PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC52600 – Etest	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director of Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

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

PURPOSE/RATIONALE:
This standard operating procedure describes how to perform the Etest.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) performing the Etest.

SAMPLE INFORMATION:

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

REAGENTS and/or MEDIA:

Type	Biomerieux ETEST strips
Stability and Storage Requirements	• Store according to the temperature specified on the packaging • Do not use reagents after the expiry date indicated on the packaging • Protect ETEST strips from moisture, heat and direct exposure to strong light at all times • Do not store ETEST strips from Single Packs that have been opened

SUPPLIES:

- Plastic Vitek tubes and caps
- Sterile saline
- Sterile swabs
- Mueller Hinton agar
- Mueller Hinton agar with 5% sheep blood
- Haemophilus Test Media
- Forceps
- Small, metric ruler

EQUIPMENT

- DensiCHEK Plus
- 35° ambient air and 37° CO_2 incubators

SPECIAL SAFETY PRECAUTIONS:

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

- Ensure that appropriate hand hygiene practices be used.
- 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 of 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. Routine Practices 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 results

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the Etest	
1	Remove the ETEST strips from refrigerator for 30 minutes and bring to room temperature.
2	Remove testing agar from the refrigerator and bring to room temperature: • For *Staphylococcus* spp., *Enterococcus* spp., Enterobacteriaceae and *Pseudomonas aeruginosa* 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 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.

REPORTING OF 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:

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.

CROSS-REFERENCES:

- MIC60020-Antibiotic Quality Control
- MIC60021-Antibiotic Quality Control Job Aid

REFERENCES:

1. Biomerieux. (2020-09). *ETEST* package insert
2. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 31st ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2021.

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	04 Apr 19	Initial Release	L. Steven
2.0	30 Jun 21	Procedure reviewed and added to NTHSSA policy template	L. Steven