MicroScan MICroSTREP *plus*® Panel Procedure

Purpose

This procedure provides directions for the MICroSTREP plus® Panel.

Policy Statements

This procedure applies to microbiologists who perform plate reading.

MICroSTREP plus® Type 1 panels are used to determine the *in vitro* antimicrobial susceptibility pattern of aerobic *Streptococcus species*. The MICroSTREP plus panel utilizes a broth dilution susceptibility method where various antimicrobial agents are diluted to concentrations within the range of clinical interest. Panels are rehydrated with Mueller-Hinton broth supplemented with 2-5% lysed horse blood. After an incubation period of 20-24 hours in non-CO_2 conditions, the (MIC) minimum inhibitory concentration is visually read based on turbidity. The MIC is recorded as the lowest concentration of antimicrobic agent showing complete growth inhibition.

Test Code

MICS

Materials

Reagents	Supplies	Equipment	Media
• 0.9% normal saline. • 25 mL Mueller-Hinton broth with 3% lysed horse blood, product number B1015-25. • Quality control organism: *Streptococcus pneumoniae*, ATCC 49619.	• MICroSTREP *plus*® panels Type 1, product number B1027-201. • MicroScan® inoculating trays, product number B1013-4. • MicroScan® cover trays, product number B1010-56B, or equivalent. • Sterile, cotton-tipped applicators. Warehouse product number 112. • MICroSTREP *plus*® panel Type 1 QC recording sheets, product number B1014-347. • MICroSTREP *plus*® panel Type 1 recording worksheets. Included in MICroSTREP *plus*® panel box. • Sterile, disposable plastic test tubes. Cardinal Health product number T1343-2.	• Microdilution viewer, product number B1010-6 or equivalent. • Type 1 RENOK® Rehydrator/Inoculator System, product number B1018-14. • 100 µL pipettor with disposable, sterile tips. • DensiChek Plus Turbidity meter • Vortex • 35° Celsius ambient air, non-CO_2 incubator	Chocolate Agar Sheep blood Agar CNA agar

Storage

- Store MICroSTREP *plus* panels at 2-25° C.
- Store Mueller-Hinton with 3% lysed horse blood at 2-8° C.
- Exposure to storage conditions other than those recommended may result in loss of potency of the antimicrobial agents. Do not use beyond the expiration the date.

Sample

Morphologically similar, well-isolated *Streptococcus* colonies from chocolate agar, sheep blood agar or CNA agar that has been incubated 16-20 hours should be used.

Special Safety Precautions

Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies:

- Biohazard Containment
- Safety in the Microbiology Laboratory
- Biohazardous Spills

Quality Control

1. Perform Quality Control using *S. pneumoniae* ATCC 49619:
    - New lot and/or shipment prior to being put into use. Record results in "MicroScan® QC notebook.
    - Monthly on the first Monday of the month. Record review of results: tech, date, "Pass/Fail" on MicroScan QC Review Log.
2. Renok fill volume is gravimetrically checked monthly. See MC 6.40 for instructions.
3. If there is a QC failure, document observation, notify Technical Specialist and notify Beckman Coulter technical service at www.beckmancoulter.com. Do not report patient results until the problem is resolved.
4. Document corrective action/problem resolution on MicroScan QC Review Log.

Procedure

A. Broth Preparation
    1. Allow the Mueller-Hinton Broth with 3% Lysed Horse Blood to come to room temperature before use.
    2. Prior to inoculating panels, ensure the broth has not been comprised by improper storage or handling by examining for the following properties.
        a. Broth should be clear and red to dark red in color.
        b. The broth may become slightly brown in color as it gets closer to the expiration date by should remain clear. The brownish color will not affect panel results/
        c. Do not use any broth that is turbid and discolored.

B. Panel Preparation
    1. Remove the panels to be used from storage. Do not use if the integrity of the packaging is compromised.
    2. Cut open the pouch and remove the panel. All opened panels should be used within the same day or discarded.
    3. Label the panel with the patient's accession number and the date of set up.

C. Inoculum Preparation
    1. Using a sterile applicator swab, touch the surface of 4-10 well isolated, morphologically similar *Streptococcus* colonies growing on a 16-20 hour old non-inhibitory agar plate media.
    2. Emulsify the selected colonies 1.8 ml of sterile, normal saline. Final turbidity should be equivalent to a 0.5 McFarland turbidity standard.
    3. Vortex for 2-3 seconds.
    4. Pipette 100ul of the standardized suspension into 25 milliliters of room temperature Mueller-Hinton broth containing lysed horse blood.
    5. Cap tightly, and invert 8-12 times to thoroughly mix the inoculum suspension, avoid bubbles.
    6. Inoculate the MICroSTREP™ plus panel within 15 minutes of adding inoculum to broth.

D. Panel Rehydration and Inoculation
    1. To ensure organism viability and purity, a portion of the inoculum should be subcultured to a sheep blood agar plate and incubated overnight.
    2. Remove transfer lid from inoculator set. Pour the inoculated Mueller-Hinton broth into the seed tray.
    3. Replace the transfer lid and gently tap lid at all four corners to remove any air bubbles.
    4. Allow the transfer lid to equilibrate for a minimum of 20 seconds in the seed tray.
    5. Attach the RENOK® Rehydrator/Inoculator to transfer lid and draw up inoculum.
    6. A priming step is recommended to minimize the possibility of uneven well filling. To perform this step, draw up the inoculum as above. While transfer lid is still seated on the seed tray, press the center release button to dispense the inoculum back into the seed tray. Then, draw up the inoculum a second time into the transfer lid.
    7. Place the RENOK® Rehydrator/Inoculator transfer lid apparatus on the MICroSTREP™ plus panel and release the inoculum into the panel.
    8. Visually inspect the volume of inoculated wells to confirm proper filling and the absence of air bubbles.

E. Incubation
1. Place a clean cover tray on top of each group of inoculated panels to prevent evaporation. Cover trays may be reused after cleaning with soap and water and allowing them to air-dry.
2. Stack the inoculated panels in groups of 3-5.
3. Incubate the Panels for 20-24 hours at 35° Celsius in a non-CO_2 incubator.

Interpretation/ Results

Panels are read manually using the MicroScan® Microdilution Viewer and results recorded on a Manual Panel Worksheet.

A. Reading the panels
1. Following 20-24 hours incubation, remove the panels from the incubator.
2. Wipe the bottom of the panel with a lint-free tissue to remove any condensation or debris that may be present.
3. Read the panel only if the control well is turbid and purity plate shows a single colony morphology. Growth appears as turbidity, a haze throughout the well, a growth button in the center of well, or a fine granular growth throughout the well. Inadequate growth appears as a slight haze or clear broth in the wells.
4. Record results on appropriate worksheet.

B. Recording MIC's
1. Record MIC results as the lowest antimicrobial concentration showing inhibition of growth.
2. When growth occurs in all concentrations of a given antimicrobic, the MIC is reported as greater than (>) the highest antimicrobic concentration.
3. When no growth occurs in any of the antimicrobial concentrations, the MIC is reported as less than or equal to (≤) the lowest antimicrobic concentration.
4. A clear well in a series of growth wells is called a skipped well and should be ignored.
5. Spot growth in isolated wells indicates contamination. Test results are invalid, and the test should be repeated.

Method Performance Specifications

1. *Streptococcus pneumoniae* isolates that are have a Penicillin MIC >0.5 should be sent to the U of M for Amoxicillin testing (except CSF isolates).

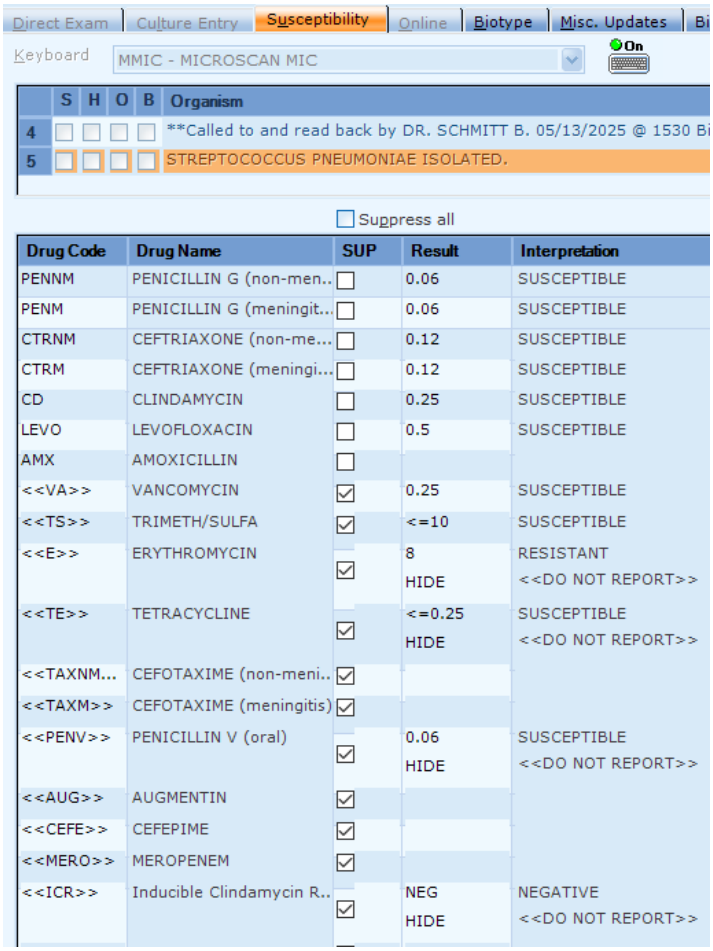
Limitations

1. This test is for *in vitro* diagnostic use only.
2. The MICroSTREP™ plus panel is only intended for testing streptococcal isolates including *S. pneumoniae.*
3. Fastidious organisms may require additional supplements and may not grow on the MICroSTREP™ plus panel.
4. The MICroSTREP™ plus panel should not be incubated in a CO_2 incubator.
5. Results obtained with mixed cultures are invalid.
6. Performance has only been established when using MicroScan® Mueller-Hinton broth supplemented with lysed horse blood. The use of any other broth media may cause misleading results.
7. Extending the time between inoculation preparation and panel inoculation beyond 15 minutes may adversely affect organism viability and susceptibility results.
8. The ability of the MICroSTREP™ plus panel to detect resistance to ampicillin, gatifloxacin, and levofloxacin among streptococcal isolates is unknown due to the lack of sufficient resistant strains at the time of comparative testing.

Result Reporting

1. Streptococci susceptibility interpretations are based on CLSI criteria.
2. Results are entered into the Sunquest system at the susceptibility tab in MRE.

3. Only penicillin, ceftriaxone, meropenem, and vancomycin should be routinely reported for CSF isolates of *S. pneumoniae*.
4. The breakpoint ranges for *S. pneumoniae* when tested against cefotaxime and ceftriaxone are different for meningitis and non-meningitis isolates, and interpretations will be changed by Sunquest when appropriate.
5. Enter results in Sunquest GUI MRE as follows:
    a) SUSC. KEYBOARD: using the dropdown arrow, chose **MMIC**
    b) ORGANISM NO.: highlight appropriate number
    c) Enter corresponding MIC value from worksheet or just return
        - The computer will continue to prompt, either enter MIC value or confirm no entry.
        - Sunquest assigns interpretations.
        - Click Summary to check entries.
        - Then click File and report.



References

1. Beckman Coulter, Inc. MicroScan® MICroSTREP™ plus Panel Procedural and QC manual. Revised August 2023, Brea, CA. ww.beckmancoulter.com

2. Clinical and Laboratory Standards Institute. (2025). Performance standards for antimicrobial susceptibility testing: Twenty-third informational supplement. M100-S35. Wayne, PA.

Training Plan	Initial Competency Assessment

Training Plan/ Competency Assessment

A. Employee must read the procedure B. Employee will observe trainer performing the procedure. C. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.	A. Direct observation

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1	Kristin Renner	11/20/2003	Initial Version
1.1	Kristin Renner	09/01/2005	Added Sunquest GUI resulting information.
2	Jessica Craig	04/09/2015	Transferred to new format.
3	Susan DeMeyere	4/12/2018	Biennial Review, changed logo
4	Susan DeMeyere	6/2/2025	Changed to monthly QC from weekly