Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

CRO Breakpoint Implementation Toolkit

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Background provided by Dr. Rauch

Key takeaways relevant to this topic

- Breakpoints are revised to ensure patient care and safety and to limit and reduce the spread of antimicrobial resistance

- Failure to use correct breakpoints during AST can result in incorrect interpretation of results, allowing for inappropriate treatment course, poor patient outcomes and continued spread of resistant infections.

- Adoption of the updated MIC breakpoints has proved challenging for clinical microbiology laboratories that use commercial MIC susceptibility testing systems

They updated the breakpoints again? Why?!

CLSI AST Rationale Documents (Free)

This package includes:

- MR01 | *Polymyxin Breakpoints for Enterobacterales, Pseudomonas aeruginosa, and Acinetobacter spp., 2nd Edition*
- MR02 | *Fluoroquinolone Breakpoints for Enterobacteriaceae and Pseudomonas aeruginosa, 1st Edition*
- MR03 | *Meropenem Breakpoints for Acinetobacter spp., 1st Edition*
- MR04 | *Azithromycin Breakpoint for Neisseria gonorrhoeae, 1st Edition*
- MR05 | Ceftaroline Breakpoints for Staphylococcus aureus, 1st Edition
- MR06 | *Daptomycin Breakpoints for Enterococci, 1st Edition*
- MR07 | *Cefazolin Breakpoints for Enterobacterales (Systemic Infections), 1st Edition*
- MR08 | *Cefazolin Breakpoints for Enterobacterales (Uncomplicated Urinary Tract Infections), 1st Edition*
- MR14 | *Piperacillin-Tazobactam Breakpoints for Enterobacterales, 1st Edition*

https://clsi.org/standards/products/packages/documents/mrpkg/

CAP will require annual review and updates

***REVISED** 09/22/2021

MIC.11380 Antimicrobial Susceptibility Test Interpretation Criteria Phase II

For antimicrobial susceptibility testing systems, there are written criteria for determining and interpreting minimal inhibitory concentration (MIC) or zone diameter sizes as susceptible, intermediate, resistant, non-susceptible, or susceptible dose-dependent. These criteria are reviewed annually.

***NEW** 09/22/2021

MIC.11385 Current Antimicrobial Susceptibility Test Interpretation Breakpoints Phase I

Effective January 1, 2024, the laboratory uses current breakpoints for interpretation of antimicrobial minimum inhibitory concentration (MIC) and disk diffusion test results, and implements new breakpoints within three years of the date of official publication by the FDA or other standards development organization (SDO) used by the laboratory.

Effective January 1, 2024 laboratory must use current breakpoints within 3 years

Where can I find current breakpoints?

At a minimum a lab must use the FDA breakpoints

Most US labs us CLSI

Google
“FDA STIC”

FDA-Recognized Antimicrobial Susceptibility Test Interpretive Criteria

Share | Tweet | LinkedIn | Email | Print

Looking for FDA-Recognized Susceptibility Test Interpretive Criteria?

Antibacterial Susceptibility Test Interpretive Criteria

Antifungal Susceptibility Test Interpretive Criteria

Looking for recent updates? Please see: Notices of Updates

Sign up to receive FDA Recognized Antimicrobial STIC Breakpoints email notifications

CLSI: M45 Breakpoints, M60 for fungi and M62 for Mycobacteria and aerobic actinomycetes

https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria
https://clsi.org/standards/products/free-resources/access-our-free-resources/

Where can I find current breakpoints?

EUCAST also available

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

- Useful when there are no FDA or CLSI breakpoints for therapies used by your providers.
- Take care to note the drug concentrations and media types as they can vary from standard US methods and impact the MIC

https://www.eucast.org/

The Problem – Fast changing breakpoints

- New CAP checklist requires that you know the current breakpoints
- Coming soon, CAP will require that your testing is up to date with current breakpoints as well
- Using the most up to date breakpoints is best for the patient and helps to standardize public health drug resistance surveillance data
- Manufacturers do not currently update their automated AST platforms to comply with the latest breakpoints for as much as 5 years after the change
- Updating is expensive and slowed down by the FDA approval process
- Relying on FDA approved verification of these instruments will result in CAP deficiencies

The Solution – Validate the new Breakpoints yourself!

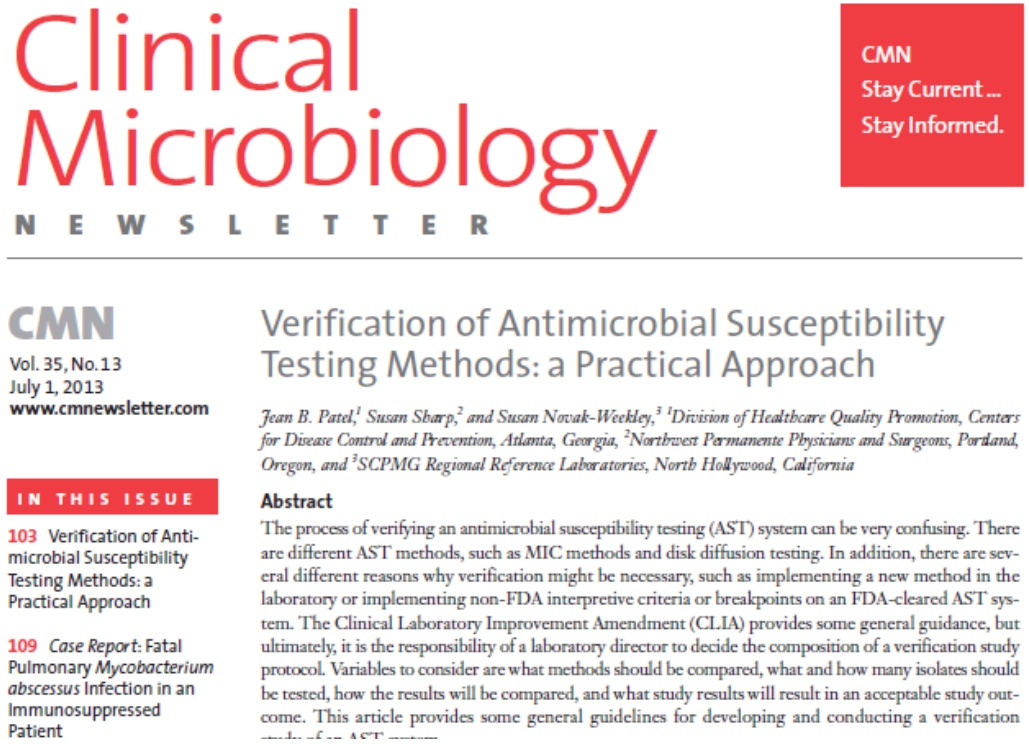
Now there’s a new problem

- Going “off-label” from the manufacturers instructions will make your test a lab developed test (LDT)
- LDTs require more extensive validation before use in patient testing
- Not all labs are experienced with LDT validations
- AST validations are some of the most complicated because there are so many bug-drug combinations and outcome interpretations

Don’t worry, there’s help!

Validation and Verification Resources

CLSI M52, 1st edition. 2015
2nd edition coming soon!



Patel, J.B., S. Sharp, and S Novak-Weekley. 2013. Clinical Microbiology Newsletter. Vol. 35 No. 13:103.

ASM Resources

- Planning a Method Verification Study in Clinical Microbiology Labs breaks down how to perform a clinical laboratory verification study with attached template (Source: ASM).
- Verifications and Validations: How to bring a new test to the lab aiming at clinical stewardship and compliance shares a real-life example of validation and verification (Source: Louis Stokes VA Medical Center).
- Cumitech 31A- Verification and Validation of Procedures in the Clinical Microbiology Laboratory. Being revised now!

https://asm.org/Articles/2022/February/Updating-Breakpoints-in-Antimicrobial-Susceptibili

APHL-ASM Antimicrobial Resistance Laboratory Workgroup

- Collaboration of Clinical and public health laboratorians whose goal is to identify and develop initiatives to improve detection and reporting of AR while fostering the relationship between clinical and public health laboratories
  - Made up of top clinical AST experts in the field. (Clinical, Public Health, CDC, and APHL)
  - Multiple projects to date including the CRO Breakpoint Implementation Toolkit

Carbapenem Resistant Organisms

- Carbapenem-resistant organisms (CROs) are a major concern for patients in healthcare facilities. Some pathogens in this family are resistant to nearly all antibiotics, leaving more toxic or less effective treatment options. The CDC's 2019 AR Threats Report categorizes carbapenem-resistant *Enterobacterales* and carbapenem-resistant *Acinetobacter* as urgent threats, while also categorizing multidrug-resistant *Pseudomonas aeruginosa* as a serious threat.
- Toolkit focuses on ***Enterobacterales***
- Future iterations will expand to include additional drug-bug combinations.

CRO Breakpoint Implementation Toolkit

1. **Introduction to the CRO Breakpoint Implementation Toolkit**
2. **About the AR Isolate Bank**
3. **Verification Template**
4. **Breakpoint Implementation Instructions**
5. **Implementation Worksheets**

https://www.aphl.org/programs/infectious_disease/Pages/CRO-Breakpoint-Implementation-Toolkit.aspx

1. One page introduction

- Can be used to inform leadership of the big pictures points
  - Why breakpoints need to be updated
  - Why the lab needs to do it's own validation and not wait for manufacturers
  - What the toolkit contains to help in this effort
  - Who created the toolkit

Introduction to the
CRO BREAKPOINT IMPLEMENTATION TOOLKIT

The Antimicrobial Resistance (AR) Laboratory Workgroup is a collaboration between public health and clinical laboratories whose goal is to identify and develop initiatives to improve the detection and reporting of AR while fostering the relationship between clinical and public health laboratories.

An important area that has been identified is assisting laboratories with the implementation of updated carbapenem susceptibility breakpoints.

Carbapenem-resistant organisms (CROs) include strains of *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter baumanii* that have high levels of resistance to antibiotics, including carbapenems. The US Food and Drug Administration and the Clinical Laboratory Standards Institute recommend decreasing carbapenem susceptibility testing breakpoints for these organisms in order to avoid mischaracterizing potentially-resistant organisims as susceptible. Failure to adopt updated breakpoints can lead to discrepancies in the interpretation of susceptibility testing, allowing for the administration of inappropriate antibiotics and subsequent poor patient outcomes, as well as the undetected spread of multidrug-resistant organisms.

However, adoption of the updated minimum inhibitory concentration (MIC) breakpoints has proved challenging for clinical microbiology laboratories using commercial MIC susceptibility testing systems, due to a verification study that must be completed according to CLIA requirements.

The AR Laboratory Workgroup has developed a toolkit to assist laboratories with this task, which contains:

- Breakpoint implementation instructions
- A verification template
- Worksheets to record consensus result for each antimicrobial agent/ organism combination generated
- Instructions on accessing isolates.

The toolkit was developed through joint collaboration between members of the Association of Public Health Laboratories and the American Society for Microbiology.

The AR LAB NETWORK

The AR Lab Network supports nationwide laboratory capacity to rapidly detect and respond to AR threats, like CROs, by identifying how transmission may be occurring, and informing local responses to prevent its spread and protect people. The AR Lab Network includes labs in 50 states, four cities and Puerto Rico, seven of which are designated as regional labs, and relies on partnership with the clinical lab community.

ARLABnetwork

The best way to detect CROs is to use the current breakpoints.

CONTACT

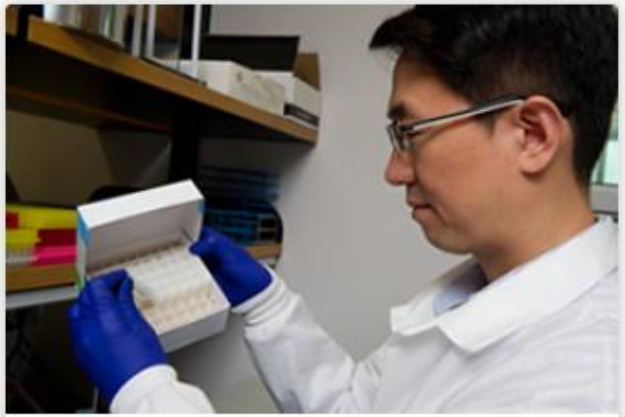
Name:
Title:
Email/Phone:

Name:
Title:
Email/Phone:

This project was 100% financed by federal funds. This publication was supported by Cooperative Agreement #NU60OE000103 funded by the US Centers for Disease Control and Prevention (CDC). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC or the US Department of Health and Human Services.

2. Where can I find isolates?

- Collaborative program between CDC and FDA
- Collection of AR organisms available to clinical and public health microbiologists, drug and diagnostic manufacturers, and researchers
- Thoughtfully curated panels laboratories can order at no cost (except shipping)
- Currently, they have 31 different Panels
    1. Enterobacterales Carbapenem Breakpoint
    2. Pseudomonas aeruginosa
    3. Acinetobacter baumannii



The AR Isolate Bank has 31 panels and 1,012 isolates as of January 2022.

https://wwwn.cdc.gov/arisolatebank/

Handling isolates from the AR Panel

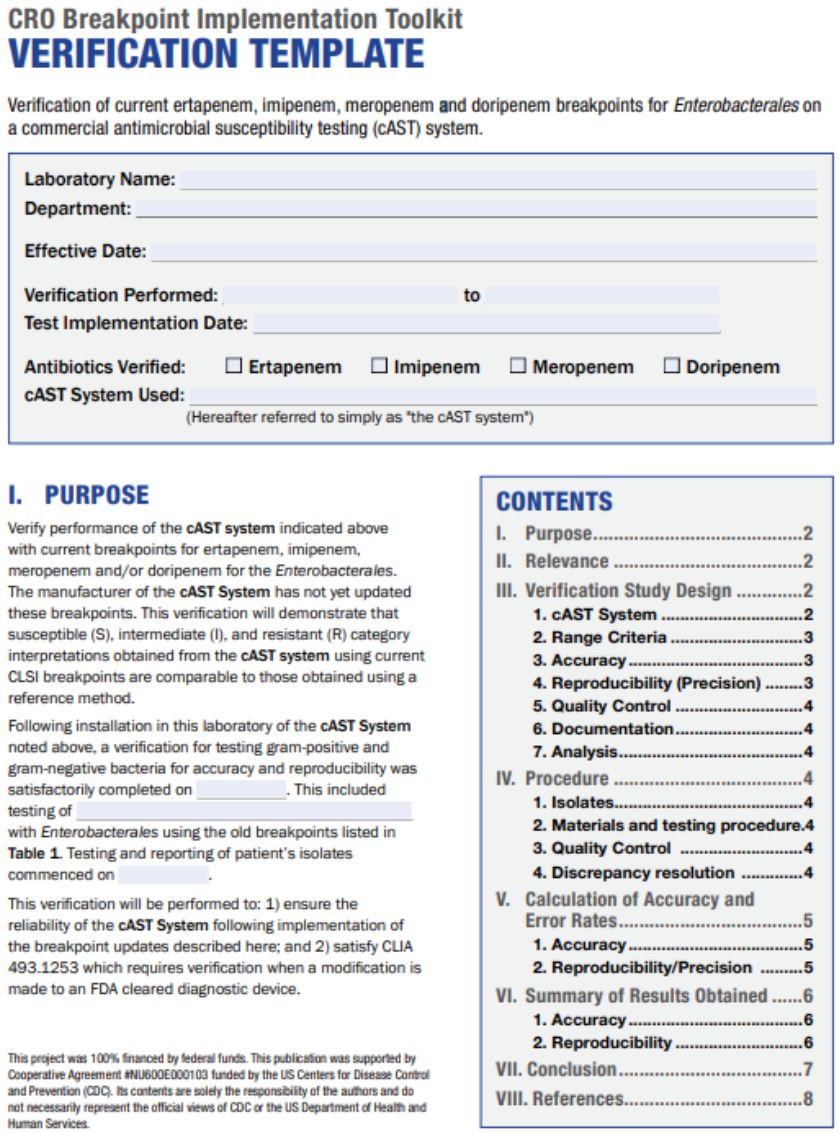
- Isolates arrive in individual tubes, shipped on dry ice
- Lyophilized powder needs to be re-suspended
  - Instructions included in the package and online
- Make your own stocks from the initial growth
  - Store at -80C
- Pass the strains at least twice before you start testing
- Do not over pass your strains, resistance can wane or be lost
- Always use fresh growth in your testing

3. Verification Template

Designed for the validation of Enterobacterales against current breakpoints on a commercial antimicrobial susceptibility testing (cAST) system.

- Ertapenem
- Imipenem
- Meropenem
- Doripenem

AR Bank Panel
Enterobacterales
Carbapenem
Breakpoint



CRO Breakpoint Implementation Toolkit

VERIFICATION TEMPLATE

Verification of current ertapenem, imipenem, meropenem and doripenem breakpoints for *Enterobacterales* on a commercial antimicrobial susceptibility testing (cAST) system.

Laboratory Name:
Department:
Effective Date:
Verification Performed: to
Test Implementation Date:
Antibiotics Verified: ☐ Ertapenem ☐ Imipenem ☐ Meropenem ☐ Doripenem
cAST System Used:
(Hereafter referred to simply as "the cAST system")

I. PURPOSE

Verify performance of the **cAST system** indicated above with current breakpoints for ertapenem, imipenem, meropenem and/or doripenem for the *Enterobacterales*. The manufacturer of the **cAST System** has not yet updated these breakpoints. This verification will demonstrate that susceptible (S), intermediate (I), and resistant (R) category interpretations obtained from the **cAST system** using current CLSI breakpoints are comparable to those obtained using a reference method.

Following installation in this laboratory of the **cAST System** noted above, a verification for testing gram-positive and gram-negative bacteria for accuracy and reproducibility was satisfactorily completed on . This included testing of with *Enterobacterales* using the old breakpoints listed in **Table 1**. Testing and reporting of patient's isolates commenced on .

This verification will be performed to: 1) ensure the reliability of the **cAST System** following implementation of the breakpoint updates described here; and 2) satisfy CLIA 493.1253 which requires verification when a modification is made to an FDA cleared diagnostic device.

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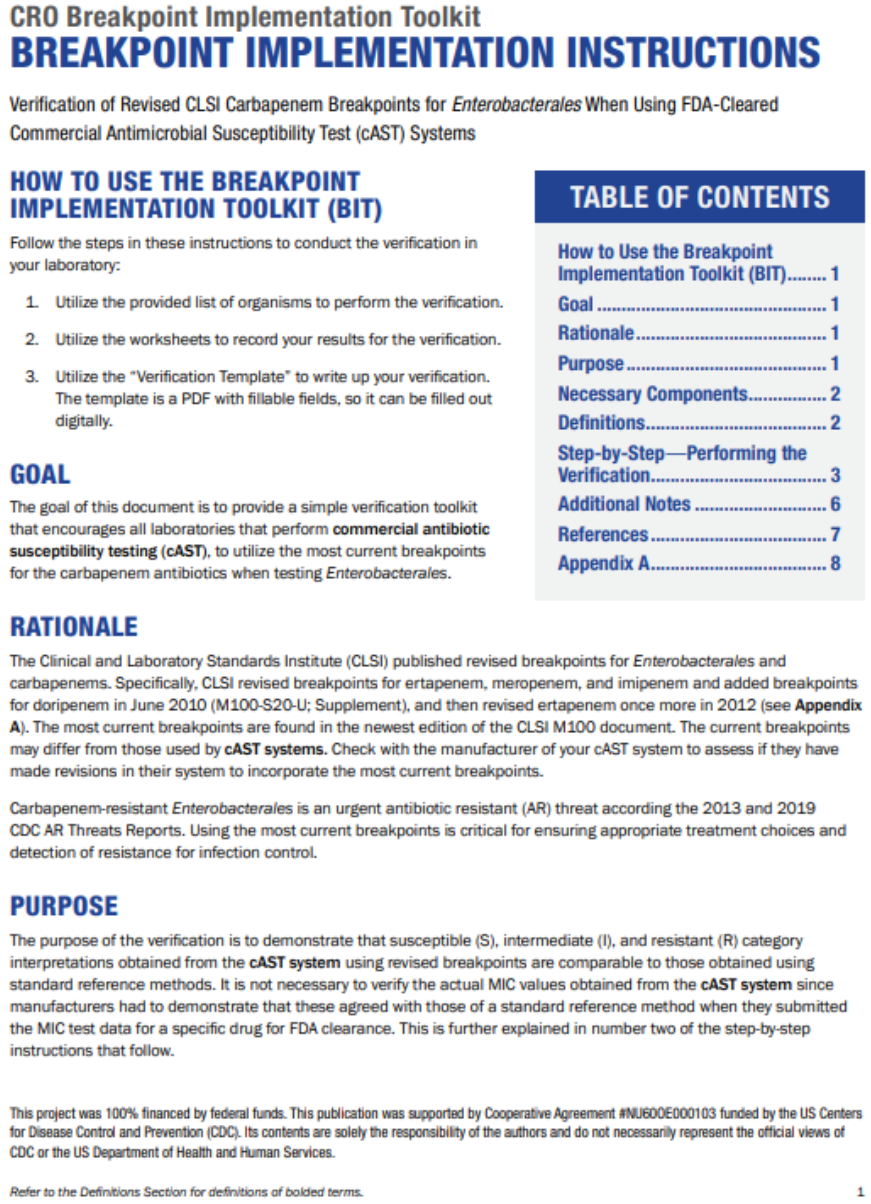
CONTENTS

4. Separate Instructions

- Includes definitions, step by step instructions, rationale and guidance



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*.

TABLE OF CONTENTS

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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Refer to the Definitions Section for definitions of bolded terms.

5. CRO Breakpoint Implementation Toolkit
APPENDIX B — IMPLEMENTATION WORKSHEETS

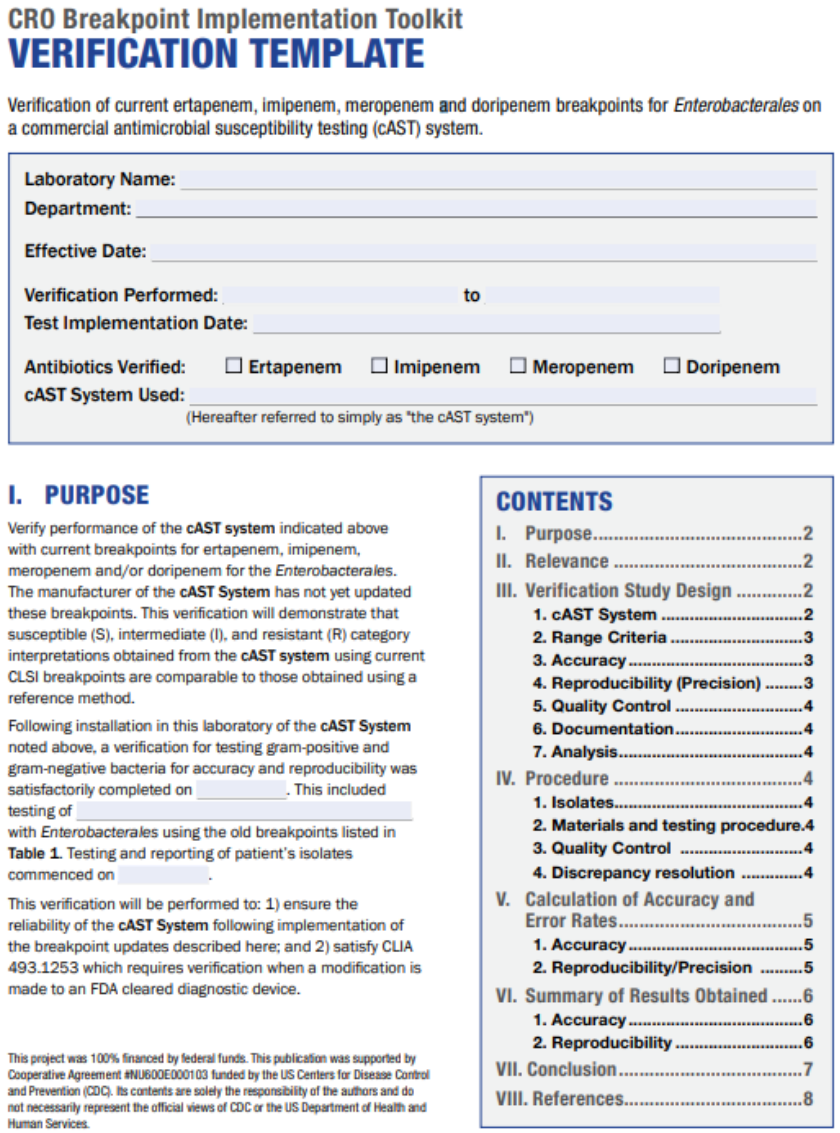
- Pre-populated with the AR panel organisms and their expected results
- Fillable electronic form
- Separate worksheets for each drug

Test Date/Tech	Isolate No.	Organism	Ertapenem			
			S, I, R		MIC	
			Reference	Observed	Reference	Observed
	0001	*Escherichia coli*	R		8	
	0002	*Enterobacter cloacae*	R		>8	
	0003	*Klebsiella pneumoniae*	R		>8	
	0004	*Klebsiella pneumoniae*	R		>8	
	0005	*Klebsiella pneumoniae*	R		>8	
	0006	*Escherichia coli*	R		>8	
	0007	*Klebsiella aerogenes*	R		2	
	0008	*Enterobacter cloacae*	R		>8	
	0009	*Klebsiella aerogenes*	R		>8	
	0010	*Klebsiella pneumoniae*	R		>8	
	0011	*Escherichia coli*	S		0.25	
	0012	*Klebsiella pneumoniae*	S		0.5	
	0013	*Escherichia coli*	S		<=0.12	
	0014	*Escherichia coli*	S		<=0.12	
	0015	*Escherichia coli*	S		0.5	
	0016	*Klebsiella pneumoniae*	S		<=0.12	
	0017	*Escherichia coli*	S		<=0.12	
	0018	*Klebsiella aerogenes*	S		<=0.12	
	0019	*Escherichia coli*	S		<=0.12	
	0020	*Escherichia coli*	S		<=0.12	
	0021	*Citrobacter freundii*	S		<=0.12	
	0022	*Citrobacter freundii*	S		<=0.12	
	0023	*Citrobacter freundii*	S		<=0.12	
	0024	*Citrobacter koseri*	S		<=0.12	
	0025	*Citrobacter koseri*	S		<=0.12	
	0026	*Providencia stuartii*	S		<=0.12	
	0027	*Serratia marcescens*	S		<=0.12	
	0028	*Klebsiella oxytoca*	S		<=0.12	
	0029	*Proteus mirabilis*	S		0.25	
	0030	*Shigella sonnei*	S		<=0.12	
	0031	*Salmonella typhimurium*	S		<=0.12	

		Number	%
Date:	Categorical Agreement		
	Very Major Errors		
Tech:	Major Errors		
	Minor Errors		

Working though the template

- Does most of the writing for you
- 11 page summary document
- Electronic fillable template
- Some “smart fields” auto-populate
- Provides flexibility for Director discretion



CRO Breakpoint Implementation Toolkit

VERIFICATION TEMPLATE

Verification of current ertapenem, imipenem, meropenem and doripenem breakpoints for *Enterobacterales* on a commercial antimicrobial susceptibility testing (cAST) system.

Laboratory Name:
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Effective Date:
Verification Performed: to
Test Implementation Date:
Antibiotics Verified: ☐ Ertapenem ☐ Imipenem ☐ Meropenem ☐ Doripenem
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(Hereafter referred to simply as "the cAST system")

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Following installation in this laboratory of the **cAST System** noted above, a verification for testing gram-positive and gram-negative bacteria for accuracy and reproducibility was satisfactorily completed on . This included testing of with *Enterobacterales* using the old breakpoints listed in **Table 1**. Testing and reporting of patient's isolates commenced on .

This verification will be performed to: 1) ensure the reliability of the **cAST System** following implementation of the breakpoint updates described here; and 2) satisfy CLIA 493.1253 which requires verification when a modification is made to an FDA cleared diagnostic device.

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CONTENTS

Provides the purpose and background

Table 1. Previously verified (old) and current MIC breakpoints (µg/ml) for Enterobacterales.

Agent	Old			Current		
	S	I	R	S	I	R
Ertapenem	≤2	4	≥8	≤0.5	1	≥2
Imipenem	≤4	18	≥16	≤1	2	≥4
Meropenem	≤4	8	≥16	≤1	2	≥4
Doripenem	None			≤1	2	≥4

II. RELEVANCE

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. The current breakpoints of our **cAST systems** for [redacted] are not up to date.

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.

Your instruments current breakpoints →

III. VERIFICATION STUDY DESIGN

1. cAST System

Panel:

Software Version:

Table 2. Current FDA Breakpoint Values for Carbapenems.
Concentrations of antimicrobial agents available for reporting and breakpoints (µg/ml) currently FDA cleared on the **cAST System**:

Agent	Concentration (µg/ml) Range	Breakpoints (µg/ml)		
		S	I	R
Ertapenem				
Imipenem				
Meropenem				
Doripenem				

2. Range Criteria

Reportable range and Reference range criteria required for a verification study as described in CLIA 493.1253 are not applicable to **cAST Systems**.

3. Accuracy

Strategy

Accuracy was assessed for Categorical Agreement only, because there will be no changes in the MIC values reported. The manufacturer of the **cAST System** previously demonstrated that MIC values generated with their system agreed with MIC values obtained from a standard reference method when they submitted the MIC test data for these drugs for FDA clearance.

It provides room for Director discretion

- Precision may already have been covered in the initial verification and does not need to be repeated
- If more testing is needed the template makes a recommendation based on field standards

4. Reproducibility (Precision)

Records from previously-performed reproducibility studies with the **cAST System** for _____ were reviewed to determine if additional reproducibility studies are warranted.

Select one of the following three options that best pertain to your laboratory's situation:

☐ It was determined that no reproducibility studies were required because adequate assessment of the carbapenem antibiotics against appropriate gram-negative organisms was already performed in the initial verification reproducibility section.

☐ A limited reproducibility assessment was performed, which included testing of one isolate(s) three times (three separate inocula) over one to three days. The following organisms were selected for testing:

- **Organism 1** (Source: Routine QC organism): _____
- **Organism 2** (Source: ☐ Routine QC ☐ AR Bank ☐ _____): _____
- **Organism 3** (Source: AR Bank *Enterobacterales* Carbapenem Breakpoint Panel): _____

☐ A comprehensive reproducibility assessment was performed, which included testing five isolates three times (three separate inocula). The following five QC and clinical isolates chosen from AR Bank *Enterobacterales* Carbapenem Breakpoint Panel:

- **Isolate 1:** _____
- **Isolate 2:** _____
- **Isolate 3:** _____
- **Isolate 4:** _____
- **Isolate 5:** _____

Setting the Acceptability Criteria

- The template allows the director to set the criteria they deem to be acceptable
- Most labs choose
  - ≥ 90-95% CA
  - < 5% VMEs (1 error)
  - < 5% MEs (1 error)
  - < 3 mE isolates

7. Analysis

- MIC results were interpreted manually utilizing the current CLSI breakpoints (M100, _____ edition)
- S, I, R results for _____, obtained from testing with _____ on the cAST System were compared to S, I, R results provided with the CDC & FDA AR Isolate Bank isolates (Reference results) and evaluated for Categoric Agreement (CA), Very Major Errors (VME), Major Errors (ME) and Minor Errors (mE).
- Accuracy is considered acceptable (each agent analyzed separately) and the current breakpoints verified based on the following criteria:
  - CA: ≥ _____ %
  - VMEs: < _____ % of total resistant isolates
  - MEs: < _____ % of total susceptible isolates
  - mEs: _____ isolate(s)
- Reproducibility is considered acceptable (each agent analyzed separately) if 95% of isolates correlate to the reference S, I, or R results.

Understanding the error types

- Categorical Agreement (CA)
  - **Overall match of S, I, and R** to reference results
  - # correct/total number tested = % correct
- Very Major Errors (VME)
  - **False susceptible:** Your test says S when isolate is R
  - # of VME/total number R tested = % VME
- Major Errors (ME)
  - **False resistant:** Test says R when isolate is S
  - # of ME/total number S tested = % VME
- Minor Errors (mE)
  - **One category off**: Test says I when isolate is S or R, or S or R when isolate is I
  - X of mE/total number of isolates tested = % mE

IV. PROCEDURE

1. Isolates

- 31 isolates of *Enterobacterales* (*Enterobacterales* Carbapenem Breakpoint panel) were obtained from the CDC & FDA AR Isolate Bank. See Appendix B1 for list of isolates and their breakpoints.
- Isolates in this set were selected to represent a variety of species, carbapenem MICs and carbapenem resistance mechanisms. Reference results for each isolate were established by CDC & FDA AR Isolate Bank criteria.

2. Materials and testing procedure

Materials required and the procedure for testing isolates of *Enterobacterales* using the cAST System are described in SOP _____.

3. Quality Control

_____ and _____ were tested each day of verification testing.

4. Discrepancy resolution

Discrepancies were resolved by:

- ☐ Repeating in triplicate
- ☐ Disk diffusion
- ☐ Sending to another laboratory for testing
- ☐ Other: _____

CRO Breakpoint Implementation Toolkit

APPENDIX B — IMPLEMENTATION WORKSHEETS

Test Date/Tech	Isolate No.	Organism	Imipenem			
			S, I, R		MIC	
			Reference	Observed	Reference	Observed
	0001	*Escherichia coli*	R		4	
	0002	*Enterobacter cloacae*	R		16	
	0003	*Klebsiella pneumoniae*	R		8	
	0004	*Klebsiella pneumoniae*	R		16	
	0005	*Klebsiella pneumoniae*	R		16	
	0006	*Escherichia coli*	R		16	
	0007	*Klebsiella aerogenes*	S		1	
	0008	*Enterobacter cloacae*	S		1	
	0009	*Klebsiella aerogenes*	R		8	
	0010	*Klebsiella pneumoniae*	R		4	
	0011	*Escherichia coli*	S		<=0.5	
	0012	*Klebsiella pneumoniae*	S		<=0.5	
	0013	*Escherichia coli*	S		<=0.5	
	0014	*Escherichia coli*	S		<=0.5	
	0015	*Escherichia coli*	S		<=0.5	
	0016	*Klebsiella pneumoniae*	S		<=0.5	
	0017	*Escherichia coli*	S		<=0.5	
	0018	*Klebsiella aerogenes*	S		1	
	0019	*Escherichia coli*	S		<=0.5	
	0020	*Escherichia coli*	S		1	
	0021	*Citrobacter freundii*	S		<=0.5	
	0022	*Citrobacter freundii*	S		1	
	0023	*Citrobacter freundii*	S		<=0.5	
	0024	*Citrobacter koseri*	S		<=0.5	
	0025	*Citrobacter koseri*	S		<=0.5	
	0026	*Providencia stuartii*	S		<=0.5	
	0027	*Serratia marcescens*	S		<=0.5	
	0028	*Klebsiella oxytoca*	S		<=0.5	
	0029	*Proteus mirabilis*	R		8	
	0030	*Shigella sonnei*	S		<=0.5	
	0031	*Salmonella typhimurium*	S		<=0.5	

		Number	%
Date:	Categorical Agreement		
	Very Major Errors		
Tech:	Major Errors		
	Minor Errors		

Test Date/Tech	Isolate No.	Organism	Imipenem			
			S, I, R		MIC	
			Reference	Observed	Reference	Observed
4/22/22 AS	0001	*Escherichia coli*	R	R	4	4
4/22/22 AS	0002	*Enterobacter cloacae*	R	R	16	16
4/22/22 AS	0003	*Klebsiella pneumoniae*	R	R	8	16
4/22/22 AS	0004	*Klebsiella pneumoniae*	R	R	16	16
4/22/22 AS	0005	*Klebsiella pneumoniae*	R	R	16	8
4/22/22 AS	0006	*Escherichia coli*	R	R	16	16
4/22/22 AS	0007	*Klebsiella aerogenes*	S	S	1	1
4/22/22 AS	0008	*Enterobacter cloacae*	S	S	1	1
4/22/22 AS	0009	*Klebsiella aerogenes*	R	R	8	8
4/22/22 AS	0010	*Klebsiella pneumoniae*	R	S	4	1
4/22/22 AS	0011	*Escherichia coli*	S	S	<=0.5	<=0.5
4/22/22 AS	0012	*Klebsiella pneumoniae*	S	S	<=0.5	<=0.5
4/22/22 AS	0013	*Escherichia coli*	S	S	<=0.5	<=0.5
4/22/22 AS	0014	*Escherichia coli*	S	S	<=0.5	<=0.5
4/22/22 AS	0015	*Escherichia coli*	S	S	<=0.5	<=0.5
4/26/22 AS	0016	*Klebsiella pneumoniae*	S	S	<=0.5	<=0.5
4/26/22 AS	0017	*Escherichia coli*	S	S	<=0.5	<=0.5
4/26/22 AS	0018	*Klebsiella aerogenes*	S	S	1	<=0.5
4/26/22 AS	0019	*Escherichia coli*	S	S	<=0.5	<=0.5
4/26/22 AS	0020	*Escherichia coli*	S	S	1	<=0.5
4/26/22 AS	0021	*Citrobacter freundii*	S	S	<=0.5	<=0.5
4/26/22 AS	0022	*Citrobacter freundii*	S	S	1	1
4/26/22 AS	0023	*Citrobacter freundii*	S	S	<=0.5	<=0.5
4/26/22 AS	0024	*Citrobacter koseri*	S	S	<=0.5	<=0.5
4/26/22 AS	0025	*Citrobacter koseri*	S	S	<=0.5	<=0.5
4/26/22 AS	0026	*Providencia stuartii*	S	S	<=0.5	<=0.5
4/22/22 AS	0027	*Serratia marcescens*	S	S	<=0.5	<=0.5

	Number	%
Categorical Agreement	30	97
Very Major Errors	1	5
Major Errors	0	0
Minor Errors	0	0

V. CALCULATION OF ACCURACY AND ERROR RATES

See **Appendix B1** for a full data set of expected breakpoints.

1. Accuracy

Drug A: _____

Calculations

Table 3-A. Accuracy Calculation for Verification of _____

CLSI Breakpoint Interpretation	Expected Reference 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]
  (___ + ___ + ___) / 31 x 100 = _____ %
- **VMEs (%)** = # isolates with false "S" results [(G / total "R" isolates tested (G+H+I), reference results) x 100]
  ___ / (___ + ___ + ___) x 100 = _____ %
- **MEs (%)** = # isolates with false "R" results [C / total "S" isolates tested (A+B+C), reference results x 100]
  ___ / (___ + ___ + ___) x 100 = _____ %
- **mEs (%)** = # of isolates with "I" result when reference is "S" or "R" + "S" or "R" result when Reference is "I"
  [(B+H+D+F)/ 31 x 100] (___ + ___ + ___ + ___) / 31 x 100 = _____ %

The template walks you through the calculations

1. Accuracy

Drug A: Imipenem

Calculations

Table 3-A. Accuracy Calculation for Verification of Imipenem

CLSI Breakpoint Interpretation	Expected Reference Interpretations		
	S	I	R
Susceptible	A: 22	D: 0	G: 1
Intermediate	B: 0	E: 0	H: 0
Resistant	C: 0	F: 0	I: 8

- **CA (%)** = # isolates with same SIR results [(A+E+I) / 31 x 100]
  (22 + 0 + 8) / 31 x 100 = 97 %
- **VMEs (%)** = # isolates with false "S" results [(G / total "R" isolates tested (G+H+I), reference results) x 100]
  1 / (1 + 0 + 8) x 100 = 5 %
- **MEs (%)** = # isolates with false "R" results [C / total "S" isolates tested (A+B+C), reference results x 100]
  0 / (22 + 0 + 0) x 100 = 0 %
- **mEs (%)** = # of isolates with "I" result when reference is "S" or "R" + "S" or "R" result when Reference is "I"
  [(B+H+D+F)/ 31 x 100] (0 + 0 + 0 + 0) / 31 x 100 = 0 %

Discrepancy Resolution, if needed

Discrepancy Resolution

- ☐ No discrepancies were found
- ☒ At least one discrepancy was found. Number of discrepancies: 1

  Isolates with a VME or ME were repeated in triplicate using the cAST System with the following resolution:

  - ☐ No further VMEs or MEs were found in any of the replicates.
  - ☒ All or some of the three replicates continued to show VMEs or MEs, therefore additional testing by a comparator method was used to confirm the reference results provided by CDC & FDA.
    Additional testing included:

    - ☒ Disk diffusion
    - ☐ Another method available in the laboratory or a referral laboratory that has been verified for current breakpoints:

- ☒ If more than one discrepancy was found or there was more than one resolution, please explain:

  Isolate was passed many times prior to testing. When a fresh isolate was tested from the stock the discrepancy resolved. The drug resistance was likely lost in passage.

Reproducibility Chart

Table 4-A. Reproducibility of Interpretations (S, I, R)

Isolates	Day 1			Day 2			Day 3		
	1	2	3	1	2	3	1	2	3

- If needed, test up to 5 isolates 1-3 times per day on over 1-3 separate days

Summary

VI. SUMMARY OF RESULTS OBTAINED

1. Accuracy

Table 6. Summary of Accuracy Results

Agent	Number of Isolates*				CA		VME		ME		MiE	
	Total	S	I	R	#	%	#	%	#	%	#	%
Ertapenem	31					94						
Imipenem	31											
Meropenem	31											
Doripenem	31											

* See **Appendix B1** for a complete list of reference results for 31 isolates of *Enterobacterales*. This list is also available in the *Enterobacterales* Carbapenem Breakpoint panel (CDC & FDA AR Isolate Bank).

Conclusion

VII. CONCLUSION

This verification study demonstrates that the cAST System _____ provides accurate susceptibility interpretations utilizing the current MIC breakpoints for _____.

This verification study has been reviewed and is acceptable for patient testing.

Reviewed by: _____ Date: _____

Signature: _____

Summary

- The new CAP checklist items will require monitoring annually and implementation of new breakpoints within 3 years of a change.
- Manufacturers are unlikely to keep up with this standard
- Labs will need to perform new breakpoint validations to stay in compliance
- Many tools exist to help in this process including the ASM-APHL CRO Breakpoint Implementation Toolkit.
- These resources can empower your lab to meet the industry standards and provide the best possible patient care.

Resources

- CLSI breakpoint rationale: https://clsi.org/standards/products/packages/documents/mrpkg/
- CAP Microbiology Checklists: https://www.cap.org/laboratory-improvement/accreditation/accreditation-checklists
- FDA STIC: www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria
- CLSI M100 (Free): https://clsi.org/standards/products/free-resources/access-our-free-resources/
- EUCAST breakpoints: https://www.eucast.org/
- CLSI M52, 1st edition. 2015
- Patel, J.B., S. Sharp, and S Novak-Weekley. 2013.Clinical Microbiology Newsletter. Vol. 35 No.13:103.
- CDC's 2019 AR Threats Report: https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf
- CRO Breakpoint Implementation Toolkit: https://www.aphl.org/programs/infectious_disease/Pages/CRO-Breakpoint-Implementation-Toolkit.aspx