

SUPPLIES:

- Plastic Vitek tubes and caps
- 0.9% sterile saline
- Sterile swabs
- DensiCHEK Plus
- Mueller Hinton agar
- Mueller Hinton agar with 5% sheep blood
- Haemophilus Test Media
- Forceps
- 35° ambient air and 37° CO_2 incubators
- Small, metric ruler

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Quality control is performed weekly.
- Refer to MIC60020 - Antibiotic Quality Control and MIC60021 - Antibiotic Quality Control Job Aid.
- A TQC order is automatically generated on Wednesdays to record the QC results.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the Etest	
1	Remove Etest strips from refrigerator for 1 hour and bring to room temperature.
2	Remove testing agar from the refrigerator and bring to room temperature: • For *Staphylococcus* spp. and *Enterococcus* spp. use Mueller Hinton agar. • For *Streptococcus* spp. use Mueller Hinton agar with 5% sheep blood. • For *Haemophilus* spp. use Haemophilus Test Media.
3	Dispense 3 mL of 0.9% sterile saline into a labelled plastic test tube. Pick several colonies from a fresh agar plate and prepare a suspension equivalent to a 0.5 McFarland standard.
4	Within 15 minutes of adjusting turbidity, dip a sterile cotton swab into the inoculum and rotate against the wall of the tube above the liquid to remove excess inoculum.
5	Swab the entire surface of the agar three times, rotating plate approximately 60° between streaking to ensure even distribution. To minimize aerosols, avoid hitting the sides of the plate. Finally, run swab around the edge of the agar to remove any excess moisture. Allow inoculated plate to stand for 3 to 15 minutes before applying strips ensuring the agar surface is completely dry.
6	Using forceps, apply the strips to the agar surface: • Make certain MIC scale is facing upward and do not touch the underside. • Put the end of the strip with the lowest concentration onto the plate first and then carefully rolling the strip onto the agar to ensure good contact with the entire length of the strip. • 2 strips may be applied to one plate. Rotate plate 180° and place the second strip in the opposite direction of the first strip. • Remove large air bubbles underneath using forceps to gently press on the strip. Small bubbles do not interfere with the test. • Do not move the strip once it makes contact with the agar surface.
7	Invert the plate and incubate within 15 minutes of the strip application: • Non-fastidious organisms in the O_2 incubator for 18 hours. • *Streptococci* spp. in the CO_2 incubator for 20 to 24 hours. • *Haemophilus* spp. in the CO_2 incubator for 18 hours.

INTERPRETATION OF RESULTS:

Step	Action
1	After incubation, read plates only if lawn of growth is confluent.
2	To read the plate, remove the cover and hold to a transmitted-light source and read the MIC at the point where growth intersects the Etest strip. Read for complete inhibition of all growth, including haze and isolated colonies.
3	If there is no inhibition of growth, report the MIC as greater than or equal to the highest concentration on the Etest strip. If the zone does not intersect the strip (zone below the strip) report MIC as less than the lowest concentration on the Etest strip. For MICs that fall between markings, use the higher value.
4	When testing hemolytic organisms, measure the diameter of the zone of inhibition of growth not the zone of inhibition of hemolysis.

Technical issues - Resistance mechanism related effects:

Effect & Action

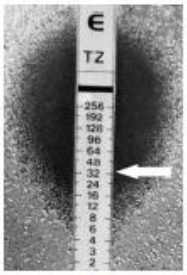

Figure 19. Small colony variants and bactericidal agents; read at complete inhibition. MIC 32 µg/mL.

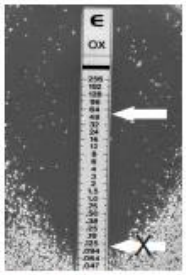

Figure 20. Isolated colonies for oxacillin represent heteroresistant subpopulations i.e. ORSA. MIC 48 µg/mL.

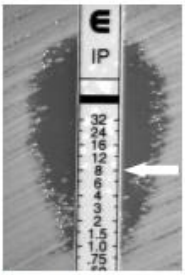

Figure 21. Isolated colonies for carbapenems may represent resistant subpopulations e.g. KPC. MIC 8 µg/mL.

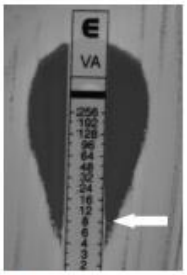

Figure 22. Trailing growth (hazes, microcolonies, macrocolonies) represent VISA/hVISA. MIC 8 µg/mL.

Technical issues - Technical and handling related effects:

Effect & Action

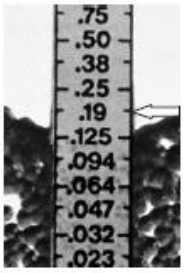

Figure 23. Intersection in-between markings, read the upper value. MIC 0.19 µg/mL.

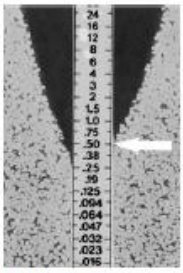

Figure 24. Uneven intersections; read the higher value. If >1 dilution, repeat the test. MIC 0.5 µg/mL.

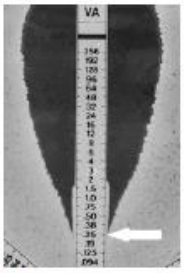

Figure 25. Ignore a thin line of growth alongside the strip. MIC 0.25 µg/mL.

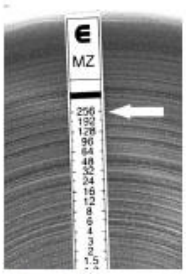

Figure 26. Complete growth around the strip. MIC ≥ 256 µg/mL.

Technical issues - Drug related effects:

Effect & Action

Figure 11. Bactericidal agents give sharp MIC endpoints. MIC 0.064 µg/mL.

Figure 12. Bactericidal agents; read at complete inhibition of hazes and microcolonies. MIC 1.5 µg/mL.

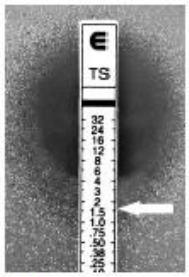

Figure 13. Bacteriostatic agents; read at 80% inhibition. MIC 1.5 µg/mL.

Figure 14. Linezolid; read at 90% inhibition (ignore finer hazes and pinpoint colonies). MIC 0.75 µg/mL.

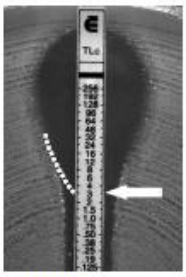

Figure 15. ß-lactamase inhibitors e.g. tazobactam; extrapolate the upper curvature to the strip. MIC 3 µg/mL.

Figure 16. Trim/sulfa; read at 80% inhibition (ignore lawn of haze within the ellipse). *Stenotrophomonas* spp. MIC 0.19 µg/mL.

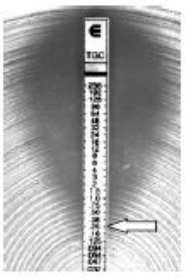

Figure 17. Tigecycline; read at 80% inhibition (ignore trailing microcolonies or hazes). MIC 0.25 µg/mL.

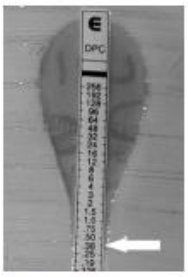

Figure 18. Polypeptides; read at the bottom of the "dip" if colonies are absent. MIC 0.38 µg/mL.

Organism related issues:

Effect & Action

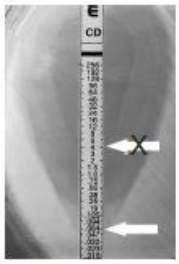

Figure 7. Ignore swarming. MIC 0.064 µg/mL

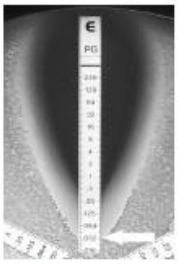

Figure 8. Ignore haemolysis; read the inhibition of growth. MIC 0.032 µg/mL.

Figure 9. Tilt plate or use a magnifying glass to see pin-point colonies and hazes, e.g. enterococci, pneumococci, fusobacteria, and *Stenotrophomonas* spp. MIC 1 µg/mL.

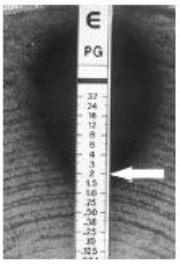

Figure 10. Scrutinise ß-lactam endpoints for pneumococci for hazes and microcolonies. MIC 2 µg/mL.

REPORTING RESULTS:

Step	Action
1	In the LIS, report Etest results under the "Breakpoint" tab in the sample screen. Both the MIC and interpretation will appear on the final report.
2	If the antibiotics have not already been generated, add them by selecting "Generate Drugs" if panel has been entered into the LIS or by selecting "Add Drug" to add drugs individually.
3	In the "Result" column add the MIC that was read from the Etest strip. The "Interpretation" column will automatically be filled out by the LIS. If the interpretation is not completed by the LIS, consult the CLSI guidelines and manually add the interpretation. Refer to the ASTM for the reporting of results.

LIMITATIONS/PRECAUTIONS:

1. Numerous factors can affect Etest MICs, such as inoculum size, rate of growth, formulation and pH of media, incubation environment and length of incubation, drug diffusion rate and measurement of endpoints.
2. Etest is an in vitro diagnostic test. Results may provide an indication of an organism's in vitro susceptibility. Use of results to guide therapy must be the sole responsibility of the physician.

REFERENCES:

- Oxoid M.I.C.Evaluator package insert, September 2010
- bioMerieux Etest package insert, 2012/01
- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	4 APR 19	Initial Release	L. Steven