CRO Breakpoint Implementation Toolkit

BREAKPOINT IMPLEMENTATION INSTRUCTIONS

Verification of Revised CLSI Carbapenem Breakpoints for *Enterobacterales* When Using FDA-Cleared Commercial Antimicrobial Susceptibility Test (cAST) Systems

HOW TO USE THE BREAKPOINT IMPLEMENTATION TOOLKIT (BIT)

Follow the steps in these instructions to conduct the verification in your laboratory:

1. Utilize the provided list of organisms to perform the verification.
2. Utilize the worksheets to record your results for the verification.
3. Utilize the "Verification Template" to write up your verification. The template is a PDF with fillable fields, so it can be filled out digitally.

GOAL

The goal of this document is to provide a simple verification toolkit that encourages all laboratories that perform **commercial antibiotic susceptibility testing (cAST)**, to utilize the most current breakpoints for the carbapenem antibiotics when testing *Enterobacterales*.

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RATIONALE

The Clinical and Laboratory Standards Institute (CLSI) published revised breakpoints for *Enterobacterales* and carbapenems. Specifically, CLSI revised breakpoints for ertapenem, meropenem, and imipenem and added breakpoints for doripenem in June 2010 (M100-S20-U; Supplement), and then revised ertapenem once more in 2012 (see **Appendix A**). The most current breakpoints are found in the newest edition of the CLSI M100 document. The current breakpoints may differ from those used by **cAST systems.** Check with the manufacturer of your cAST system to assess if they have made revisions in their system to incorporate the most current breakpoints.

Carbapenem-resistant *Enterobacterales* is an urgent antibiotic resistant (AR) threat according the 2013 and 2019 CDC AR Threats Reports. Using the most current breakpoints is critical for ensuring appropriate treatment choices and detection of resistance for infection control.

PURPOSE

The purpose of the verification is to demonstrate that susceptible (S), intermediate (I), and resistant (R) category interpretations obtained from the **cAST system** using revised breakpoints are comparable to those obtained using standard reference methods. It is not necessary to verify the actual MIC values obtained from the **cAST system** since manufacturers had to demonstrate that these agreed with those of a standard reference method when they submitted the MIC test data for a specific drug for FDA clearance. This is further explained in number two of the step-by-step instructions that follow.

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NECESSARY COMPONENTS

To implement the breakpoint verification study you will need:

1. The *Enterobacterales* Carbapenem Breakpoint panel (found in the CDC & FDA AR Isolate Bank). The panel is made up of 31 isolates of Enterobacterales with resistance mechanisms confirmed by a variety of phenotypic and molecular methods. These include isolates that are carbapenem-resistant due to a carbapenemase gene, isolates that are carbapenem-resistant due to mechanisms other than carbapenemases, and isolates that are carbapenem susceptible.

2. Worksheet (**Appendix B1**) that includes:

   a. Listing of the S, I, or R AR Bank reference method result for each antimicrobial agent/organism combination generated for doripenem, ertapenem, imipenem and meropenem, and the resistance mechanism, if known is listed.

   b. Fields for recording observed AST results obtained from the **cAST system** and fields for documenting percentage agreement with reference AST results.

DEFINITIONS

CLSI Standard Reference AST Method

Broth or agar dilution MIC test as described in CLSI M07 or disk diffusion test as described in CLSI M02 standards.

Commercial AST System (cAST)

For the purpose of the BIT verification kit, this is the specific FDA-cleared **cAST system** on which the laboratory will verify the revised CLSI breakpoints.

Categoric Agreement (CA)

Agreement of interpretive category results (S, I, R) between the **cAST system** under evaluation and a standard reference AST method.

% CA = (# of S, I, R results from **cAST system** that agree with S, I, R results from standard reference AST method / Total # of organisms tested) x 100

Very Major Error (VME)

The standard reference AST category result is R and the **cAST system** result is S.

% VME = (# **VME** / Total # resistant organisms by standard reference AST method) x 100.

Major Error (ME)

The standard reference AST category result is S and the **cAST system** result is R.

% ME = (# **ME** / Total # susceptible organisms by standard reference AST method) x 100.

Minor Error (mE)

The standard reference AST category result is R or S and the **cAST system** result is I; or the standard reference result is I and the **cAST system** result is R or S.

% mE = (# **mE** / Total # organism tested) x 100.

STEP-BY-STEP—PERFORMING THE VERIFICATION

Note: The step-by-step plan for this verification should be approved by the Laboratory Director before verification testing is initiated.

1. Determine which antimicrobial agents will be verified.

In order to implement the most current CLSI breakpoints for *Enterobacterales*, the lower concentrations encompassing these breakpoints must be available on the cAST panel. Changing panel type to accommodate the newest and more appropriate carbapenem antibiotic ranges should be considered, if necessary.

2. Understand your laboratory's current cAST system breakpoints.

a. Assess software status to determine if the manufacturer has made updates that align with CLSI breakpoints for the carbapenems or any other antibiotics you may be assessing. You will only need to verify those antibiotics where your **cAST system** does not align with CLSI breakpoints.

b. Determine if and how the **cAST system**'s software will be able to accommodate the revised breakpoints. This may require discussions with the manufacturer's technical staff. The manufacturer cannot provide written instructions or software, or perform the software manipulations since they are required to use only FDA breakpoints. However, in most cases the manufacturer will be able to provide general guidance to accommodate alternative breakpoints.

3. Determine the number of isolates that will be tested and acquire isolates.

The *Enterobacterales* Carbapenem Breakpoint panel contains 31 isolates. Thirty is the minimum number of isolates that should be considered for a cAST verification of this type. If acceptable accuracy rates are not attained, additional isolates may be tested. In order to achieve acceptable accuracy (≥90%), there can be no more than 3 errors (28/31 = 90.3%).

Quality Control Organisms: QC organism(s) should be run each day during the verification testing. Choose QC organisms per cAST system manufacture's guidelines. *Pseudomonas aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 are recommended by CLSI for routine QC of carbapenems when testing *Enterobacterales*; note that *E. coli* ATCC 25922 will have off scale endpoints in most cAST systems.

4. Determine if Reproducibility/Precision will be assessed.

This should be determined based on the original verification and the drug/organism combinations assessed at that time. Three options to consider:

a. No Reproducibility Assessment: If reproducibility studies were robust during the initial or subsequent verification(s) of the cAST (e.g. the panels were modified), including assessment of one or more carbapenems, then no reproducibility studies may be needed at this time.

b. Limited Reproducibility Assessment: If reproducibility studies were performed during the initial or subsequent verification, but no carbapenems were assessed or were minimal, then a limited reproducibility assessment is warranted. Most laboratories will find this option adequate for the purposes of this verification.

   i. Test 1-3 isolates 3 times (separate inoculum preparations) on one or more days.

   ii. The isolates may represent at least one appropriate QC organism, and can include isolates from the AR Bank Enterobacterales Carbapenem Breakpoint panel.

c. Comprehensive Reproducibility Assessment: If reproducibility was not evaluated (or if documentation is unavailable) during the initial verification (or subsequent verifications if panels were modified) for ertapenem, imipenem, meropenem and doripenem on the cAST system, or as a Laboratory Director requirement, a more comprehensive reproducibility should be performed.

   i. Test a minimum of 5 isolates (separate inoculum preparations) on one or more days.

   ii. The isolates may represent appropriate QC organisms, and can include isolates from the AR Bank Enterobacterales Carbapenem Breakpoint panel.

d. For the purposes of this verification of reproducibility, it is suggested that S, I, R be assessed even though there are no standard guidelines in CLSI M52 describing acceptable reproducibility of S, I, or R results (reproducibility is typically assessed by comparing MIC values where 95% must fall within +/- one two-fold dilution [essential agreement]).

e. Utilize reproducibility table. Prepare a table for each antibiotic being assessed.

Reproducibility of Interpretations (S,I, R).

Isolates	Day 1			Day 2			Day 3		
	1	2	3	1	2	3	1	2	3
Isolate 1									
Isolate 2									
Isolate 3									
Isolate 4									
Isolate 5									

5. Determine acceptance criteria for the verification of each antimicrobial agent.

This should take into consideration:

a. Acceptable **CA** (recommended ≥90%)

b. Acceptable **VME** (recommended none; <3 % of total resistant isolates)

c. Acceptable **ME** (recommended none or 1; <3% of total susceptible isolates)

d. Acceptable **mE**: there is no defined **mE** acceptable rate, however thoroughly investigate a **mE** rate that exceeds 10% (when 31 isolates are tested, no more than 3 **mEs** are allowed).

e. Note: When testing 31 isolates, only one **ME** and one **VME** can be accepted, therefore, additional isolates may need to be tested to show acceptable performance if the initial testing does not meet acceptance criteria.

f. Acceptable Reproducibility/Precision ≥95% of the isolates tested correlate to the reference S, I or R.
Note: If using *P. aeruginosa* ATCC 27853 for reproducibility studies, it would not be unlikely to obtain both S and I results for imipenem among repetitive tests since the acceptable QC range for this isolate is 1 µg/ml (S) to 4 µg/ml (R).

6. Determine how discrepancies will be resolved.

It is advised to retest isolates with **VME** and **ME**.

a. Expression of resistance mechanisms may vary following storage, freeze/thaw cycles, or other manipulations of bacteria. Although all of the 31 BIT AR Bank isolates were tested after two passages from frozen stocks, it is possible that certain resistance characteristics may have been lost. Therefore, if results obtained with a laboratory's commercial cAST system differ from those listed for any BIT AR Bank isolate; it may be useful to retest the isolate with a reference method (e.g. disk diffusion) to help resolve the discrepancy.

b. Optimal repeat testing is done in triplicate.

c. If there are discrepancies after repeat testing, a different method for AST should be used, optimally a CLSI reference MIC method, or disk diffusion (if available/applicable). Isolates may also be referred to another laboratory.

d. If **CA** is ≥90% and there is only one **ME** that persists on repeat testing, the current breakpoints canl be considered verified

e. If **CA** is <90%, >0 **VME** or >1 **ME** persists, it is up to the discretion of the laboratory director to determine a path forward

f. If **mE** rate exceeds 10% (when 31 isolates are tested, no more than 3 **mEs** are allowed):

   i. Determine if any of the **mEs** are within +/- one two-fold dilution of the breakpoint. These errors are considered acceptable providing not all errors are in the same direction (e.g. for meropenem, **cAST System** result = I and Reference [AR Bank] = S for all **mEs**).

   ii. If **mEs** are greater than +/- one two-fold dilution of the breakpoint or in the same direction, repeat once. If error persists, consult with laboratory director to determine path forward.

g. Record repeat results and re-analyze the data (See example table at right).

h. See CLSI M52 for additional suggestions for troubleshooting discrepant results.

Note that all results obtained (from initial and any repeat tests) should be recorded and a discussion of the possible source of errors should be documented in the verification write-up (See the verification template, page 5 under Discrepancy Resolution).

Table example for documentation of repeat testing.

Antibiotic: Meropenem		
Isolate #	Reference AST Result	Commercial AST System Result
Initial results		
Replicate 1	S	R
Replicate 2	S	S
Replicate 3	R	R
Repeat results		
Replicate 1	S	S
Replicate 2	S	S
Replicate 3	R	R
Comments		
Repeat testing resolved discrepancy.		

7. Test with cAST system.

Test the 31 isolates and QC organism(s) using the cAST system, following manufacturer's instructions.

8. Record MIC values.

Record the MIC values generated by the cAST system in the "Observed MIC" field of **Appendix B1**. Manually interpret these MICs as S, I, or R using the interpretive criteria listed in **Appendix A**. Disregard the interpretation automatically generated by the **cAST system**'s software.

Note: **Appendix B1** may be printed out to conveniently record data in the laboratory.

9. Calculate CA, VME, ME, and mE

CLSI Breakpoint Interpretation	Expected Interpretations		
	S	I	R
Susceptible	A	D	G
Intermediate	B	E	H
Resistant	C	F	I

CA (%) = # isolates with same SIR results (A+E+I) / 31 x 100

VMEs (%) = # isolates with false "S" results (G) / number of "R" isolates (G+H+I, Reference results) x 100

MEs (%) = # isolates with false "R" results (C) / number of "S" isolates tested (A+B+C, Reference results) x 100

mEs (%) = # of isolates with "I" result when Reference is "S" or "R" and **mEs** = # of isolates with "S" or "R" result when Reference is "I": (B+H+D+F)/ 31 x 100

10. Determine if results are acceptable.

Use the laboratory's predefined acceptance criteria described in step 5.

11. Resolve any discrepancies.

Follow predefined protocol for addressing discrepancies, described in step 6.

12. Verification write-up.

If verification is successful, complete the verification write-up using the Verification Template, attaching all results and worksheets generated from all testing performed.

13. Post-verication Steps

Additional Steps after verification has been signed by laboratory director include:

a. Follow your laboratory's standard protocols for implementing procedural changes in AST reporting, and modify the **cAST system**'s software to accommodate revised CLSI breakpoints.

b. Ensure that appropriate changes have been made to the LIS to accommodate new breakpoint interpretations.

c. Communicate changes to clinicians, especially infectious disease clinicians.

d. Ensure that new procedure is communicated to staff and staff are trained properly to implement new procedure.

e. Consider review of ASM's IQCP resources and update accordingly (if applicable).

ADDITIONAL NOTES

1. The BIT can be used to verify antibiotics other than the carbapenems, such as the cephalosporins.

   Recommendation: Save the data for other antibiotics so that data can be used for other breakpoint implementations

2. Extensive testing of the AR Bank isolates provided in this kit has not been performed with all FDA- cleared cAST systems. However, all isolates have been tested multiple times using in house frozen broth microdilution panels per CLSI guidelines and the modal MIC is provided.

3. Reference results for imipenem and AR-0029 (*Proteus mirabilis*) were not reproducible. These organism/antimicrobial agent combinations should not be evaluated.

4. Imipenem results for *Proteus/Providencia/Morganella* may be problematic as wild type strains of these genera typically have elevated imipenem MICs that approach the revised breakpoint. This should be considered when evaluating results for imipenem from AR-0026 (*Providencia stuartii*).

5. Several organism/antimicrobial agent combinations resulted in a consensus result of S and I or I and R by reference methods. This occurred mostly with cefazolin and wild type strains. A result other than that listed for the reference result will be considered a minor error (e.g., you answer R if the reference result is S, I).

6. MIC results for each antimicrobial agent for an isolate may commonly be ± 1 log2 (doubling dilution) different than what is posted on the FDA-CDC AR Bank website because this is the normal technical variability of antimicrobial susceptibility testing (see Jorgensen. 1993). MICs obtained by a user could possibly show an even greater difference than this, depending on the methodology, the modal MIC of the isolate, and if the MIC on the website is at a very low or very high testing range. However, major (resistant versus susceptible) or very major (susceptible versus resistant) interpretive category difference for a given drug-organism result should be uncommon (i.e., this could occur in the rare occasions where the susceptible and resistant breakpoints are within a single doubling dilution). In other cases a major or very major error likely indicates a problem with either the organism (e.g., losing a resistance mechanism upon passage) or the testing method being applied.

7. Reference results for many antimicrobial agents beyond the carbapenems (e.g., cephalosporins, fluoroquinolones, polymyxins, beta-lactam combination agents and other newly approved agents) are available for the 31 isolates recommended in this verification procedure. Those results can be accessed here. For efficiency, laboratories should assess the need for verification of additional agents and test methods when planning their carbapenem breakpoint verification studies.

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4. FDA requirements for Clearance of FDA AST devices: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/u cm080564.htm

5. FDA Guidance Document for Updating Labeling (to include updating breakpoints): http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidanc es/UCM169359.pdf

6. IDSA Guidelines for Revised Breakpoints: https://www.idsociety.org/public-health/antimicrobial-resistance/archive-antimicrobial-resistance/antimicrobial-susceptibility-testing/

APPENDIX A

Revised CLSI Interpretive Criteria—M100 30th Edition (2020)

Agent	Interpretive Criteria					
	MIC (µg/mL)			Disk Diffusion (mm)		
	Susc	Int	Res	Susc	Int	Res
Doripenem	≤1	2	≥4	≥23	20-22	≤19
Ertapenem	≤0.5	1	≥2	≥22	19-21	≤18
Imipenem	≤1	2	≥4	≥23	20-22	≤19
Meropenem	≤1	2	≥4	≥23	20-22	≤19