PROCEDURE: TREK SENSITITRE® MIC PROCEDURE

I. PRINCIPLE

Dilution tests are used to determine the minimum concentration of an antibiotic required to inhibit or to kill an organism. Serial dilutions of the antimicrobial agent are inoculated with the organism and incubated. The minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial without visible organism growth. The minimum bactericidal concentration (MBC) is the lowest concentration of drug yielding a reduction of viability of greater than 99.9%. The following procedure is for SENSITITRE® lyophilized MIC panels.

II. AVAILABILITY

A. MIC panels will be set up as needed for all culture types.

III. TEST CODES

A. GNX2F – Trek MIC GNX2F Gram Negative Sensititre® Panel
B. STP6F – Trek MIC STP6F Strep Pneumo Sensititre® Panel
C. GPN3F – Trek MIC GPN3F Gram Positive Sensititre® Panel

IV. SPECIMEN

A. Specimens should be collected, transported, stored, and then plated onto primary isolation medium according to established microbiological laboratory practice to give isolated colonies.
B. Non-Fastidious aerobic organisms: Isolate growth should be fresh (visible growth capable of producing adequate growth control wells and acceptable colony count plates) and on primary agar plates.
C. Fastidious organisms: Pure isolate growth from an overnight culture.

V. MATERIALS AND EQUIPMENT

A. Sensititre® plates
B. Adhesive seal
C. Sensititre® demineralized water
D. Sensititre® cation adjusted Mueller-Hinton (MH) broth with TES buffer (CAMHBT)
E. Sensititre® cation adjusted MH broth with TES buffer and lysed horse blood (CAMHBT + LHB)
F. Sensititre® dosing heads
G. Sensititre AIM Auto-inoculator
H. Sensititre® Vizion
I. 0.5 McFarland turbidity standard
J. 1µl calibrated loop
K. Quality control strains
L. Agar plates
M. Incubator 34-36°C, non-CO_2
N. Vortex mixer

VI. STORAGE AND HANDLING

A. The plates should be stored at room temperature (15-25°C) away from direct sunlight and direct heat.
B. Each plate is packaged in foil with a silica gel desiccant. Do not use the plate or broth if past its expiration date, the desiccant color is not blue, or the foil pouch is damaged.
C. Inoculate plate within 5 hours of removal from pouch.

VII. QUALITY CONTROL

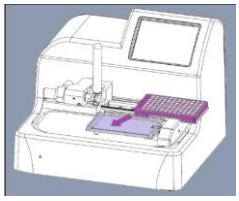
A. Quality Control
1. Ranges – Refer to www.trekds.com/techinfo for QC barcodes.
2. Frequency – The laboratory's Individualized Quality Control Plan (IQCP) for Trek MIC Panels contains complete details of the QC data and QA plan approved by the Director. Refer to IQCP document for compete details.
3. All Sensititre® plates include positive control wells. Tests are invalid unless there is distinct growth in all positive control wells.
4. Some plate formats also include a "negative growth" well. This well is used for calibration of the AutoReader® and is not required for manual reading.
5. Inoculum should be cultured onto a suitable medium to check for purity and colony count. Test results are invalid if a mixed culture is detected or if colony count is not within acceptable range. Acceptable Colony Counts are:
    a. Gram Negative Rods: inoculum of 1x105cfu/mL, (range $5x10^4$ – $5x10^5$), approximately 10-100 colonies. *Proteus* isolates $1x10^4$cfu/mL (range $5x10^3$ – $5x10^4$), approximately 1-10 colonies.
    b. *Streptococcus* spp should have an inoculum of $5x10^5$cfu/mL (Range $2x10^5$ – $7x10^5$), approximately 10-100 colonies. This check is important for *Streptococcus* spp., as inocula can vary depending on the conditions of incubation of the primary plate.
    c. Isolates should have an inoculum of $1x10^5$ cfu/mL, (range $5x\ 10^4$ - $5x\ 10^5$). Approximately 10-100 colonies on the colony count plate.
6. QC will be performed as part of the scheduled weekly QC and recorded in the MIC QC binder. QC Organisms:
    a. GNX2F: *P. aeruginosa* ATCC 27853; *E. coli* ATCC 25922; *E. coli* ATCC 35218
    b. GPN3F: *S. aureus* ATCC 29213; *E. faecalis* ATCC 29212
    c. STP6F: *S. pneumoniae* ATCC 49619
7. Patient results should not be reported if QC results are outside the acceptable ranges.
8. Contact Thermo Fisher Scientific for assistance in the event that quality control discrepancies cannot be resolved.

VIII. TEST PROCEDURE

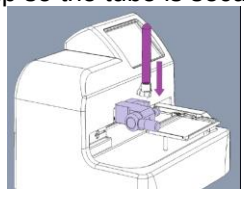
A. **DEN-1 Densitometer**
1. Calibration – must occur before each use and/or after the unit is switched on/off.
    a. Densitometer must be switched "ON" 15 minutes before use.
    b. Press SELECT button on rear of densitometer.
    c. When flashing "0.5" appears on display, insert 0.5 McFarland standard**.**
    d. Press INSTALL to save that calibration point**.**
    e. When flashing "1.0" appears on display, insert 1.0 McFarland standard.
    f. Continue or any additional McFarland standards that you wish to calibrate (at least two standards are required for calibration) and INSTALL to save or SELECT to skip a point.
    g. After the 5.0 McFarland calibration point is saved or skipped, the calibration is done.
2. Standardizing an Inoculum
    a. Densitometer must be switched "ON" 15 minutes before use.
    b. Emulsify (by vortex) bacterial colonies from a fresh, pure, overnight growth on an agar plate into 4-5 mL of suitable diluent (refer to appropriate sections below to determine correct diluent for the organism growing).
    c. With no tube inserted, "0.0" indicates densitometer is ready for use, "CC" indicates it needs calibration, "EE" indicates there is operator error (densitometer needs to be shut off and switched on again)

   d. Insert the tube containing bacterial suspension into the densitometer.
   e. A correct bacterial suspension will read 0.5.
   f. Inoculate broth into the plate within 30 minutes of inoculum preparation.
3. Gram Negative Rods (GNX2F Panel)
   a. Follow instructions above to achieve standardized inoculum using demineralized water as the diluent.
   b. Transfer 10 µl of the suspension into an 11 mL tube of cation adjusted Mueller-Hinton (MH) broth with TES buffer to give an inoculum of 1 X 105 cfu/mL.
   c. Vortex or invert the tube 8-10 times.
   d. Replace the tube cap with a Sensititre® single-use dosing head
   e. Follow the instruction listed in section **B. Sensititre® AIM (Auto-inoculator)** to deliver 50 µL of inoculated MH to each well. Alternatively, a multichannel pipette may be used to deliver 50 µL of inoculated MH to each well.
4. *Streptococcus* species (STP6F Panel)
   a. Follow instructions above to achieve standardized inoculum using 5 mL CAMHBT as the diluent.
   b. Transfer 100 µl of the *Streptococcus* spp. suspension into 11 mL CAMHBT+LHB.
   c. Vortex or invert the tube 8-10 times.
   d. Replace the tube cap with a Sensititre® single-use dosing head.
   e. Follow the instruction listed in section **B. Sensititre® AIM (Auto-inoculator)** to deliver 100 µL of inoculated CAMHBT+LHB to each well. Alternatively, a multichannel pipette may be used to deliver 100 µL of inoculated CAMHBT+LHB to each well.
5. *Staphylococcus* spp. for Vancomycin (GPN3F Panel)
   a. Follow instructions above to achieve standardized inoculum using 5 mL demineralized H_2O as the diluent.
   b. Transfer 30 µL of the suspension into an 11 mL tube of cation adjusted Mueller-Hinton broth with TES buffer.
   c. Vortex or invert the tube 8-10 times.
   d. Replace the tube cap with a Sensititre® single-use dosing head.
   e. Follow the instruction listed in section **B. Sensititre® AIM (Auto-inoculator)** to deliver 50 µL of inoculated MH to each well. Alternatively, a multichannel pipette may be used to deliver 50 µL of inoculated MH to each well.

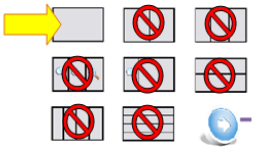
B. **Sensititre® AIM (Auto-inoculator)**
1. Label the side of the MIC panel with order and isolate number.
2. Place the appropriate MIC panel into the panel holder ensuring the panel is positioned the correct way (lettering upright and to the left side of the panel; barcode will face toward user)



3. Insert the tube containing the standardized inoculum of organism *with attached dosing head* into the dosing clamp.
4. Close the dosing clamp so the tube is secure. Do not overtighten.



5. Use the touchpad to select the correct dosing pattern:



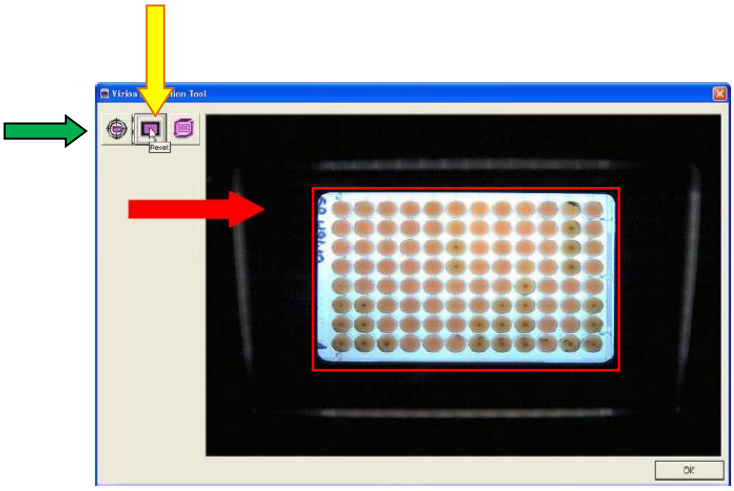
6. Once the correct dosing pattern is selected, the screen will display the chosen pattern and the dosing volume per well. The volume can be changed by touching the volume amount displayed in the center of the screen.

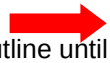
7. To begin inoculation, touch displayed green inoculation area.
8. When inoculation is complete, remove the test tube/dosing head combo from the AIM within 30 seconds of dosing a plate. Discard appropriately.
9. Inoculate a colony count/growth control plate using 1µL green looped dipped into the appropriate growth control well.
10. Cover all wells using adhesive seal provided with MIC panels. Avoid creases in the seal.
11. All non-fastidious aerobic organisms should be incubated at 34-36°C in a non-CO_2 incubator for 18-24 hours.
12. Up to 3 plates can be stacked.

C. **Sensititre® Vizion® (**It is never a requirement to remove the plate seal)

1. <u>Calibration</u>

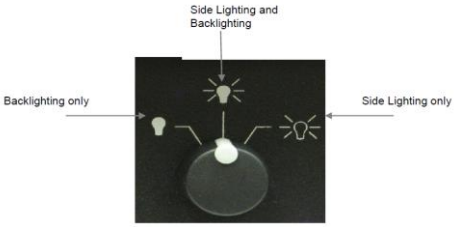
a. Open SWIN software on the computer.
b. Place an inoculated and incubated MIC tray in the Vizion®.
c. Go to the **Tools Menu** and select **Utilities**.
d. Select **Vizion® Calibration Tool**



e. Click the middle reset button indicated by  to reset the displayed image.
f. Position the mouse pointer over the location indicated by 
g. Click and hold the left mouse button and drag the **RED** outline until it includes all of the wells.

h. Press the Calibrate button indicated by the  to crop the image
i. If the image is satisfactory, close the window.
j. Once the Vizion® is setup and calibrated, and the purple light on the front of the instrument is illuminated, it is ready for use.

2. Light Settings



a. There are no required light settings when reading Sensititre® MIC Panels.
b. The intensity of lighting can be adjusted using the dials on the front of the instrument.
c. Suggestions: use light background for non-fastidious organisms; dark background for light growers and fastidious organisms; YeastOne use both back and side light.

3. Reading of MIC values

a. Check the control wells first.
b. Visible growth in the positive control wells is indicated by selecting the  Resistant Button.
c. No growth in the negative control well (if present on panel) is indicated by selecting the  Accept Controls Button.
d. If the controls do not perform as expected, the test is not valid.
e. For each antibiotic, select the MIC by clicking on the first well with no visible growth. (Most organism/antimicrobic combinations give distinct end points. With some combinations there may be a gradual fading of growth over 2 to 3 wells. The end points should be taken as the first well that inhibits visible growth, except sulphonamides when the MIC must be read as an 80-90% decrease in growth compared to the control well.)
f. Reading faint growth on the Vizion® can be improved by adjusting the lighting.
g. For drugs that are fully resistant, click 
h. You can restart reading the plate by clicking the Clear Grid Button 
i. Once the results have been correctly entered, the results can be accepted by clicking the Accept Read Results Button 
j. To transfer results, select the green check mark on the next screen.
k. Refer to User manual for additional options and instructions.
l. Alternately, a mirror viewer can be used to interpret the results of the plate.
m. Refer to **ANTIBIOTIC BATTERIES** Procedure and LIS Procedures for instructions on reporting susceptibility results

IX. INTERPRETATION

A. For interpretation of results, refer to the CLSI MIC Interpretive guidelines.

X. LIMITATIONS AND NOTES

A. All Sensititre® plates and strips include positive control wells. Tests are invalid unless there is distinct growth in all positive control wells. Some plate formats also include a “negative growth” well. This well is not required for manual reading.

B. A number of factors influence MICs including organism state, inoculum density, temperature, and broth.
C. Instrumentation should not be used if there is evidence of damage or excessive wear to electrical connections.
D. The drawer of the Vizion® must be completely closed to help ensure accurate results.
E. It is suggested that the entire plate be reviewed with a range of lighting options before reading begins. Certain isolates, such as *Acinetobacter spp*., may require more lighting adjustments.
F. Dirty tubes will affect the turbidity detected by the nephelometer.
G. Contamination may result in growth in a well bordered by wells showing no growth. Such a single well contamination can be ignored, but if multiple well contaminants are suspected, the test should be repeated.
H. Occasionally a “skip” may be seen - a well showing no growth bordered by wells showing growth. There are variety of explanations including contamination, mutation, creased seal and misaligned dosing. A single skip can be ignored. However, in order to ensure effective antimicrobic therapy NEVER read the skip well as the MIC; always read the lowest well concentration above which there is consistently no growth.
I. Poor growth of non-enterococcal strains of streptococci in Mueller-Hinton broth may give unreliable results with aminoglycosides.

XI. TECHNICAL SUPPORT

Refer to www.trekds.com/techinfo
(800) 642-7029

XII. REFERENCES

A. Trek Diagnostics Document: MIC susceptibility plates and JustOne® strips for testing Haemophilus influenzae and Streptococcus species
B. Trek Diagnostics Document: MIC susceptibility plates and JustOne® strips for testing Gram negative and Gram positive non-fastidious isolates
C. www.trekds.com/techinfo
D. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, M7 Approved Standard. The Clinical and Laboratory Standards Institute.
E. Performance Standards for Antimicrobial Susceptibility Testing: Informational Supplement M100. The Clinical and Laboratory Standards Institute.
F. User Manuals for Sensititre® Nephelometer, AIM and Vizion®.

XIII. REVISIONS

A. 6/20/24 Updated densitometer calibration/instructions for use