

MICROSCAN® GRAM NEGATIVE MIC/COMBO PANEL PROCEDURE

Purpose

This procedure provides directions for the performance of MicroScan Gram Negative MIC/Combo Panel.

Policy Statements

This procedure applies to microbiologists who perform plate reading.

The antimicrobial susceptibility tests are miniaturizations of the broth dilution susceptibility test which have been dehydrated. Various antimicrobial agents are diluted to concentrations bridging the range of clinical interest. After inoculation and rehydration with a standardized suspension of organism and incubation at 35°C, the minimum inhibitory concentration (MIC) or a qualitative susceptibility (Susceptible, Intermediate, or Resistant) for the test organism is determined by observing the lowest antimicrobial concentration showing inhibition of growth.

Modified conventional and chromogenic tests are used for the identification of fermentative and non-fermentative gram negative bacilli. Identification is based on detection of pH changes, substrate utilization, and growth in the presence of antimicrobial agents after 16-42 hours incubation at 35°C.

Workup Code

MICS or MID

Materials

Reagents	Supplies	Equipment	Media
<ul style="list-style-type: none"> • 0.45% Sterile Saline • Inoculum Water with PLURONIC®, 25 ml (B1015-7) • Kovac's indole (IND). Dade Behring MicroScan® product number B1010-41A. • 40% potassium hydroxide (VP1). Dade Behring MicroScan® product number B1010-43A. • α-naphthol (VPA), Becton, Dickinson and Company product number 261192. • 0.8% sulfanilic acid (NIT1). Dade Behring MicroScan® product number B1010-44A. • 0.5% N, N-dimethyl-alpha-naphthylamine (NIT2). Dade Behring MicroScan® product number B1010-45A. • 10% ferric chloride (TDA). Dade Behring MicroScan® product number B1010-48A. • Mineral Oil 30 mL (B1010-40) • Oxidase reagent 	<ul style="list-style-type: none"> • MicroScan® NUC101 Gram Negative Combo Panel Beckman Coulter, Cat. No. C89643 • MicroScan® inoculating trays, product number B1013-4. • Cover Trays • 12 X 75 Sterile FALCON® Tubes • Sterile Cotton-tipped applicators • Manual Report Forms, MIC • Quality Control Report Forms • Quality Control Organisms (Refer to QC section) • Seal Strips (B1010-51) 	<ul style="list-style-type: none"> • 100 µl pipette with disposable sterile tips • DensiChek Turbidity Meter • Vortex Mixer • RENOK® Rehydrator/Inoculator (B1018-14) • Microdilution Viewer (B1010-6) • 35° C Ambient Air Incubator 	<ul style="list-style-type: none"> Sheep Blood Agar CNA agar MacConkey Agar

Storage

- Store MicroScan® Dried panels at 2-25° C.
- Store Inoculum Water with PLURONIC® at 15-30° 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

- Rapidly growing gram-negative bacilli
- Need 4-5 large or 5-10 small morphologically similar, well isolated colonies from a non-inhibitory agar plate that has been incubated for 18-24 hours.

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. STERILITY- a sterility control well is provided on each panel. If this well grows, the test has been contaminated and must be repeated.
2. GROWTH – A growth well is provided on each panel to demonstrate how well the organism will grow in the test medium. If there is no growth in this well, the test must be repeated.
3. ANTIBIOTIC POTENCY – The acceptability of the antimicrobial agents should be checked by testing organisms with known MIC ranges. The recommended American Type Culture Collection (ATCC) control organisms are:
 - *E. coli* ATCC 25922
 - *P. aeruginosa* ATCC 27853
 - *K. pneumoniae* ATCC 700603
 - *E. coli* ATCC 35218

Deterioration of an antimicrobial would result in endpoints at higher concentrations than the acceptable values. Variation of more than one well from the accepted values for any antimicrobial indicates that the antimicrobial is out of control and should not be reported.

4. Perform MIC and ID QC with each new lot or shipment before put into service.
5. Perform MIC QC monthly on the first Monday of each month.
6. Document QC set-up on MicroScan QC Review Log. Record results in MicroScan® QC notebook. Record review of results: tech, date, "Pass/Fail" on MicroScan QC Review Log.
7. If there is a QC failure, do not report patient results until the problem is resolved.
8. Investigate potentially affected patient results performed since the last successful QC event.
9. If there is a QC failure, document observation, notify Technical Specialist or designee and call MicroScan® technical service if necessary at 1- (800) 854-3633.
10. Document corrective action/problem resolution on MicroScan QC Review Log.
11. Each month QC data will be reviewed and assessed by the Micro Technical Specialist or designee. Persons assessing the results will initial the log for monthly review and notify Lab Director of any ongoing of critical issues.

12. Perform a RENOK Rehydrator/Inoculator Dispenser Volume check monthly and record on the Desk 3 Maintenance board.

RENOK Rehydrator/Inoculator Dispenser Volume Check:

1. Obtain the gravimetric scale from Send-outs.
2. Equipment: seed trough, a cover tray, and a tube of pluronic water.
3. Turn on the scale, place the cover tray on the scale, and zero the scale.
4. Inoculate the seed trough with the tube of Pluronic water.
5. Use the RENOK to obtain inoculum just as would normally be done to inoculate with an isolate except this will be un-inoculated Pluronic water.
6. Dispense into the cover tray (not while on the scale) and record the weight.
7. Weight should be between 10.1 and 12.0 grams.
8. Record on the Desk 3 Maintenance Checklist.
9. If the fill volume is not within the desired range, try repeating the above steps if an obvious error can be determined. If an obvious error is not detected try replacing the rubber seal.

Procedure

A. 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.

B. Inoculum Preparation

1. Using a sterile cotton-tipped applicator, touch the surface of 4-5 large or 5-10 small morphologically similar, well-isolated colonies from an 18-24 hour non-inhibitory agar plate.
2. Emulsify in 1.8 ml of 0.45% sterile saline. The final turbidity should be equivalent to a 0.5 McFarland standard.
3. Vortex the suspension for 2-3 seconds.
4. Pipette 100ul of the standardized suspension into 25 mL of Inoculum Water with PLURONIC®. Cap tightly. Invert 8-10 times to mix.

C. Panel Rehydration and Inoculation

- The RENOK® rehydrator/inoculator is a manual pipettor that simultaneously rehydrates and inoculates MicroScan® panels. It is used in conjunction with disposable MicroScan® inoculator sets.
- An inoculator set consists of a transfer lid (to hold and dispense the inoculum) and a seed trough (to contain the inoculum).
 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. Pour the prepared inoculum into a seed trough. Rock the trough gently to distribute the inoculum evenly throughout the trough.
 3. Replace the transfer lid over the seed trough.
 4. Gently tap the transfer lid in all four corners to ensure absence of bubbles. Allow the lid to equilibrate for a minimum of 20 seconds in the seed trough.
 5. Pick up the RENOK® unit from its stand and place unit on top of the transfer lid.

6. Fully lift the center lever of the unit to draw inoculum into the transfer lid, then release hand pressure. The mechanical catch will hold the center lever in place.
7. Depress the center release button of the unit to return the inoculum water to the seed tray.
8. Repeat step 5. This “priming” of the inoculator tray reduces bubble formation in the panels.
9. Pick up the unit with the transfer lid attached, holding it level, position the lid onto a MicroScan® panel.
10. Depress the center release button of the unit to inoculate panel. There should be a smooth, even release of the inoculum without hesitation or sticking.
11. Return the lid to the seed trough. Release lid by depressing the pick-up lever.
12. Place the RENOK® unit back on its stand.
13. **Additional steps for use of the panel for Identification.**
 - Perform Oxidase test. Refer to [MC 3.19 Oxidase DrySlide Test](#) for instructions.
 - Using a dropper bottle of mineral oil, overlay the GLU, URE, H₂S, LYS, ARG, ORN and DCB with 3 drops of mineral oil (these wells are underlined on the panel). The media must be completely covered but the oil should not overflow the wells.
 - For oxidase positive organisms, place a seal strip over the CIT, MAL, ONPG, TAR, ACE, CET, OF/G, OF/B and DCB wells.
14. The panel is then ready for incubation.

D. Incubation

1. To ensure even thermal distribution during incubation, stack the panels in groups of 3-5.
2. Place a clean Cover Tray on top of each group of panels to prevent evaporation. Cover Trays may be reused. Do not decontaminate the Cover Trays with alcohol. They may be cleaned with soap and water. Rinse well and allow to air dry.
3. Incubate the panels for 16-20 hours at 35°C in a non-CO₂ incubator.

Interpretation/ Results

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

- A. Following 16-20 hours incubation, remove the panels from the incubator.
- B. Check purity plate for contamination or mixed populations. Do not continue if mixed or contaminated.
- C. Wipe off the bottom of the panel with a lint-free tissue to remove any condensation or debris that may be present.
- D. Read the panel only if the control well is clear and the growth well is turbid. Growth in the wells appears as turbidity, which may take the form of a white haze throughout the well, a white button in the center of the well, or a fine granular growth throughout the well. Inadequate growth is defined as a slight whiteness in the well or the broth is clear.
- E. The results are recorded on the appropriate worksheet.
 - Worksheets are available in SharePoint > Lab > Microbiology > MicroScan Documents > NUC101 > MicroScan NUC 101 Result form and QC form.
 - Please print on the color printer as the interpretations are colored boxes.
 - Pre-printed worksheets are found in the drawer by Desk 2.
- F. Reading Antimicrobial Susceptibilities (MIC's)
 1. Read all MIC's against a black (indirectly lighted) background.
 2. Read the tray from lowest concentration to the highest concentration and record the MIC as the first well showing no growth.
 3. When growth occurs in all concentrations, the MIC is recorded as greater than (>) the highest concentration.

4. When no growth occurs in any of the concentrations, the MIC is recorded as less than or equal to (\leq) the lowest concentration.
5. A clear well in a series of growth wells is called a skipped well and should be ignored.
6. Spot growth in isolated wells indicates contamination. The test should be repeated.
7. Clinical isolates of *Klebsiella oxytoca*, *Klebsiella pneumoniae* and *Escherichia coli* with increased MICs (≥ 2 ug/ml) of ceftazidime and ceftriaxone should be suspected of harboring an extended-spectrum beta-lactamase.
8. Clinical isolate of *Proteus mirabilis* with increased MIC's of ceftazidime should be suspected of harboring an extended-spectrum beta-lactamase.
9. A clinical isolate is considered positive by the ESBL confirmation test if there is a ≥ 3 two-fold dilution drop (i.e. a 3 well decrease) in an MIC value for the antibiotic tested with clavulanic acid as compared to the MIC value of that antibiotic tested alone. A positive result (a ≥ 3 two-fold dilution drop in MIC) with either antibiotic combination is considered ESBL phenotypic-confirmation positive.
 - Example: Confirmation positive CAZ=8 CAZCLA \leq 0.25/4. CFT=16 CFTCLA=4/4
 - Example: Confirmation negative CAZ=4 CAZCLA=2/4. CFT=16 CFTCLA=4/4
 - ESBL Dilution Sequences that are unable to interpret: CAZ>16 CAZCLA >2/4. CTF>32 CFTCLA >4/4.

G. Reading Identification Substrates

1. Read all the identification substrates with a white background except for the CET and the antimicrobials used for identification (Cl₄, Cf₈, P₄, K₄, Fd₆₄, To₄) which should be read against a black (indirect lighted) background.
2. The following substrates are always read at 16-24 hours: GLU, SUC, SOR, RAF, RHA, ARA, INO, ADO, MEL, P₄, K₄, Cl₄, Fd₆₄, Cf₈, To₄.
3. Re-incubate the panel for an additional 24 hours before adding reagents if the following conditions are not met:
 - GLU or SUC or SUC positive or
 - ARG positive and OF/G positive or
 - ARG positive and CET positive or
 - LYS positive or
 - ORN positive or
 - OXI negative and OF/G positive and Fd₆₄ positive and MAL positive.
4. Add reagents and read the remaining substrates at 16-24 hours of incubation if any of the above listed conditions are true.
 - Add 1 drop of 40% Potassium Hydroxide (KOH) and 1 drop of Alpha Naphthol to the VP well. Wait at least 20 minutes for the VP reaction to develop.
 - Add 1 drop of 10% Ferric Chloride to the TDA well. The color will develop immediately.
 - Add 3 of Kovac's reagent to the IND well. The color will develop immediately.
 - Add 1 drop of 0.8% Sufanilic Acid then 1 drop of 0.5% N, N-dimethyl-alpha-naphthylamine to the NIT well. Wait at least 5 minutes prior to reading for the NIT reaction to develop.
 - Refer to the chart in the product inset for interpretations to the reactions.
5. Circle positive reactions on the table at the bottom of the Neg Urine Combo Panel Worksheet.
6. Determine a Biotype number by adding up the score for each column and putting the calculated in number in the boxes above the table.
7. Use the Biotype number in the Biotype Lookup Program on the Beckman Coulter website to determine the identification of unknown test organisms. The program lists the organism identification and the relative probabilities, in the order of the highest probability. [Beckman Coulter Inc. - Biotype Lookup](#)

Limitations

- A. *Haemophilus* and other fastidious gram-negative bacteria will not grow in Mueller-Hinton broth that is not supplemented with growth enrichment.
- B. This procedure is suitable only for rapid growing aerobic organisms.
- C. **Nitrofurantoin will not be reported from the panel as the antibiotic did not pass verification testing.**
- D. The following antibiotic/organism combinations are product limitations and results will not be reported:
 - *Acinetobacter* spp. and Piperacillin-Tazobactam
 - *B. cepacia* complex and Ceftazidime
 - *B. pseudomallei* and Ceftazidime
 - *M. morganii* and Ceftazidime
 - *Providencia* spp. and Ceftazidime
- E. Additional tests may be necessary to determine the final identification when a low probability (<85%) is obtained.
- F. Biotype numbers should not be used for phenotype identification of strains isolated from various specimens from the same patient.
- G. Elevated MIC's with beta-lactam antimicrobials may be observed if panels are over-inoculated. Inoculum concentration is critical with these antimicrobials as their mechanism of action involves disruption of bacterial cell wall synthesis.
- H. Isolates of *Providencia stuartii* that provide a MIC's of 4 and 8 µg/mL with ceftazidime/avibactam should be retested using an alternate method.
- I. Isolates of *P. aeruginosa* that provide a MIC's of 8 µg/mL with ceftazidime/avibactam should be retested using an alternate method.
- J. The ability of the MicroScan Gram Negative Panels to detect resistance to Ceftazidime avibactam is unknown with *C. freundii* complex, *C. koseri*, *M. morganii*, and *P. mirabilis* because resistant strains were not available. If such isolated are observed, test on an alternate method.
- K. The ability of the MicroScan Gram Negative Panels to detect resistance to Meropenem vaborbactam is unknown with *C. koseri*, *K. aerogenes*, *K. oxytoca*, *M. morganii*, *P. mirabilis*, *Providencia* species and *S. marcescens* because resistant strains were not available. If such isolated are observed, test on an alternate method.
- L. The ability of the MicroScan Gram Negative Panels to detect resistance to Ciprofloxacin is unknown with *C. koseri* and *P. vulgaris* because resistant strains were not available. If such isolated are observed, test on an alternate method.
- M. The ability of the MicroScan Gram Negative Panels to detect resistance to Levofloxacin is unknown with *C. koseri*, *P. vulgaris* and *P. agglomerans* because resistant strains were not available. If such isolated are observed, test on an alternate method.
- N. The ability of the MicroScan Gram Negative Panels to detect resistance to Meropenem is unknown with *C. koseri* and *P. vulgaris* because resistant strains were not available. If such isolated are observed, test on an alternate method.
- O. The ability of the MicroScan Gram Negative Panels to detect resistance to Ceftazidime is unknown with *P. vulgaris* and *Serratia* spp. because resistant strains were not available. If such isolated are observed, test on an alternate method.

Method Performance Specifications

- A. A "trailing effect" may be observed in some drug/organism combinations such as *Proteus* with ceftolozane-tazobactam (C/T), cefuroxime (Crn) and Imipenem (Imp), *Serratia* with piperacillin-tazobactam (P/P) and piperacillin (Pi). Trailing may also be observed with Trimethoprim/ Sulfa (T/S), Trimethoprim (T), and Sulfamethoxazole (Sx) with the use of the RENOK® system due to the inoculum concentration. The endpoint should be read as the lowest concentration which when compared to the growth well shows:
 1. Approximately 80% reduction of growth (T/S, T, Sx) compare to the growth well.
 2. A white button which is less than 2mm in diameter, or
 3. A white button which is semi-translucent

**Susceptibility
Reporting
Guidelines for
ESBL Positive
isolates**

- The MicroScan NUC101 panel can be used to test or confirm ESBL.
 - If using to confirm Vitek ESBL results, enter result in Workups and the VITMIC keyboard.
 - Enter the ESBL results on the MMIC keyboard if reporting the susceptibilities from the MicroScan.
 - For *Proteus mirabilis*, result the ESBL result on the MMIC keyboard.

Organism #5 - (C)ESCHERICHIA COLI ISOLATED.		
- MMIC -		
SS	PIPT(8),CP(0.25),GM(2),TS(40),ETP(0.5)	
POS	ESBLP(POS)	
R	AM(32),CFZ(32),AS(32),CAX(32),CAZ(32)	
HIDE	TO(1-SS),MERO(1-SS),CEFE(32-R),CZA(8-SS),MVM(4-SS),AZTR(R)	

- Change the following antibiotics to 'R' if not already resistant.

Penicillin's	Cephalosporin's
Ampicillin	Cefazolin
	Cefepime
	Cefotaxime
Monobactams	Ceftazidime
Aztreonam	Ceftriaxone
	Cefpodoxime

- Beta-Lactamase inhibitor combinations and Cephamycins are not affected by ESBL; do not edit the susceptibility results.

β-lactamase inhibitor combinations	Cephamycins
Amoxicillin-clavulanic acid	Cefoxitin
Ampicillin-sulbactam (Unasyn)	Cefotetan
Piperacillin/tazobactam (Zosyn)	
Ticarcillin/clavulanic acid (Timentin)	

**Result
Reporting into
Sunquest**

- Use criteria specified by CLSI to interpret the MIC's.
- Record results and workups in Sunquest GUI MRE using the susceptibility tab.
- To enter MIC results:
 - SUSC. KEYBOARD: using the dropdown arrow, chose **MMIC**
 - ORGANISM NO.: highlight appropriate number
 - 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.

Keyboard MMIC - MICROSCAN MIC On

S H O B Organism

4 ☐ ☐ ☐ ☐ **Called to and read back by DR. SCHMITT B. 05/13/2025 @ 1530 Biof

5 ☐ ☐ ☐ ☐ **ESCHERICHIA COLI ISOLATED.**

☐ Suppress all

Drug Code	Drug Name	SUP	Result	Interpretation
AM	AMPICILLIN	<input type="checkbox"/>	4	SUSCEPTIBLE
CFZ	CEFAZOLIN	<input type="checkbox"/>	2	SUSCEPTIBLE
PIPT	PIPERACILLIN/TAZOBA...	<input type="checkbox"/>	2	SUSCEPTIBLE
CP	CIPROFLOXACIN	<input type="checkbox"/>	0.25	SUSCEPTIBLE
GM	GENTAMICIN	<input type="checkbox"/>	2	SUSCEPTIBLE
TS	TRIMETH/SULFA	<input type="checkbox"/>	40	SUSCEPTIBLE
ESBLP	ESBL	<input type="checkbox"/>	NEG	NEGATIVE
<<TO>>	TOBRAMYCIN	<input checked="" type="checkbox"/>	1	SUSCEPTIBLE
			HIDE	<<DO NOT REPORT>>
<<AS>>	AMP/SULBACTAM	<input checked="" type="checkbox"/>	4	SUSCEPTIBLE
			HIDE	<<DO NOT REPORT>>
<<CAX>>	CEFTRIAXONE	<input checked="" type="checkbox"/>	0.5	SUSCEPTIBLE
			HIDE	<<DO NOT REPORT>>
<<CAZ>>	CEFTAZIDIME	<input checked="" type="checkbox"/>	0.5	SUSCEPTIBLE
			HIDE	<<DO NOT REPORT>>
<<ETP>>	ERTAPENEM	<input checked="" type="checkbox"/>	0.5	SUSCEPTIBLE
			HIDE	<<DO NOT REPORT>>
<<MERO>>	MEROPENEM	<input checked="" type="checkbox"/>	1	SUSCEPTIBLE
			HIDE	<<DO NOT REPORT>>
<<AZTR>>	AZTREONAM	<input checked="" type="checkbox"/>	1	SUSCEPTIBLE
			HIDE	<<DO NOT REPORT>>
<<CEFE>>	CEFEPIME	<input checked="" type="checkbox"/>	1	SUSCEPTIBLE
			HIDE	<<DO NOT REPORT>>
<<CZA>>	CEFTAZIDIME AVIBACTAM	<input checked="" type="checkbox"/>	1	SUSCEPTIBLE
			HIDE	<<DO NOT REPORT>>

A. For workups:

Enter workup #, media, description, and ID. Work-up code is MID (ID and MIC) or MICS (MIC only)

W1 MAC NLF GNR

Wkp: MICS: MIC susceptibility

MID: MIC/ID

B. If additional information is available after the exam has been finalized, remove the final status and send out a supplementary report using the code SRPT in SREQ or CULTURE RESULTS. Re-final the culture when testing is complete.

C. If an exam requires a correction, the code CORR (corrected report) must be used in CULTURE RESULTS. Refer to the procedure [MCVI 5.0 Micro Specimen Ordering and Receiving](#) or [MCVI 5.1 Mislabeled & Unlabeled Specimens](#).

**Result
Reporting for
ESBL Positive
isolates**

1. When reporting, append the Sunquest code **ESBL** onto the organism code, i.e., **KLPN-ESBL**.
2. Also include the code **DRO** on a separate line.
3. Call the patient's caregiver with the result and document in the patient's report
4. Report ESBL positive organisms resistant to all Penicillin's, Cephalosporin's and Aztreonam.

References

1. Beckman Coulter Diagnostics. 250 South Kraemer Boulevard. Brea, CA 92821-6232 USA, MicroScan® Dried Gram Negative (8/2022).
2. Clinical and Laboratory Standards Institute (CLSI) M100 Performance Standards for Antimicrobial Susceptibility Testing, 35th edition, 2025.

Training Plan/ Competency Assessment

Training Plan	Initial 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	Effective Date:	Written/Revised by:
1	08/02/2004	Peggy Winston (formatted to PC version)
1.2	12/18/2006	Peggy Winston (added information regarding extraintestinal Salmonella)
1.3	06/25/2009	Brian Howell (PROCEDURE > B. Inoculum Preparation > 2. & 3. Have been removed, as these are no longer supported by the manufacturer).
2	04/29/2015	Jessica Craig / Becky Carlson (reformatted for CMS load. Renumbered from MC 1102)
3	9/24/2015	Becky Carlson- Added MicroScan QC Review Log for reviewing QC results
4	1/1/2017	Becky Carlson- update for new panel types NC 68 and PC29
5	5/23/2017	Susan DeMeyere-Removed naladixic acid testing for <i>Salmonella</i>
6	4/12/2018	Susan DeMeyere- biennial review
7	1/22/2024	Susan DeMeyere-updated for new panel NUC101
8	6/2/2025	Changed to monthly quality control from weekly