Resources for Validations and Verifications to Implement Updated Breakpoints

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Disclosures

- **Amelia S. Bhatnagar*:** None.

*The findings and conclusions in the report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Objectives

- Discuss practical strategies for both verification and validation studies to implement updated breakpoints
- Review and define the performance characteristics of a validation and verification
- Identify and integrate key resources that describe validation and verification studies and processes

Where do I start?

- Identify whether your breakpoints are need to be updated

  Review! See Romney's talk for details!

- Discuss and prioritize breakpoint updates with your antimicrobial stewardship team and the device manufacturer

  See Romney's talk for details!

- Determine whether a validation or verification is needed
- Perform the study and assess performance

How do I determine if my breakpoints are updated?

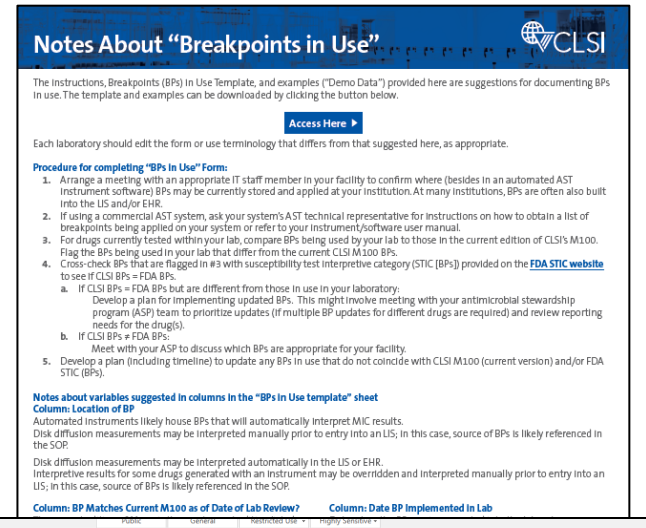
- Review CLSI M100 annually
  - Attend annual CLSI webinar!
- Check FDA STIC website for updates. Manufacturers can only use breakpoints published on the FDA STIC website.
  - Sign up to receive updates!
- Review commercial AST device's IFU at least annually for updates

In Oct 2021, CAP Checklist items require laboratories to document breakpoints in use (MIC.11380) and implement updated breakpoints within 3 years of publication on FDA STIC (MIC.11385).

Labs must implement updated breakpoints by Jan 1, 2024 for breakpoints updated prior 2021.

How do I determine if my breakpoints are updated and comply with the CAP checklist items?

- CLSI “Breakpoints in Use”
  - Released in June 2022 CLSI AST Newsletter. Available **free** from CLSI at: https://clsi.org/standards/products/microbiology/companion/bpiu/
  - Compare breakpoints used with your laboratory’s test system(s) to the breakpoints most recently published by CLSI or FDA (at your institution’s preference)


Notes About “Breakpoints in Use”

CLSI

The instructions, Breakpoints (BPs) in Use Template, and examples (“Demo Data”) provided here are suggestions for documenting BPs in use. The template and examples can be downloaded by clicking the button below.

Access Here

Each laboratory should edit the form or use terminology that differs from that suggested here, as appropriate.

Procedure for completing “BPs in Use” Form:

1. Arrange a meeting with an appropriate IT staff member in your facility to confirm where (besides in an automated AST instrument software) BPs may be currently stored and applied at your institution. At many institutions, BPs are often also built into the LIS and/or EHR.
2. If using a commercial AST system, ask your system’s AST technical representative for instructions on how to obtain a list of breakpoints being applied on your system or refer to your instrument/software user manual.
3. For drugs currently tested within your lab, compare BPs being used by your lab to those in the current edition of CLSI’s M100. Flag the BPs being used in your lab that differ from the current CLSI M100 BPs.
4. Cross-check BPs that are flagged in #3 with susceptibility test interpretive category (STIC [BPs]) provided on the FDA STIC website to see if CLSI BPs = FDA BPs.
   a. If CLSI BPs = FDA BPs but are different from those in use in your laboratory:
      Develop a plan for implementing updated BPs. This might involve meeting with your antimicrobial stewardship program (ASP) team to prioritize updates (if multiple BP updates for different drugs are required) and review reporting needs for the drug(s).
   b. If CLSI BPs ≠ FDA BPs:
      Meet with your ASP to discuss which BPs are appropriate for your facility.
5. Develop a plan (including timeline) to update any BPs in use that do not coincide with CLSI M100 (current version) and/or FDA STIC (BPs).

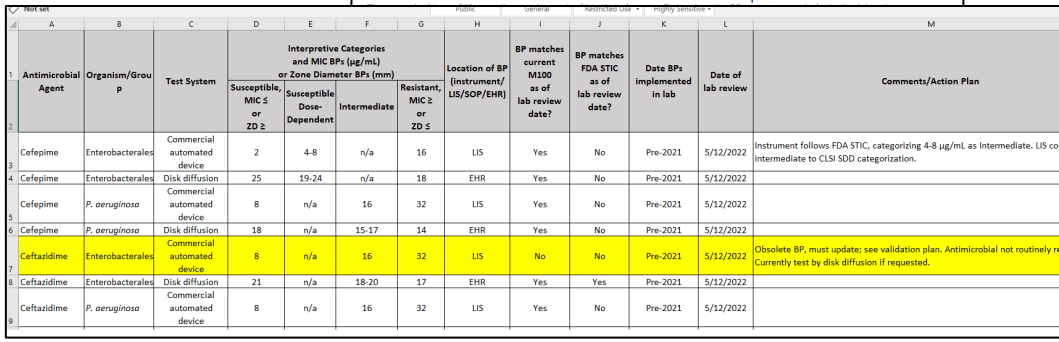
Notes about variables suggested in columns in the “BPs in Use template” sheet

Column: Location of BP

Automated instruments likely house BPs that will automatically interpret MIC results.
Disk diffusion measurements may be interpreted manually prior to entry into an LIS; in this case, source of BPs is likely referenced in the SOP.

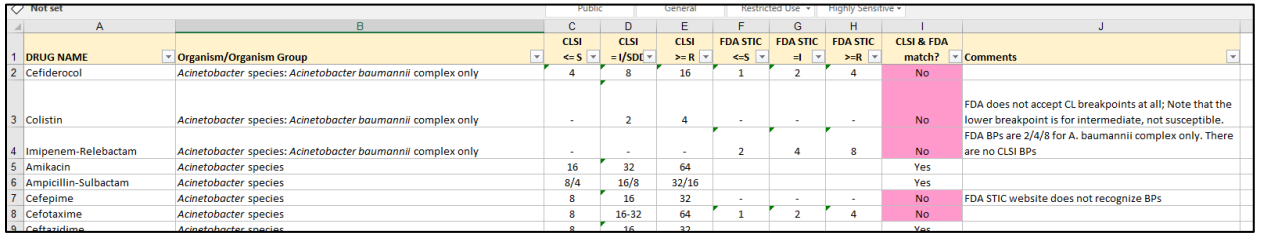
Disk diffusion measurements may be interpreted automatically in the LIS or EHR.
Interpretive results for some drugs generated with an instrument may be overridden and interpreted manually prior to entry into an LIS; in this case, source of BPs is likely referenced in the SOP.

Column: BP Matches Current M100 as of Date of Lab Review? **Column: Date BP Implemented in Lab**



Antimicrobial Agent	Organism/Group	Test System	Interpretive Categories and MIC BPs (µg/mL) or Zone Diameter BPs (mm): Susceptible, MIC ≤ or ZD ≥	Susceptible Dose-Dependent	Intermediate	Resistant, MIC ≥ or ZD ≤	Location of BP (instrument/ LIS/SOP/EHR)	BP matches current M100 as of lab review date?	BP matches FDA STIC as of lab review date?	Date BPs implemented in lab	Date of lab review	Comments/Action Plan
Cefepime	Enterobacterales	Commercial automated device	2	4-8	n/a	16	LIS	Yes	No	Pre-2021	5/12/2022	Instrument follows FDA STIC, categorizing 4-8 µg/mL as Intermediate. LIS co
Cefepime	Enterobacterales	Disk diffusion	25	19-24	n/a	18	EHR	Yes	No	Pre-2021	5/12/2022	
Cefepime	*P. aeruginosa*	Commercial automated device	8	n/a	16	32	LIS	Yes	No	Pre-2021	5/12/2022	
Cefepime	*P. aeruginosa*	Disk diffusion	18	n/a	15-17	14	EHR	Yes	No	Pre-2021	5/12/2022	
Ceftazidime	Enterobacterales	Commercial automated device	8	n/a	16	32	LIS	No	No	Pre-2021	5/12/2022	Obsolete BP, must update; see validation plan. Antimicrobial not routinely r Currently test by disk diffusion if requested.
Ceftazidime	Enterobacterales	Disk diffusion	21	n/a	18-20	17	EHR	Yes	Yes	Pre-2021	5/12/2022	
Ceftazidime	*P. aeruginosa*	Commercial automated device	8	n/a	16	32	LIS	Yes	No	Pre-2021	5/12/2022	

- CLSI MS Excel table for comparison of 2023 CLSI breakpoints to FDA breakpoints
  - Part of forthcoming **free** CLSI Breakpoint Implementation Toolkit resource



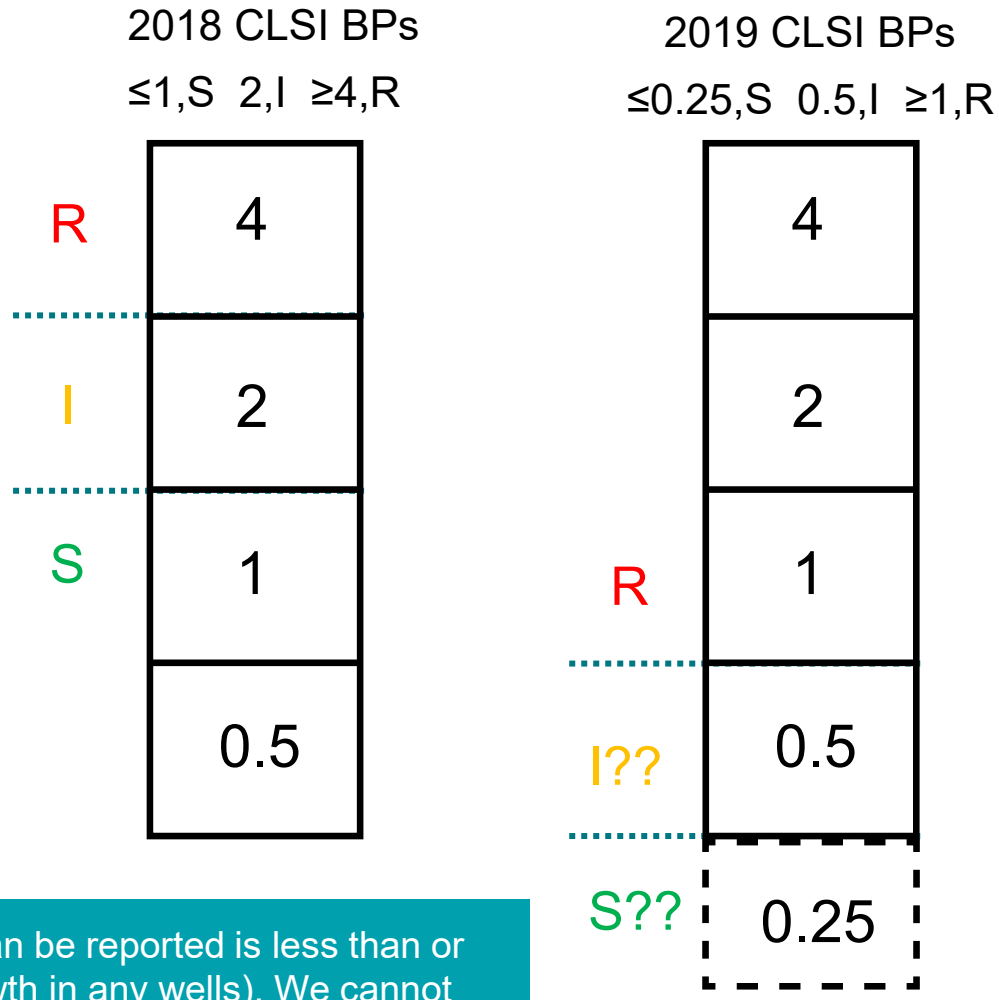
DRUG NAME	Organism/Organism Group	CLSI <= S	CLSI = I/SDD	CLSI >= R	FDA STIC <=S	FDA STIC =I	FDA STIC >=R	CLSI & FDA match?	Comments
Cefiderocol	*Acinetobacter* species: *Acinetobacter baumannii* complex only	4	8	16	1	2	4	No	
Colistin	*Acinetobacter* species: *Acinetobacter baumannii* complex only	-	2	4	-	-	-	No	FDA does not accept CL breakpoints at all; Note that the lower breakpoint is for intermediate, not susceptible.
Imipenem-Relebactam	*Acinetobacter* species: *Acinetobacter baumannii* complex only	-	-	-	2	4	8	No	FDA BPs are 2/4/8 for A. baumannii complex only. There are no CLSI BPs
Amikacin	*Acinetobacter* species	16	32	64				Yes	
Ampicillin-Sulbactam	*Acinetobacter* species	8/4	16/8	32/16				Yes	
Cefepime	*Acinetobacter* species	8	16	32	-	-	-	No	FDA STIC website does not recognize BPs
Cefotaxime	*Acinetobacter* species	8	16-32	64	1	2	4	No	

Draft, CLSI 2023 Breakpoint Implementation Toolkit

We've identified breakpoints needing to be updated, is it possible to implement based on the device?

- If the device is a panel or card, check to see if the concentrations on the panel/card can accommodate the new breakpoints
- If yes, proceed with validation or verification
- If no, you have some options:
  - Discuss with appropriate stakeholders on the clinical need and importance of the antimicrobial
  - Consider only reporting interpretations that are covered by the panel/card, use a validated/verified back up method if the MIC falls in the non-distinguishable range

Panel X: Ciprofloxacin, Enterobacterales



The lowest concentration that can be reported is less than or **equal to (≤)** 0.5 µg/mL (no growth in any wells). We cannot distinguish between an MIC 0.5 (I) or 0.25 (S)!

How do I determine whether a validation or verification is appropriate?

- Understand the difference and risk between validation and verification

Review! See Romney's talk for details!

- Look at the device IFU or contact the manufacturer to determine whether the device is FDA-cleared for the updated breakpoints
  - It is possible a device may be FDA-cleared for an antimicrobial agent/organism but cleared for a breakpoint no longer recognized by CLSI or FDA! Manufacturers are not mandated to update their device's breakpoints.

Updated BP Status	Commercial AST System Status	Performance Assessment Required
CLSI = FDA	CLSI BPs are FDA cleared and available on panel/software	Verification 10-15 isolates/drug
CLSI = FDA	Device manufacturer has notified customers that device has received FDA clearance with updated CLSI/FDA BPs and how to implement BPs within their panels/software, if necessary	Verification 10-15 isolates/drug
CLSI = FDA	Device manufacturer has not received FDA clearance of the device with updated CLSI/FDA BPs	Validation (if desire to use CLSI BPs) 30 isolates/drug
CLSI ≠ FDA	Manufacturer must provide FDA BPs; use of CLSI BPs would be off label	Validation (if desire to use CLSI BPs) 30 isolates/drug

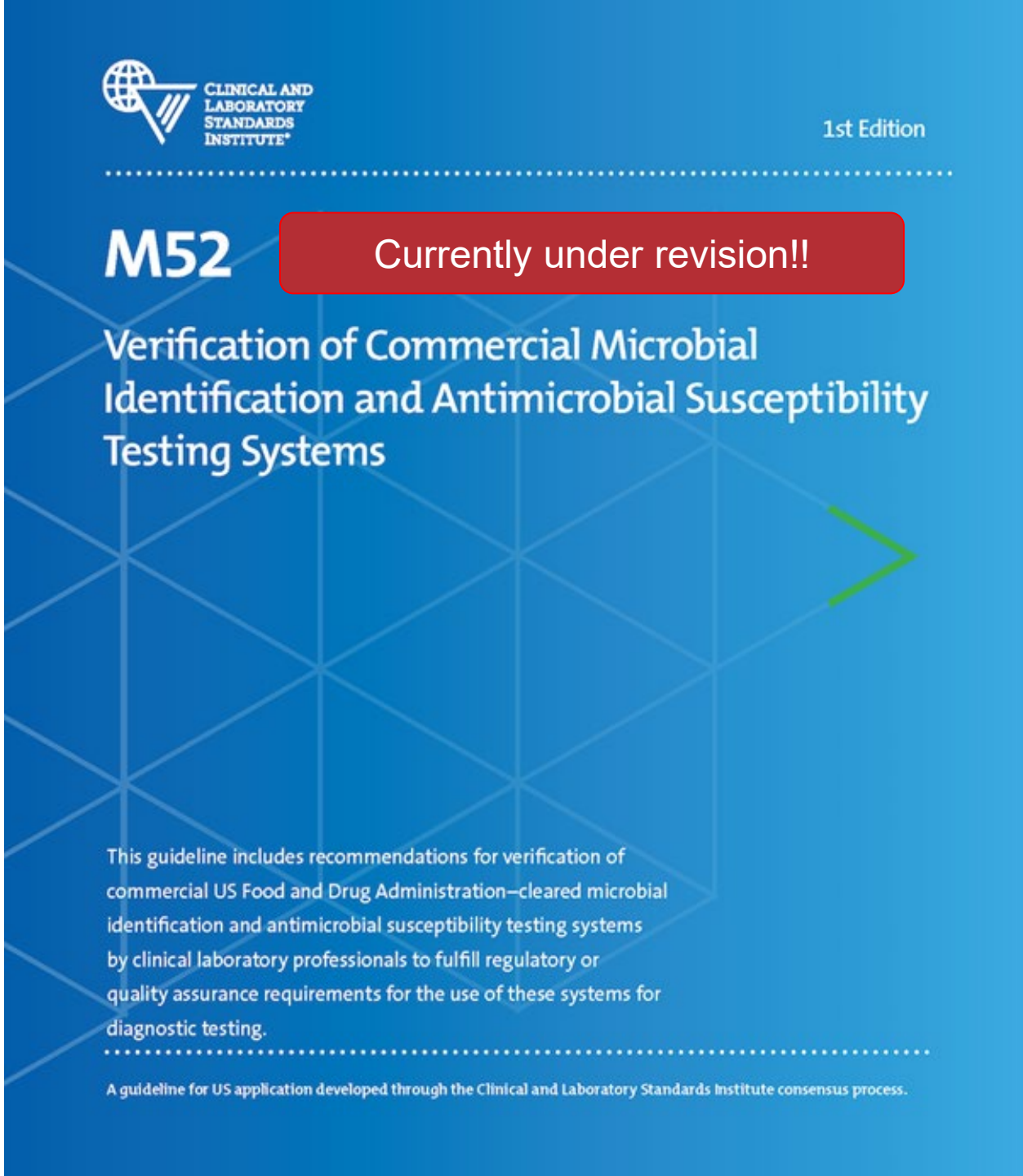
Draft, CLSI 2023 Breakpoint Implementation Toolkit

- Record lab's action plan in "Breakpoints in Use" spreadsheet!

Where is validation and verification guidance located?

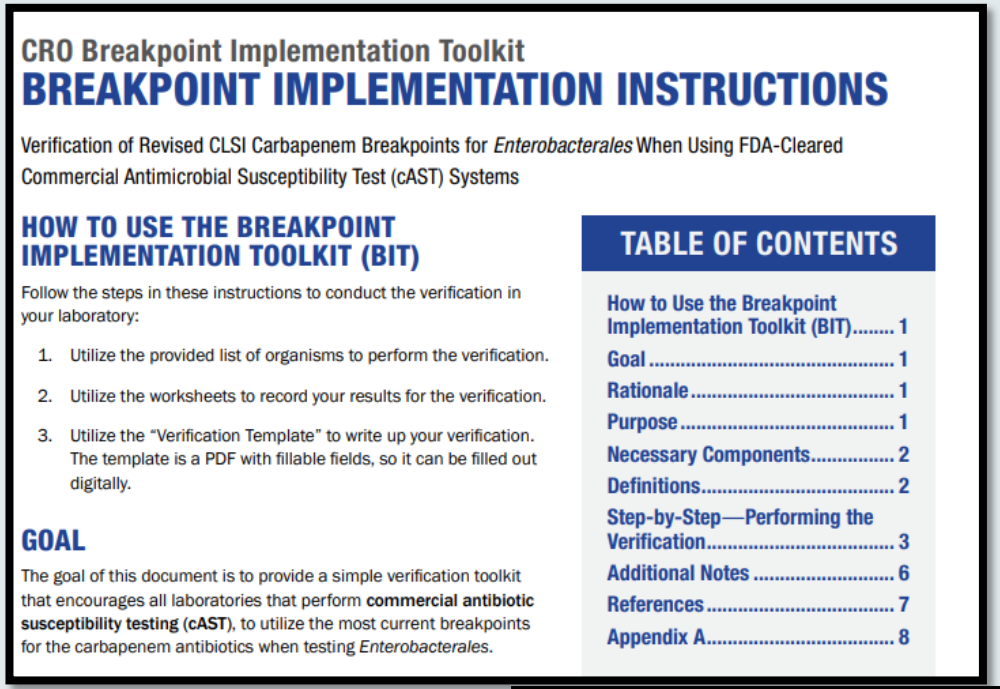
- **CLSI M52 1st Edition**: One stop shop for AST verification (and validation) needs
  - Entire process (pre-, post- study)
  - Isolates
  - Comparator method
  - Study Design (accuracy, precision, QC)
  - Acceptance Criteria
  - Discrepancy Testing

Generally geared towards **verification**, but Appendix B describes a **validation** for breakpoint updates.



Where is validation and verification guidance located?

- **ASM-APHL's Carbapenem Breakpoint Implementation Toolkit:** Toolkit specifically for verifying or validating updated Enterobacterales carbapenem breakpoints
  - Collaboration between APHL, ASM, and CDC
  - CLSI M52 guideline used as a basis
  - Includes detailed instructions, templates, worksheets, and isolate list
  - Available for **free** from APHL at: https://www.aphl.org/programs/infectious_disease/Pages/CRO-Breakpoint-Implementation-Toolkit.aspx



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)

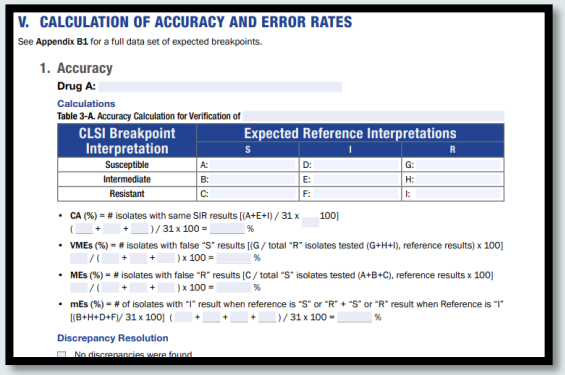
Follow the steps in these instructions to conduct the verification in your laboratory:

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



V. CALCULATION OF ACCURACY AND ERROR RATES

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

1. Accuracy

Drug A:

Calculations

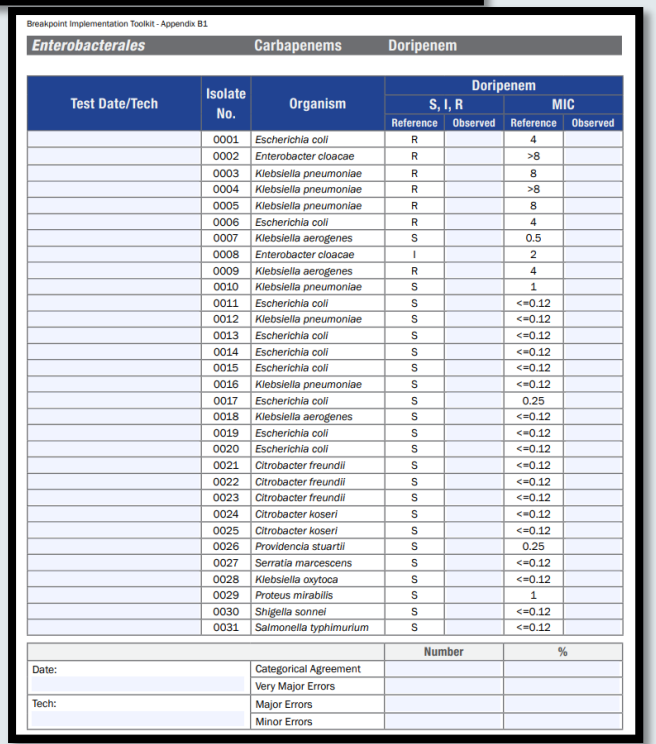
Table 3-A. Accuracy Calculation for Verification of

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 = %

Discrepancy Resolution

☐ No discrepancies were found



Breakpoint Implementation Toolkit - Appendix B1

Enterobacterales Carbapenems Doripenem

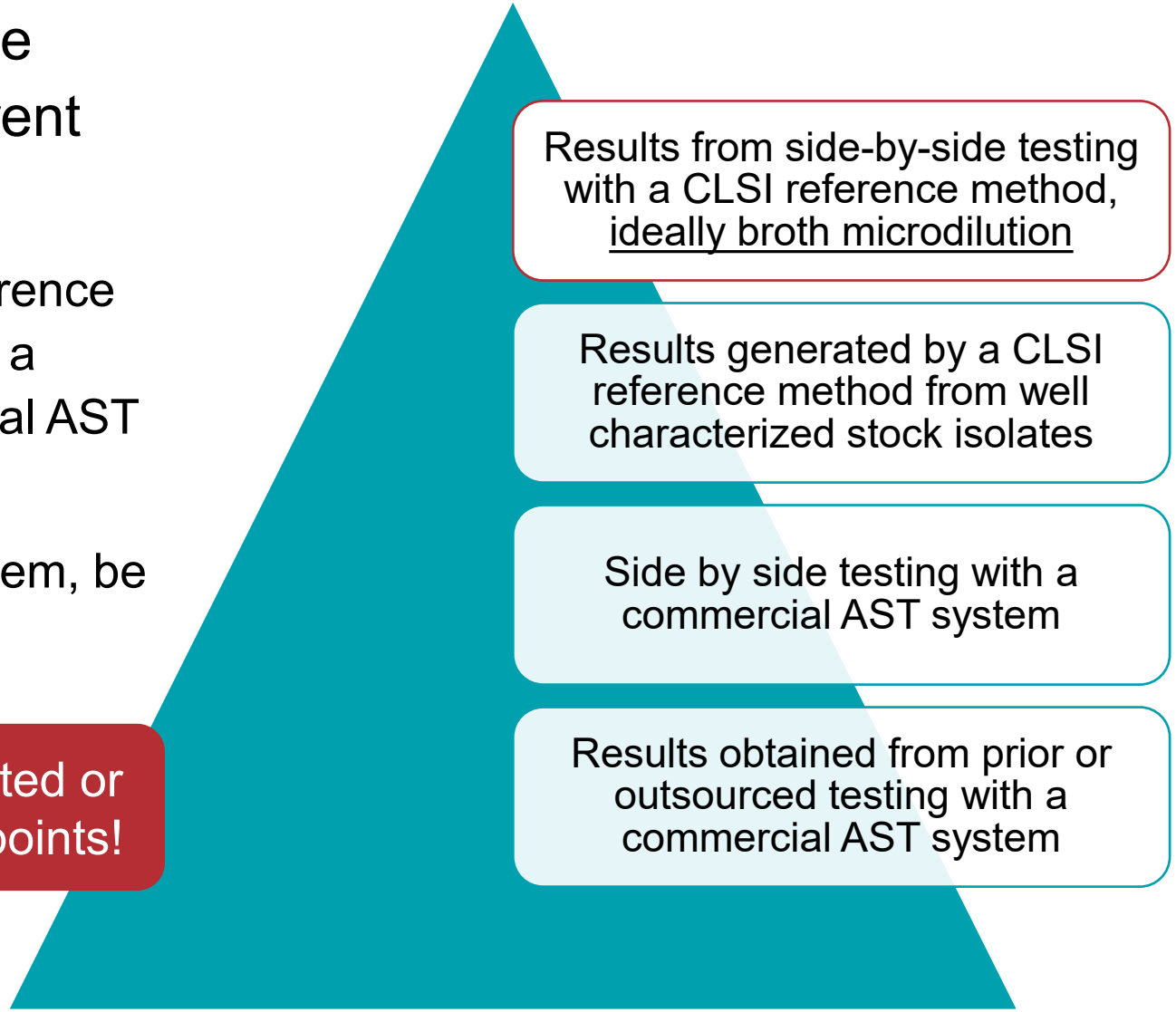
Test Date/Tech	Isolate No.	Organism	Doripenem S, I, R Reference	Doripenem S, I, R Observed	Doripenem MIC Reference	Doripenem MIC Observed
	0001	*Escherichia coli*	R		4	
	0002	*Enterobacter cloacae*	R		>8	
	0003	*Klebsiella pneumoniae*	R		8	
	0004	*Klebsiella pneumoniae*	R		>8	
	0005	*Klebsiella pneumoniae*	R		8	
	0006	*Escherichia coli*	R		4	
	0007	*Klebsiella aerogenes*	S		0.5	
	0008	*Enterobacter cloacae*	I		2	
	0009	*Klebsiella aerogenes*	R		4	
	0010	*Klebsiella pneumoniae*	S		1	
	0011	*Escherichia coli*	S		<=0.12	
	0012	*Klebsiella pneumoniae*	S		<=0.12	
	0013	*Escherichia coli*	S		<=0.12	
	0014	*Escherichia coli*	S		<=0.12	
	0015	*Escherichia coli*	S		<=0.12	
	0016	*Klebsiella pneumoniae*	S		<=0.12	
	0017	*Escherichia coli*	S		0.25	
	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		0.25	
	0027	*Serratia marcescens*	S		<=0.12	
	0028	*Klebsiella oxytoca*	S		<=0.12	
	0029	*Proteus mirabilis*	S		1	
	0030	*Shigella sonnei*	S		<=0.12	
	0031	*Salmonella typhimurium*	S		<=0.12	

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

What do I compare to?

- "Comparator" method or result: The method or result that the lab's current system being compared to.
  - CLSI standard reference method (reference broth microdilution or disk diffusion) or a previously validated/verified commercial AST system
  - When comparing to a commercial system, be aware of potential bias on bias

The comparator must be already validated or verified for use with the updated breakpoints!



How many isolates do I test for a breakpoint update?

- **Verification**: 10-15 isolates per drug
  - "Limited Verification" in CLSI M52
- **Validation**: 30 isolates per drug
  - Appendix B in CLSI M52

For breakpoint updates, it's important to test isolates that have results that span the range of the test, covering the interpretations, **including some resistant organisms**!

Tip: If performing multiple verifications or validations, select isolates that can be used for multiple antimicrobials.

Where can I get isolates?

- Lab's own isolates, fresh or frozen
- Another lab's (clinical or public health) isolate collection
  - Some labs may have their own well characterized isolates
- At cost isolate banks/repositories (ATCC, Laboratory Specialists, Inc [LSI])
- The Antibacterial Resistance Leadership Group (ARLG) maintains a virtual biorepository
- Emerging Infections Program (EIP)'s Active Bacterial Core surveillance (ABCs) biorepository
- Obtain isolates from the CDC & FDA Antimicrobial Resistant Isolate Bank (AR Bank)

Laboratory Specialists, Inc. (labspec.org)

Drug-resistant Bacteria | ATCC

ARLG VB Strain Catalogue (arlgcatalogue.org)

ABCs Isolate Bank Overview | CDC

FDA-CDC Antimicrobial Resistance Isolate Bank

What is the AR Bank?

- Interagency collaboration between CDC & FDA to address objectives in National Action Plan for Combatting Antibiotic Resistant Bacteria (aka “CARB”)
- Established in 2015 to provide isolates to labs free of charge
- Repository of organisms with a variety of resistance mechanisms and range of MIC results generated by reference BMD
- Curated panels of isolates to address various needs
  - Specific drug validation/verification studies
  - Resistant strains
  - Emerging or uncommon resistance mechanisms

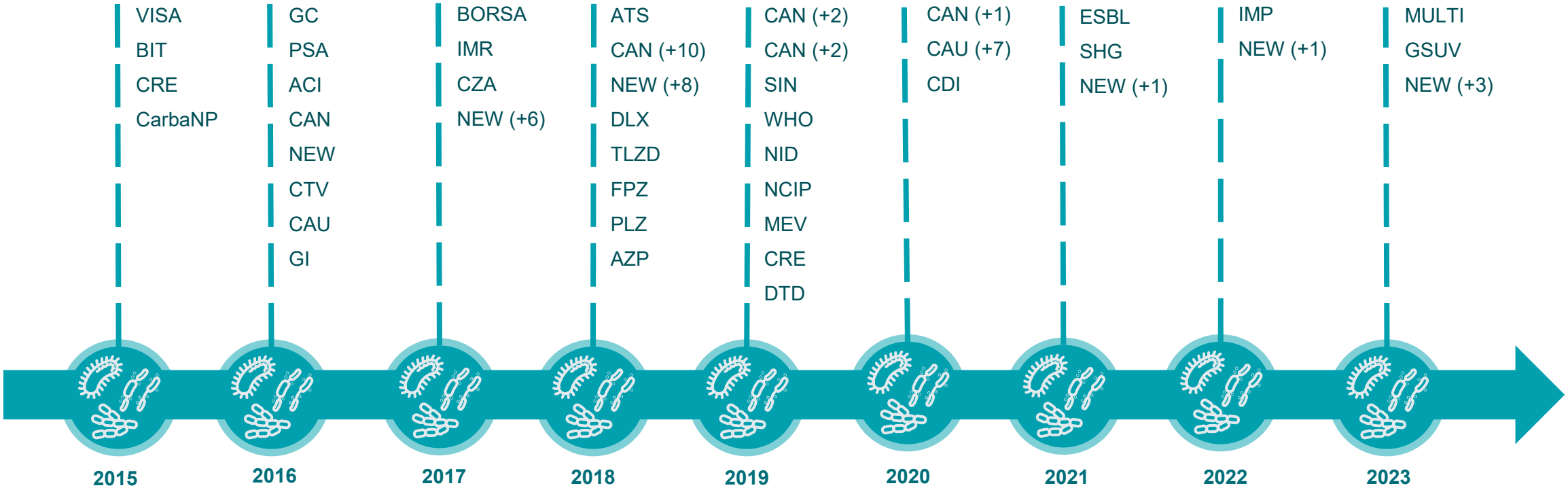
Photo: https://www.cdc.gov/drugresistance/resistance-bank/index.html

Important Links

Link to website: https://wwwn.cdc.gov/ARIsolateBank/
Link to FAQs: https://wwwn.cdc.gov/arisolatebank/QA

AR Bank over the years...

- 33 panels and >1000 isolates
- The AR Bank continues to grow, adding more panels or isolates based on the needs of the customers (fill out those surveys!)
- Movement towards using existing isolates for multiple purposes



Data provided courtesy of CDC & FDA AR Isolate Bank; does not include custom panels utilizing existing AR Bank strains (e.g., FDC, FPA). Refer to AR Bank website for full panel names. Numbers in parentheses indicate number of isolates added to an existing panel.

Recent survey of AR Bank orders

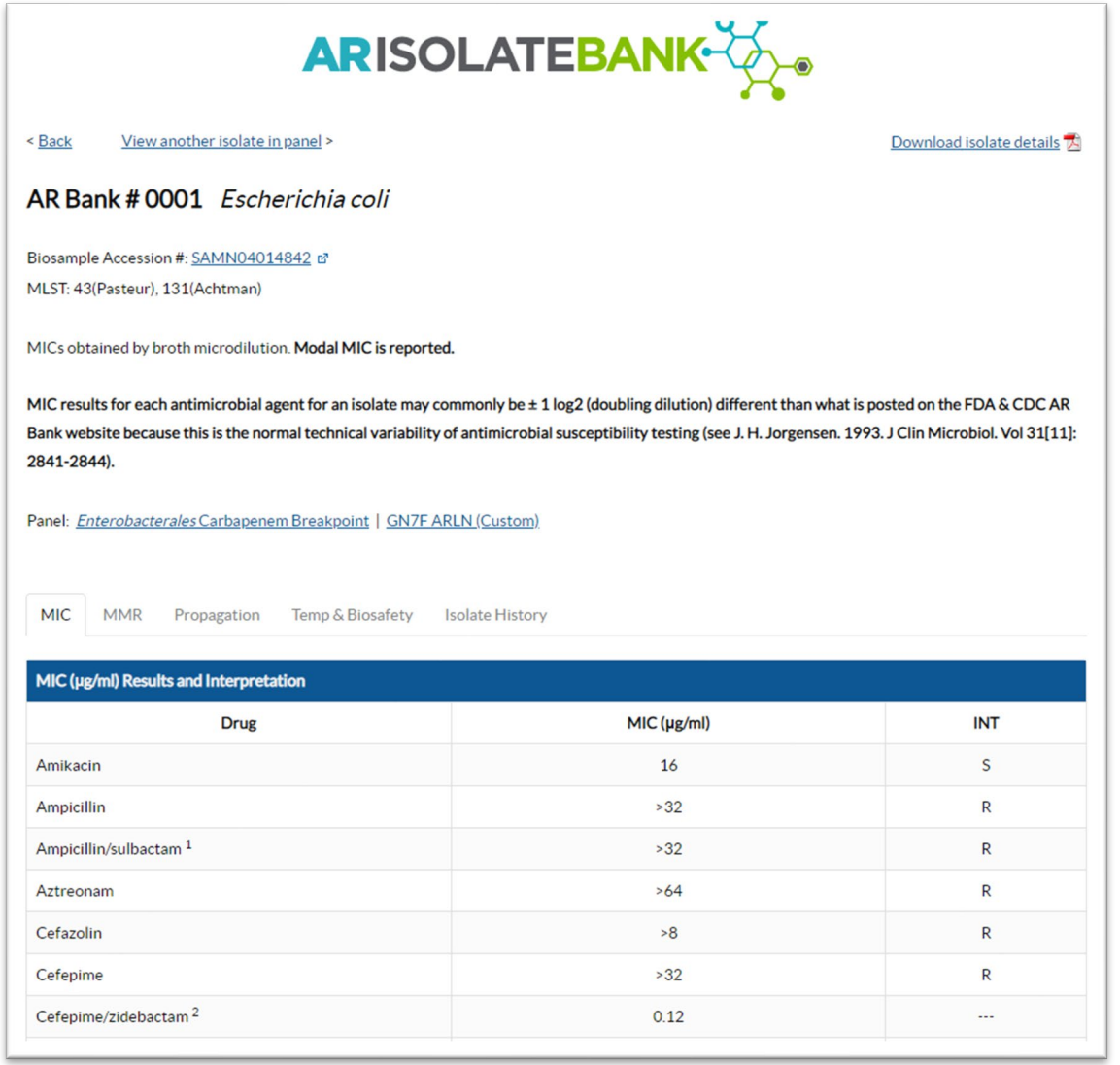
- AR Bank largely serves clinical laboratories
- Verification, validation, or proficiency testing is the most common use of AR Bank isolates.
  - Most frequent reason across all years, ~70% of users

Use of AR Bank Materials for delivered orders, n=569 (2022)	
Regulatory Submssion to FDA	**No. of Customers**
Yes	74
No	495
Use of Materials	**No. of Orders**
To aid in the development of a diagnostic test	85
To aid in the development of a new drug	32
Verification, validation or proficiency testing	386
Research	47
Other purpose	19
Type of Organization	**No. of Organizations**
Academic	42
Clinical Laboratory	386
Diagnostic Company	51
Pharmaceutical company	20
Federal Agency	4
Public Health Laboratory	48
Other	18

Data provided courtesy of CDC & FDA AR Isolate Bank

What data are available from AR Bank?

- The modal MIC result generated from replicate testing (≥ 3 datapoints) by **reference BMD method** per CLSI M07
- Phenotypic data (e.g., mCIM, BMD MBL screen, BMD ESBL screen)
- Whole genome sequencing (uploaded onto NCBI Biosample No. linked)
- Collection year and specimen source, country/region are added to some newer isolate panels
- MIC and WGS data can be **downloaded** to MS Excel file
  - By panel or for the entire collection of AR Bank isolates
- The **range** of observed MICs can be requested and provided on an ad-hoc basis for troubleshooting



What about isolates do I need?

- CLSI worked with AR Bank to curate specific isolate sets of gram-negative organisms (n= 30-35) and breakpoint updates.
  - Isolates from the most frequently ordered panels were prioritized.
  - Reproducibility was reviewed and some antimicrobials were refereed with commercial systems (piperacillin/tazobactam).

AR Bank Isolate Set Name	Reporting Group	Antimicrobials
BIT-1 (BIT)	Enterobacterales	Ertapenem, meropenem, imipenem, cefazolin (systemic), aztreonam, cefepime, ceftazidime, cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin
BIT-2 (PTA)	Enterobacterales	Piperacillin/tazobactam, gentamicin, tobramycin, amikacin, colistin
BIT-3 (FPA)	*Pseudomonas aeruginosa*	Meropenem, imipenem, aztreonam, cefepime, ceftazidime, piperacillin/tazobactam, ciprofloxacin, levofloxacin, colistin
BIT-4 (ACI)	*Acinetobacter baumannii*	Meropenem, imipenem, colistin

Details coming soon in the CLSI Breakpoint Implementation Toolkit…

How do I order isolates from the AR Bank?

1. Review BIT set and determine whether isolates need to be ordered
2. Register with AR Bank
3. Start shopping!
   - Add panels to cart and submit order
   - Only panels can be ordered, not individual isolates (exception for the Isolates with New or Novel Mechanism panel [NEW])
4. Complete any necessary forms (Simple Letter Agreement and Biosafety Compliance Agreement)
5. Pay for shipping, isolates are free
6. Receive frozen aliquots and store in -80°C freezer
   - Follow AR Bank instructions for proper storage and handling

What we've determined so far...

- Which breakpoints are obsolete
- What type of study we need to perform based on our device's current FDA-clearance status
- The comparator method or result
- Number and type of isolates to test
- Where to get these isolates

Now, let's start planning the study!

M52, 1st ed.

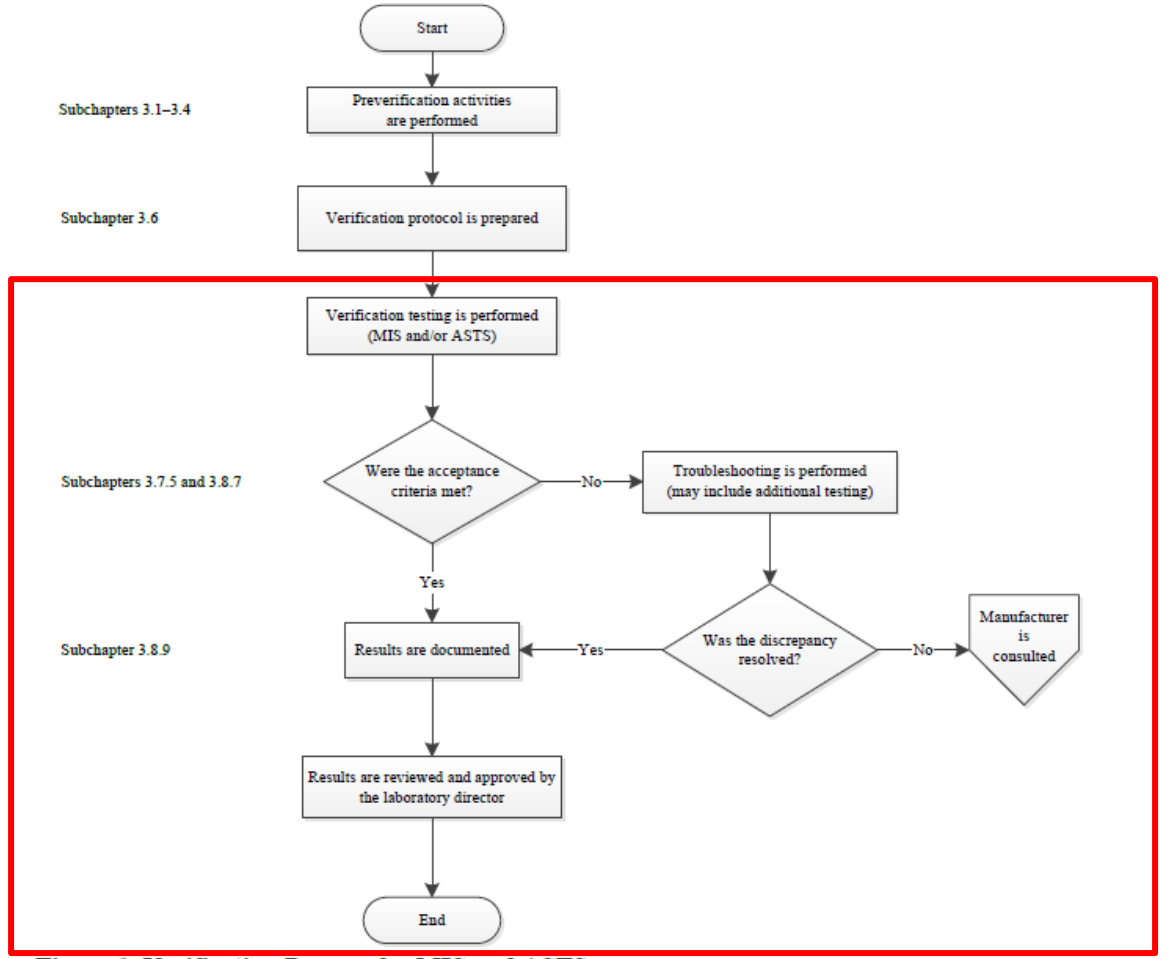
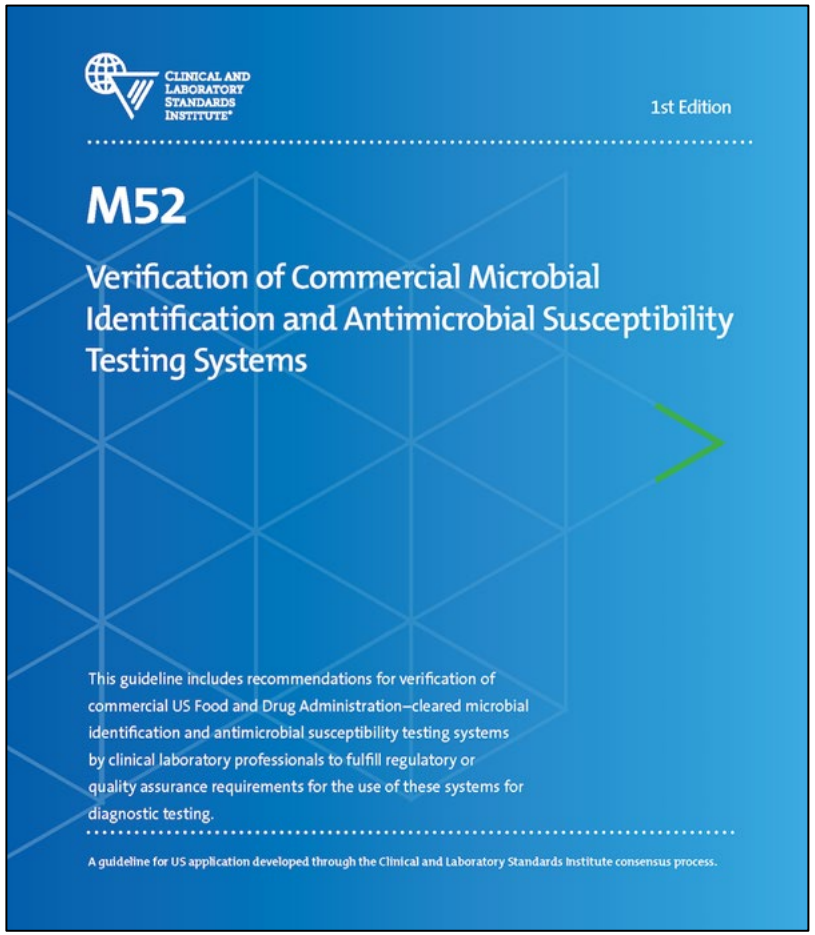


Figure 1. Verification Process for MIS and ASTS
Abbreviations: ASTS, antimicrobial susceptibility testing system(s); MIS, microbial identification system(s).

What performance characteristics do I measure for an AST validation or verification?

- Typical verification* for **new** devices
  - Accuracy (30 isolates per drug)
    - ➢ Essential Agreement
    - ➢ Categorical Agreement + Errors
  - Precision/Reproducibility (3-5 isolates)
    - Can use QC strains to create data for IQCP
  - QC Testing
    - Test QC each day of testing
  - Training
    - Competency assessments

Modifications to assessing performance characteristics when implementing updated breakpoint on an existing system!



*For validation, test more isolates to establish the same performance characteristics

Quick review of definitions: Accuracy (Agreement)

Accuracy: The agreement of the results from the commercial test system with the results generated by the comparator method for the same isolate. Inherent variability of ASTs is incorporated into accuracy (typically* ±1 doubling dilution for bacteria or ±2 doubling dilutions for fungi).

CLIA Performance Specifications for new Breakpoint Validation	Definition
Essential Agreement (EA) (qualitative measure)	When the MIC result obtained by the commercial AST system is within one doubling dilution step (two-fold serial dilution) from the comparator result. • >8 • >8 • 8 • 8 Note: When comparing gradient diffusion strips to a MIC from a BMD system, always **round up** the 'in-between' results from the gradient strip!
Category Agreement (CA) (qualitative measure)	When the S, I, SDD, or R interpretation agree between results generated the commercial AST system and comparator method. • S and S are in CA • S and I are <u>not</u> in CA

Not necessary to summarize EA for validations or verifications to implement updated breakpoints!

Quick review of definitions: Accuracy (Error types)

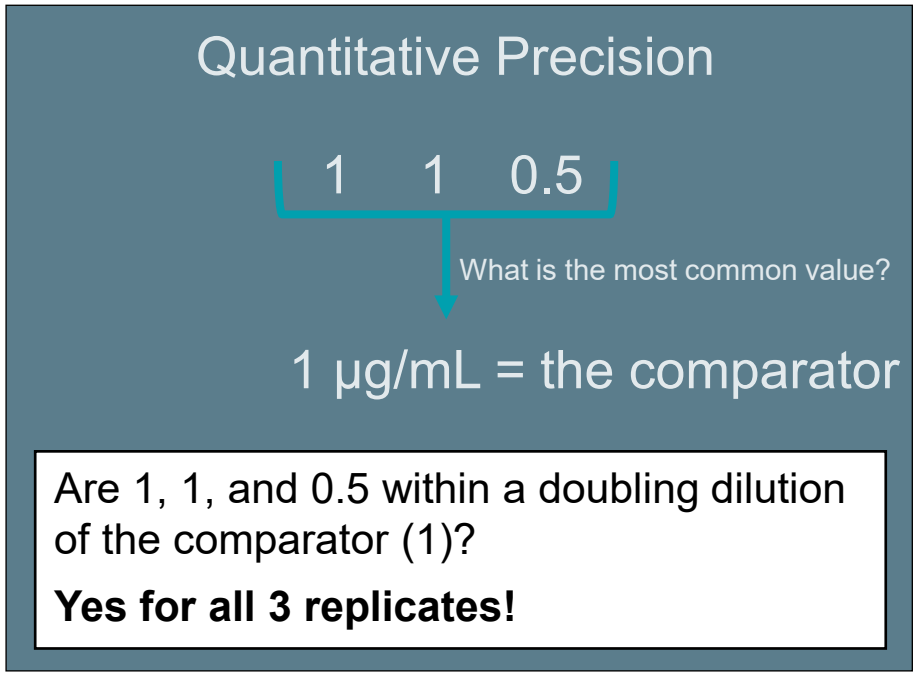
Error types provide more information on what's driving imperfect categorical agreement

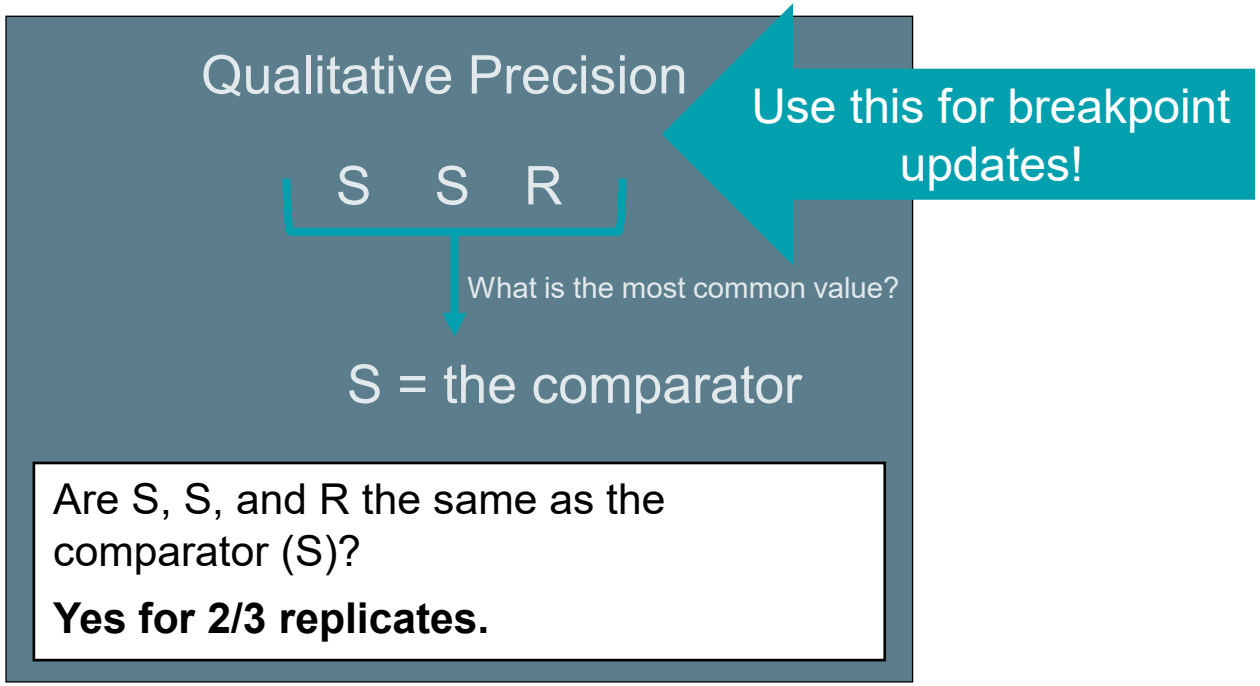
Error Type	Definition
Minor	Error when either the comparator result's or commercial AST result's interpretation is intermediate (or SDD) and the other is susceptible or resistant. The overall minor error frequency is expressed as a percentage of results with a minor error divided by the **total number** of results.
Major	Error when the **comparator** result's interpretation is **susceptible** and the **commercial AST's** result is **resistant**. The overall major error frequency is expressed as a percentage of results with a major error divided by the **total number of susceptible** comparator results.
Very Major	Error when the **comparator** result's interpretation is **resistant** and the **commercial AST's** result is **susceptible**. The overall major error frequency is expressed as a percentage of results with a major error divided by the **total number of resistant** comparator results.

Quick review of definitions: Precision/Reproducibility

Precision (or reproducibility): the closeness of agreement between results (MIC or interpretation) obtained by independent replicate measurements (separate inoculum preparations) on the test system under the same conditions. The "comparator" result is the mode (or median if mode is not available) of all replicate results that occurred per isolate/drug (n=3).

Precision is independent of the comparator method… you can have a precise inaccurate device! Often, precision is performed after accuracy of the device has been established.





Note: For AST systems, the terms precision and reproducibility are used interchangeably. For the purpose of the presentation, precision will be used.

How to Design Your Breakpoint Update Study: Validation vs. Verification

Device Clearance Status for Updated Breakpoints	Study Type	Accuracy						Precision	QC Needed	Competency Assessment Needed
		# Isolates per drug	CA	EA	mE	ME	VME			
Current device is <u>not</u> FDA-cleared for new BP. Modified FDA-cleared test.	Validation	30	✓	X	✓	✓	✓	Yes	No, follow current QC/IQCP protocols	Not specifically for the validation
Current device is FDA-cleared for new BP. Unmodified FDA-cleared test.	Verification	10-15	✓	X	✓	✓	✓	Yes	No, follow current QC/IQCP protocols	Not specifically for the verification

What is considered acceptable for accuracy and precision?

Study Component	Characteristic	The Math	Acceptable Criteria
Accuracy	Category Agreement	$\frac{\text{\# with same S, I, SDD, or R result by reference}}{\text{Total isolates tested}} \times 100$	≥ 90%, but it **can** be less in certain circumstances
	Minor Error (minE)	$\frac{\text{\# with minE}}{\text{Total isolates tested}} \times 100$	No criteria, recommended ≤10%
	Major Error (ME)	$\frac{\text{\# with ME}}{\text{Total "S" tested}} \times 100$	< 3%, it can be more depending on denominator N*
	Very Major Error (VME)	$\frac{\text{\# with VME}}{\text{Total "R" tested}} \times 100$	< 3%, it can be more depending on denominator N*
Precision		$\frac{\text{\# equivalent replicates compared to the mode or median category}}{\text{Total isolates tested}} \times 100$	95% of all data points by antimicrobial

*An approach could be to set a threshold to a number of errors, rather than a percentage. This was used in the APHL CRO Toolkit.

When do I start testing?

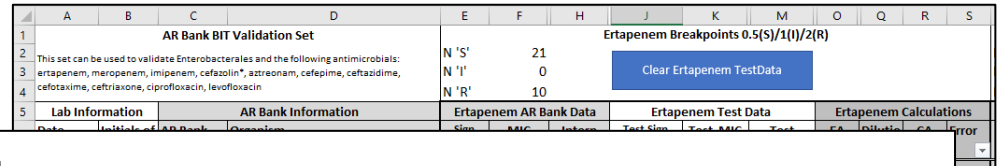
- ✓ Identify which breakpoints are obsolete
- ✓ Determine the type of study we need to perform based on our device's current FDA-clearance status
- ✓ Prioritize updates with antibiotic stewardship and clinical teams
- ✓ Select the comparator method or result
- ✓ Specify the number and type of isolates to test
- ✓ Determine how to get isolates
- ✓ Define acceptance criteria
- ✓ Submit the verification/validation protocol* to your medical director for approval
- ✓ Engage IT staff

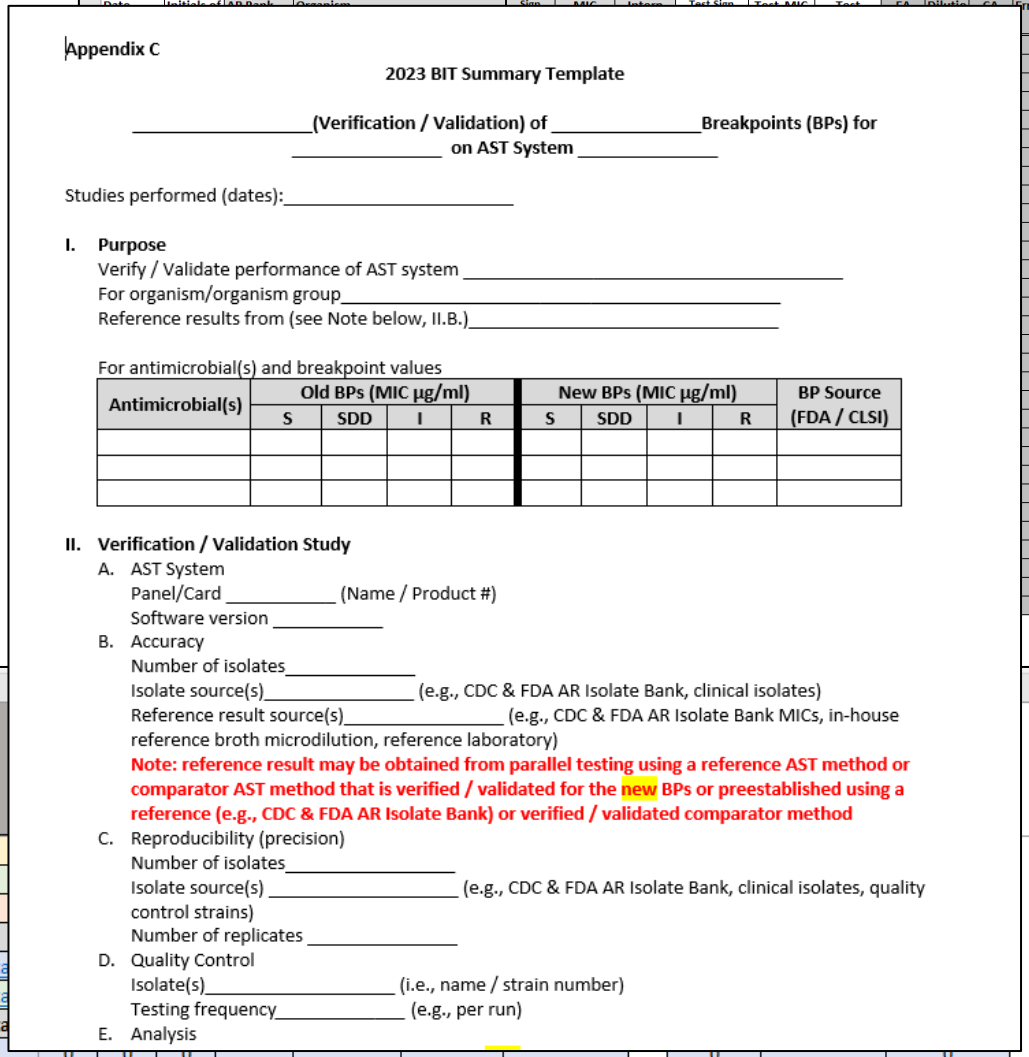
Let's start testing!

*The full protocol should include purpose, description of the system and comparator method, policy considerations, and description of testing components (performance characteristics, QC testing, acceptability, discrepancy resolution procedures, method used to analyze the results). CLSI M52ED1 Section 3.6.

Are there any tools to help with documentation and calculations? There will be soon...

- Part of the new 2023 CLSI Breakpoint Implementation Toolkit includes an *interactive* **MS Excel Workbook** aligned with AR Bank "BIT" sets.
  - AR Bank comparator data already filled in
  - Calculations for accuracy and precision automatically performed
  - Summary of aggregated data
- Record results in validation/verification template





Appendix C

2023 BIT Summary Template

_____(Verification / Validation) of _____Breakpoints (BPs) for _____ on AST System _____

Studies performed (dates):_____

I. **Purpose**

Verify / Validate performance of AST system _____
For organism/organism group_____
Reference results from (see Note below, II.B.)_____

For antimicrobial(s) and breakpoint values

Antimicrobial(s)	Old BPs (MIC µg/ml)				New BPs (MIC µg/ml)				BP Source (FDA / CLSI)
	S	SDD	I	R	S	SDD	I	R	

II. **Verification / Validation Study**

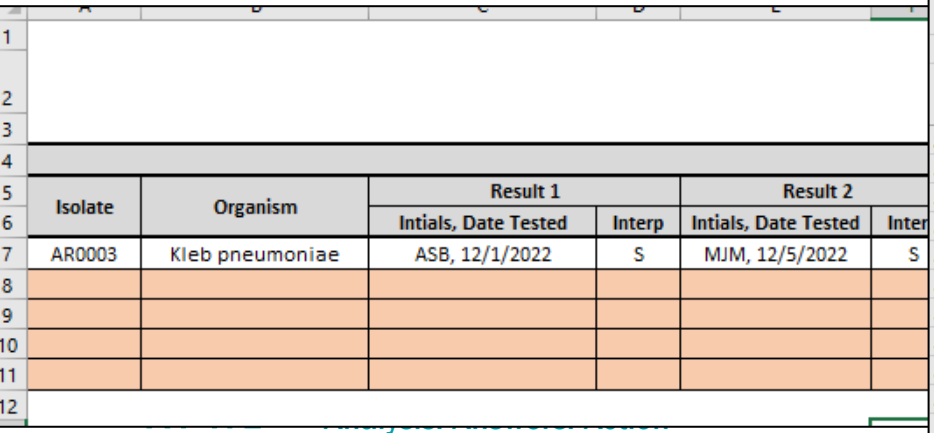
A. AST System
Panel/Card _____ (Name / Product #)
Software version _____

B. Accuracy
Number of isolates_____
Isolate source(s)_____ (e.g., CDC & FDA AR Isolate Bank, clinical isolates)
Reference result source(s)_____ (e.g., CDC & FDA AR Isolate Bank MICs, in-house reference broth microdilution, reference laboratory)
Note: reference result may be obtained from parallel testing using a reference AST method or comparator AST method that is verified / validated for the new BPs or preestablished using a reference (e.g., CDC & FDA AR Isolate Bank) or verified / validated comparator method

C. Reproducibility (precision)
Number of isolates_____
Isolate source(s) _____ (e.g., CDC & FDA AR Isolate Bank, clinical isolates, quality control strains)
Number of replicates _____

D. Quality Control
Isolate(s)_____ (i.e., name / strain number)
Testing frequency_____ (e.g., per run)

E. Analysis



Isolate	Organism	Result 1		Result 2	
		Intials, Date Tested	Interp	Intials, Date Tested	Inter
AR0003	Kleb pneumoniae	ASB, 12/1/2022	S	MJM, 12/5/2022	S

Set	Reporting Group	Antimicrobial											
BIT-1	Enterobacterales	Meropenem											
BIT-3	*Pseudomonas aeruginosa*	Meropenem											
BIT-4	*Acinetobacter baumannii*	Meropenem											
NA	**Combined**	**Meropenem**											
BIT-2	Enterobacterales	Piperacillin/tazobacta											
BIT-3	*Pseudomonas aeruginosa*	Piperacillin/tazobacta											
NA	**Combined**	**Piperacillin/tazobacta**											
BIT-2	Enterobacterales	Tobramycin	0	0	0						0		0
BIT-3	*Pseudomonas aeruginosa*	Tobramycin	0	0	0						0		0
NA	**Combined**	**Tobramycin**	**0**	**0**	**0**						**0**		**0**

How do I approach discrepancy testing?

Troubleshoot to determine if discrepancy testing is necessary.

Generally, ME and VME are always repeated.

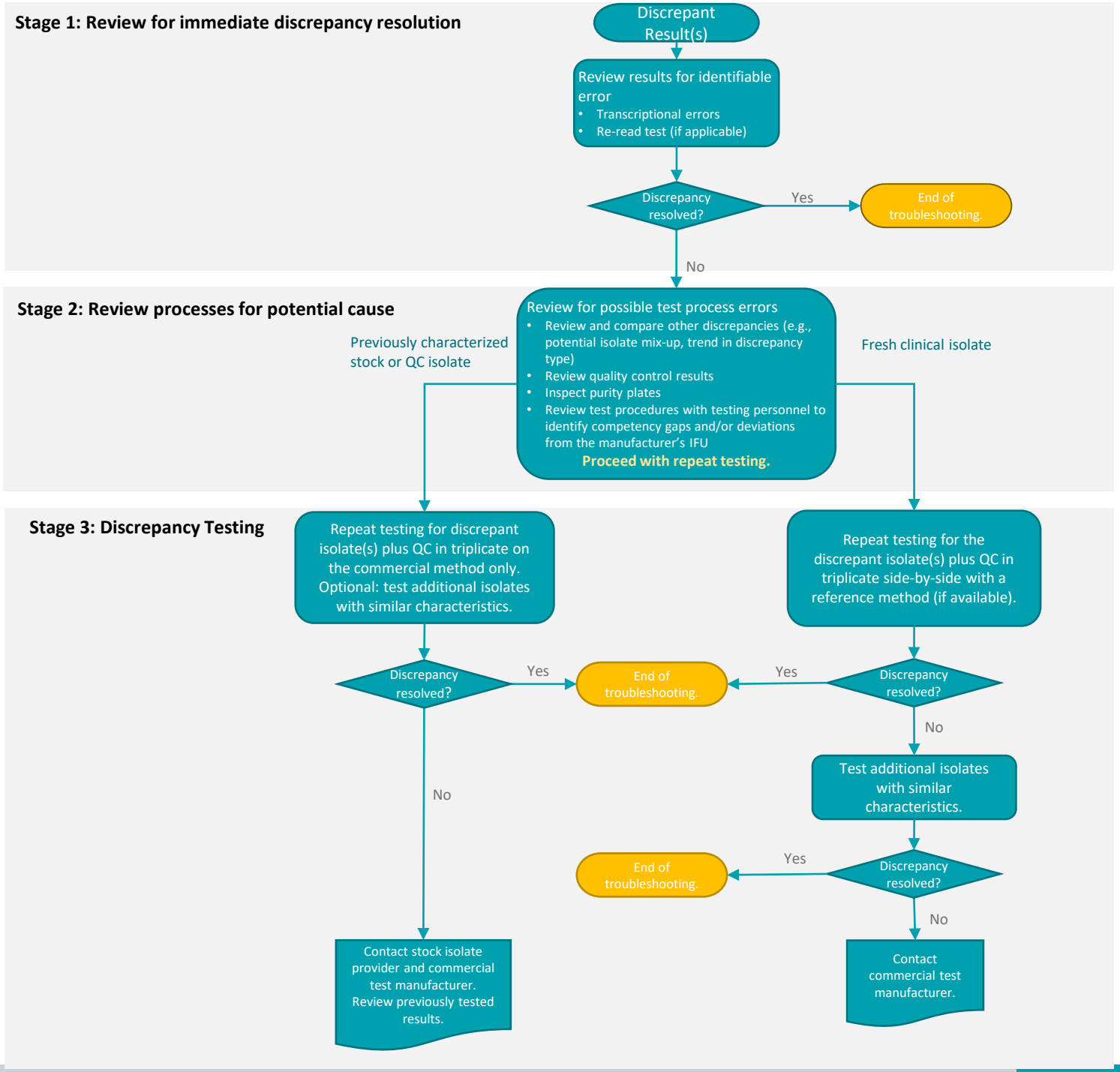
1. Review for immediate discrepancy resolution.
2. Review process for potential cause.
3. Conduct discrepancy testing.

Tip: Review your data as isolates are being tested to proactively identify discrepancies.

Conducting Discrepancy Testing

- Triplicate testing performed side by side (same inoculum preparation) with the new and comparator or reference system
  - Use the modal value
- Test another validated or verified method side-by-side to arbitrate
- Replace discrepant result with the new result
- If discrepancy reproduces, contact stock isolate provider (if using stock isolates) and/or manufacturer
  - Manufacturer may request the isolate to test under their optimal conditions
  - Consider replacing the isolate with a similar isolate, test more isolates
- Consider utilizing result range (i.e., MIC range, zone of inhibition range), if available, for justification or acceptability of a discrepancy

CLSI M52 3.8.4 Precision
Some clinical isolates, especially those producing ESBLs or other β-lactamases, may produce MIC values that vary over **four or more dilutions** for reference broth dilution testing."



Is <90% CA really a 'failure'? NO!

- **<90% agreement may be considered acceptable.** Analyze what is driving failures. Are you seeing very good EA? What type of errors are driving the CA? If there are high minE, are those MICs on-scale and within a dilution? Are you seeing errors in a certain direction? If errors are in certain direction, did the manufacturer note this too? Did your QC pass throughout the study? Is it one drug that is not performing well or most drugs? Would the CA be <90% with old breakpoints?
- If errors are in EA, their CA may be considered acceptable as this not a true indicator of poor performance, but as a corollary of the MIC result to the breakpoint location.
- Look at number of ME and VME, as these denominators are S or R by reference, respectively. Looking solely at % ME and VME is misleading (1 VME for where there are only 4 resistant isolates is 25% VME).
- Weigh the risks of the type of error with new breakpoints versus continuing to use old breakpoints.

At the end of the day, acceptable criteria is up to the medical director.

Sources

CLSI Methods Development and Standardization Working Group Best Practices for Evaluation of Antimicrobial Susceptibility Tests

Authors: Romney M. Humphries, Jane Ambler, Stephanie L. Mitchell, Mariana Castanheira, Tanis Dingle, Janet A. Hindler, Laura Koeth, Katherine Sei, and on behalf of the CLSI Methods Development and Standardization Working Group of the Subcommittee on Antimicrobial Susceptibility Testing | AUTHORS INFO & AFFILIATIONS

"There is a directly proportional relationship between the categorical error rate and the percentage of isolates that hover around the intermediate MIC breakpoint by 1 $\log_2$ dilution (i.e., intermediate MIC plus 1 $\log_2$ dilution and intermediate MIC minus 1 $\log_2$ dilution) or the susceptible/resistant breakpoint (if no intermediate category is recognized)."

These sources can be used to justify the verification of a drug displaying low category agreement driven by minor errors. You're not alone, CDC has done this in the past!

Guidance for Industry and FDA

Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems

Tables 8 and 9 show the discrepancy rate and minimum acceptable EA rate we recommend. Discrepancy rates will vary depending on the proximity of the MIC of the organisms tested to the interpretative categories. FDA considers the following to be acceptable performance for the clinical data for AST devices for all organisms appropriate for testing:

- Percent essential and category agreement > 89.9 %. A CA of < 90% may be acceptable under certain circumstances (e.g., very good EA of the evaluable test results with the majority of the discrepancies as minor discrepancies).
- A maj rate of ≤ 3% based on the number of susceptible organisms tested.
- A vmj rate based on the number of resistant organisms tested. Table 8 lists the numbers of very major discrepancies as a function of the total number of resistant organisms tested with proposed statistical criteria for acceptance that include an upper 95% confidence limit for the true vmj rate of ≤ 7.5% and the lower 95% confidence limit for the true vmj ≤ 1.5%.
- Growth failure rates in the system < 10% for any genus or species tested.

Justification language we've used:

Modified Summary Results based on Revised Acceptance Criteria

We propose a modified summary of results based on revised acceptance criteria featuring lowered minimum CA caused by higher minor error (mE) rate allowance. The rationale for accepting the revised criteria is described below:

Higher mE rates were driven by the high proportion of isolates displaying MICs near or at the breakpoints, where inherent variation for EA would cause mEs. In the sets of clinical samples tested, these isolates are over-represented, and they would likely not occur at this frequency in routine testing.

The method is performing as expected, based on EA ([range], [overall EA]) for all clinical isolates tested and reproducibility (X%) for a selected group of X clinical isolates. In addition, errors are not systematically higher or lower.

Option1: We did not observe any ME or VME – the overall risk for these errors should be low based on the test performance (EA and reproducibility).

Option2: We did observe few ME or VME, but no more than one of each per drug/organism group – the overall risk for these errors should be low based on the test performance (EA and reproducibility).

Am I done yet?

- After your validation or verification summary has received approval, start utilizing the new breakpoints.
  - Ensure your IT team has been engaged throughout the process!
- Don’t forget to notify appropriate stakeholders (e.g., antimicrobial stewardship team, clinical staff, pharmacy)!
- Continue to monitor CLSI and FDA for breakpoint updates… they are continually revised.

Resources Summary

Regulatory

- CLIA standard § 493.1253(b)(1) for verification of test systems
- CLIA standard § 493.1253(b)(2) for validation of test systems
- CAP requirements for recording (MIC.11380) and implementing updated breakpoints (MIC.11385) in e-LAB Solutions Suite.
- On demand CLSI and CAP Webinar entitled “Breakpoints Matter: Understanding CLSI Efforts and New CAP Requirement to Ensure Antimicrobial Treatment for all Patients”

Process/Implementation

- CLSI M52ED1 Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems
- APHL-ASM’s CRO Breakpoint Implementation Toolkit
- CLSI 2023 Breakpoint Implementation Toolkit (*pending release*)
- Refer to slides for isolate resources.

Most Recent Breakpoints

- FDA’s Antibacterial Susceptibility Test Interpretive Criteria Website
  - Link to sign up for email updates
- CLSI M100 Performance Standards for Antimicrobial Susceptibility Testing
  - Annual M100 update webinar

Device’s Current Breakpoint Clearance

- Your device’s most recent IFU
- FDA’s 510(k) Premarket Notification database

Resources in the queue ...

Timeline

- CLSI ad-hoc working group for breakpoint implementation (Outreach Working Group)'s Breakpoint Implementation Toolkit (collab with ASM, APHL, CAP)
  - Summarizes all steps described in this talk in one 'toolkit'
    - Flowchart, introduction narrative, documentation for breakpoints in use (Excel), list comparing 2023 CLSI breakpoints to FDA or FDA-recognized breakpoints (Excel), verification/validation template, curated and refereed custom AR Bank isolates sets to address specific breakpoints, and tool to aid in result documentation and calculations for MIC tests (Excel)
  - Will include short 'how to' videos
- Revised CLSI M52: revision will address only verification
- (Potential) New CLSI validation guideline: A proposal is being reviewed for a separate CLSI document to address validations (pending consensus process)

References addressing potential negative impact of using obsolete BPs

- Ambrose PG, Bhavnani, SM, Andes, DR et al. Old in vitro antimicrobial breakpoints are misleading stewardship efforts, delaying adoption of innovative therapies, and harming patients. *Open Forum Infectious Diseases*, Volume 7, Issue 7, July 2020, ofaa084, https://doi.org/10.1093/ofid/ofaa084.

- Bartsch SM, Huang SS, Wong KF, Slayton RB, McKinnel JA, Sahm DF, Kazmierczak K, Mueller LE, Jernigan JA, Lee BY. 2016. Impact of Delays between Clinical and Laboratory Standards Institute and Food and Drug Administration Revisions of Interpretive Criteria for Carbapenem-Resistant *Enterobacteriaceae*. J Clin Microbiol 54: 2757-62.

- Humphries RM, Hindler JA, Epson E, et al. Carbapenem-resistant Enterobacteriaceae detection practices in California: what are we missing? Clin Infect Dis. 2018 Mar 19;66(7):1061-1067. https://doi.org/10.1093/cid/cix942. PMID: 29099915.

- Johnson, WM, JA Clark, K Olney et al. 2022. Changing times: The impact of gram-negative breakpoint changes over the previous decade. Antimicrob Stewardship & Healthcare Epidem, 2(1), E165. https://doi.org/10.1017/ash.2022.301.

- Shugart A, Walters MS, Weiner LM, Lonsway D, Kallen AJ. 2018. Hospital microbiology laboratory practices for Enterobacteriaceae; Centers for Disease Control and Prevention National Healthcare Safety Network (NHSN) annual survey, 2015 and 2016. Infect Control Hosp Epidemiol 39: 1115-7.

- Yarbrough ML, Wallace MA, Potter RF, D'Souza AW, Dantas G, Burnham CD. Breakpoint beware: reliance on historical breakpoints for Enterobacteriaceae leads to discrepancies in interpretation of susceptibility testing for carbapenems and cephalosporins and gaps in detection of carbapenem-resistant organisms. Eur J Clin Microbiol Infect Dis. 2020 Jan;39(1):187-195. https://doi.org/10.1007/s10096-019-03711-y. Epub 2019 Nov 2. PMID: 31679102.

References addressing why BPs were updated

- Humphries RM, Abbott AN, Hindler JA. Understanding and addressing CLSI breakpoint revisions: a primer for clinical laboratories. J Clin Microbiol. 2019 May 24;57(6): e00203-19. doi: https://doi.org/10.1128/JCM.00203-19. PMID: 30971460; PMCID: PMC6535595.

- Newitt, VN. AST and safety at core of microbiology checklist changes. *CAP Today.* October 2021. https://www.captodayonline.com/ast-and-safety-at-core-of-microbiology-checklist-changes/ Accessed February 21, 2023.

- Prinzi, Andrea. Updating Breakpoints in Antimicrobial Susceptibility Testing. ASM Articles. February 2022. https://asm.org/Articles/2022/February/Updating-Breakpoints-in-Antimicrobial-Susceptibili Accessed March 3, 2023.

- Simner, PJ, CA Rauch, IW Martin et al. 2022. Raising the Bar: Improving Antimicrobial Resistance Detection by Clinical Laboratories by Ensuring Use of Current Breakpoints. Open Forum Infectious Diseases. *Open Forum Infectious Diseases*, Volume 9, Issue 3, March 2022, ofac007, https://doi.org/10.1093/ofid/ofac007

Presentation Wrap Up

- Validation and verification studies are needed when implementing updated breakpoints.
- Acceptance criteria for validation and verification data may be modified in certain scenarios.
- Guidance for validation and verification studies are currently available and targeted resources for breakpoint updates will soon be available through CLSI.

Acknowledgements

- CLSI ad-hoc Working Group for Breakpoint Implementation
- Maria Machado (CDC), CDC & FDA AR Isolate Bank
- CLSI Document Development Committee for M52 Revision
- Co-panelists

Thank you!

Questions?

Supplemental Slides

Do not share in workshop packet.

Example of Accuracy data

Antimicrobial: Imipenem (1/2/4)

Lab Information		Isolate Information		Comparator Results		Test System XZY Results		Essential Agreement (Y/N)	Category Agreement (Y/N)	If Category Agreement is No, what is the type of error?
Date Tested	Initials of Tester	Isolate	Organism	MIC	S,I,R	MIC	S,I,R			
1/10/2023	ASB	1	E. coli	>16	R	>8	R	YES	YES	
1/10/2023	ASB	2	E. cloacae	8	R	8	R	YES	YES	
1/10/2023	ASB	3	K. pneumoniae	4	R	2	I	YES	NO	minE
1/10/2023	ASB	4	K. pneumoniae	1	S	0.5	S	YES	YES	
1/10/2023	ASB	5	E. coli	2	I	1	S	YES	NO	minE
1/10/2023	ASB	6	K. aerogenes	≤0.5	S	≤0.5	S	YES	YES	
1/10/2023	ASB	7	K. pneumoniae	>16	R	1	S	NO	NO	VME
1/10/2023	ASB	8	K. pneumoniae	>16	R	>8	R	YES	YES	
1/10/2023	ASB	9	E. coli	≤0.5	S	≤1	S	YES	YES	
1/10/2023	ASB	10	E. coli	16	R	>8	R	YES	YES	

Panel Details

Download panel MICs and WGS

Download MICs, Biosafety and WGS disclaimers

< Back View another panel > Download this page Download MIC & gene data Download all isolate details

Enterobacterales Carbapenem Breakpoint

This panel was assembled to challenge tests that assess carbapenem susceptibility of *Enterobacterales.* Isolates in this collection demonstrate a range of carbapenem susceptibility. The carbapenem-resistant isolates also have different resistance mechanisms (i.e., some isolates produce a carbapenemase and others do not).

Add Panel to Cart

Panel ID: BIT

MICs obtained by broth microdilution. **Modal MIC is reported.**

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 J. H. Jorgensen. 1993. J Clin Microbiol. Vol 31[11]: 2841-2844).

Showing 1 - 25 of 31 records Search:

AR Bank #	Organism	Resistance Mechanisms	Biosample Accession #
0001	*Escherichia coli*	aac(6')-Ib-cr, aadA5, ACRF, catB4, dfrA17, **KPC-3**, MDF(A), mph(A), OXA-1, sul1, tet(A), tet(R)	SAMN04014842

Quick view to AR mechanisms and hyperlink to NCBI

AR0346-*E.coli (ESBL)*-Ceftolozane/tazobactam

MIC (ug/mL)	N Observed
1	1
2	2
4	1
8	4
16	2
>16	1

6 dilution spread across all categories

AR0300 *(A. baumannii)*-Amp/sulbactam

MIC (ug/mL)	N Observed	Int
8	3	S
16	8	I
32	3	R

Nice 3 dilution spread, but spans around the breakpoint. AR0300 was the only *Acinetobacter baumannii* with an intermediate interpretation.

AR0075 *(K. pneumoniae)*-Tigecycline

MIC (ug/mL)	N Observed	Int
2	4	S
4	6	I

Tight 2 dilution spread,
but evenly seen as S and I

AR0010 (*E. coli*)-Cefepime

MIC (ug/mL)	N Observed	Int
4	8	SDD
8	13	
16	17	R

Uneven 3 dilution spread.

AR0082 *(P. rettgeri)*-Meropenem

MIC (ug/mL)	N Observed	Int
1	2	S
2	3	I
4	7	R
8	16	

4 dilution uneven spread

Is it just AR Bank isolates?

No! This also happens with QC strains (their ranges are established by CLSI Tier 2 studies: ≥7 sites and >200 datapoints).

- 700603's acceptable QC range for ampicillin/sulbactam is 8/4 – 32/16 ug/mL. It crosses all three breakpoints (8/16/32).
- 700603's acceptable range for piperacillin/tazobactam is 8/4 – 32/4 ug/mL. It crosses S-I breakpoints. (BPs: 16/32-64/128)
- 27853's acceptable range for levofloxacin is 0.5 – 4 ug/mL It crosses all three breakpoints (BPs: 1/2/4) and is also a 4-dilution range.
- 27853's acceptable range for imipenem is 1 – 4 ug/mL. It crosses S-I breakpoint.

Isolates used in verifications are held to a **higher** standard than strains that QC the actual test (which have gone through rigorous testing)!

Considerations

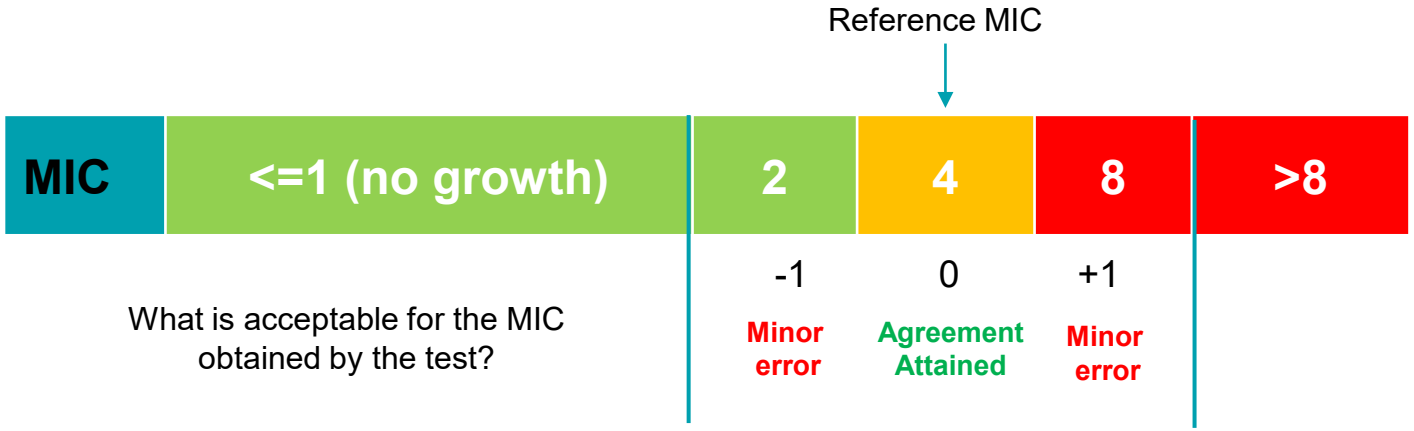
- **Side-by-side testing with a reference methods is ideal, but not always feasible. Using pre-characterized isolates, like those in AR Isolate Bank, is the next best option as long as its limitations are acknowledged.**
  - The single MIC posted- Number of observations not posted
  - Reproducibility data is not as strong as reproducibility behind development of QC ranges (i.e. multi-site testing)
  - Some isolates/drugs combinations may be more reproducible than others.

Please note:

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 J. H. Jorgensen. 1993. J Clin Microbiol. Vol 31[11]: 2841-2844). 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. The MIC results provided for each isolate were generated using frozen broth microdilution panels unless otherwise specified.

Relationship of essential agreement, categorical agreement, and assay/organism variability

Example: Imipenem for a *P. aeruginosa*



A test MIC of 2 µg/mL falls within the inherent variability allowance but does not attain categorical agreement.

"The MIC or True MIC from a single test is a myth. There is no such thing as a true MIC, each MIC test value is actually a single sample from a probability distribution."
-Katherine Sei, CLSI 2017

- It is possible to have essential agreement but not categorical agreement (minor errors for S/I/R or ME/VME for S/R, S/NS).
- We believe essential agreement is the better indicator for actual test performance (that the test can accurately reproduce the quantitative result of the reference AST method).
  - Can be difficult for drugs with short dilution schemes

My validation failed! What do I do?

Don't give up!

Acceptability is at the discretion of the Medical Director.

- Labs may need to be flexible for some bug/drug combinations that do not meet acceptable criteria.
- This likely occurs when then CA falls just shy of 90% or minor errors just exceed 10%.
- Consider the risk of a VME or ME with the new breakpoints versus risk of treatment failure if keeping the old breakpoints.
  - ME are thought to be 'conservative', since it will result in false-resistance (drug not used), not false-susceptible (drug might be used).
- If failure is dramatic (ex: CA of 50%), troubleshoot the verification study.

- Other Considerations:
- - Was it all drugs that failed or just one?
  - Ex: Carbapenem breakpoints- validation passed for Meropenem and Ertapenem but failed with Imipenem.
  - Is the drug (ex: Imipenem) used? Would suppression be an option? Could the lab test by request only using disk diffusion?
  - Did QC pass for Imipenem? Could there be an issue with Imipenem for that lot/panel?
- - Did the validation fail with just the new breakpoints? Or would the validation have failed if using the old breakpoints, too?
  - Suggests there may be a larger problem- instrument, panel or isolate issue?
  - Where did the errors occur? Were they near or span the new breakpoint?
  - Was there certain organism(s) that gave the errors?

CLSI/CAP Webinar 11/20/19. "Rational Approach to Antibacterial and Antifungal Breakpoints: What can and should my laboratory update?"

It happens to manufacturers too...

Table 4. Performance of *Enterobacteriaceae* Clinical and Challenge Isolates, Read Using VIZION

	Tot	EA N	EA %	Eval Tot	Eval EA N	Eval EA %	CA Tot	CA %	No. R	No. S	Min	maj	vmj
Enterobacteriaceae [a], ≤2(S), 4(I), ≥8 (R)													
Clinical	268	253	94.4	195	180	92.3	258	96.3	7	250	10	0	0
Challenge	82	75	91.5	69	62	89.9	64	78.0	25	41	17	0	1
Total	350	328	93.7	264	242	91.7	322	92.0	32	291	27	0	1

[a]Includes *E. coli*, *E. cloacae*, *K. pneumoniae* and *P. mirabilis*

Table 5. Performance of *Enterobacteriaceae* Clinical and Challenge Isolates, Read Using OptiRead

	Tot	EA N	EA %	Eval Tot	Eval EA N	Eval EA %	CA Tot	CA %	No. R	No. S	Min	maj	vmj
Enterobacteriaceae [a], ≤2(S), 4(I), ≥8 (R)													
Clinical	268	250	93.3	193	175	90.7	258	96.3	7	250	10	0	0
Challenge	82	75	91.5	69	62	89.9	59	72.0	25	41	22	0	1
Total	350	325	92.9	262	237	90.5	317	90.6	32	291	32	0	1

[a]Includes *E. coli*, *E. cloacae*, *K. pneumoniae* and *P. mirabilis*

Table 6. Performance of *P. aeruginosa* Clinical and Challenge Isolates, Read Using VIZION

	Tot	EA N	EA %	Eval Tot	Eval EA N	Eval EA %	CA Tot	CA %	No. R	No. S	Min	maj	vmj
P. aeruginosa, ≤1(S), 2(I), ≥4 (R)													
Clinical	60	58	96.7	58	56	96.6	59	98.3	0	58	1	0	0
Challenge	21	21	100	21	21	100	18	85.7	0	20	3	0	0
Total	81	79	97.5	79	77	97.5	77	94.8	0	78	4	0	0

Table 7. Performance of *P. aeruginosa* Clinical and Challenge Isolates, Read Using OptiRead

	Tot	EA N	EA %	Eval Tot	Eval EA N	Eval EA %	CA Tot	CA %	No. R	No. S	Min	maj	vmj
P. aeruginosa, ≤1(S), 2(I), ≥4 (R)													
Clinical	60	58	96.7	59	57	96.6	58	96.7	0	58	2	0	0
Challenge	21	21	100	21	21	100	17	81.0	0	20	4	0	0
Total	81	79	97.5	80	78	97.5	75	92.6	0	78	6	0	0

Thermofisher Sensititre 510(k) clearance data for cefiderocol

- Looking at the Enterobacterales challenge sets, where higher N of R isolates are tested (30% versus 2% for fresh clinical isolates), CA is poor (in the low 80s/70s), but driven by minor errors.
- The weight of the very susceptible clinical isolate population lets the overall CA pass.

“Not appreciating the possibility of inherent variability leads to assuming a single test MIC is THE MIC or TRUE MIC and may lead to erroneous conclusions about the performance of an antimicrobial agent or test method.”

-Katherine Sei, CLSI 2017