"Preparing for the Ministry of Magic Regulations"

Implementing Accepted CLSI Revised Breakpoints

Ministry of Magic/College of American Pathologists (CAP) Regulation:

"Revised MIC.11380 9/22/2021 Antimicrobial Susceptibility Test Interpretation Criteria
(Previously MIC.21930 (Susceptibility Test Endpoint Determination) 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."

- Laboratories must know what BPs are in use in their laboratory.
- Laboratories must review and document the BPs applied in their laboratory annually.
- Laboratories should discuss breakpoints in use with their antimicrobial stewardship team, as appropriate.

"New MIC.11385 9/22/2021 Current Antimicrobial Susceptibility Test Interpretation Breakpoints
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 laboratories must use current BPs for MIC and disk diffusion tests.
- At minimum, US laboratories must use current FDA BPs, but laboratories may choose to use current CLSI or EUCAST BPs.
- It will be UNACCEPTABLE for laboratories to use BPs that are no longer recognized by either FDA, CLSI, or EUCAST.
- In rare cases, a laboratory can use alternative BPs (including old breakpoints), if justified. This would require documentation that would optimally include input from the institution's antimicrobial stewardship team.

https://clsi.org/about/blog/ast-news-update-june-2022-updating-breakpoints-new-developments-from-cap/

Introduction

Jorn Bansberg

Overview:
The 2023 BIT Tool (Breakpoint Implementation ToolKit)

Coaxed Into Existence By CAP,

But Birthed By CLSI, APHL, CDC, and ASM.

2023 BIT Introduction

- Use this tool and you won't become a tool of he who must not be named, causing innumerable souls to no longer exist in the flesh.
- Watch and do not weep through the YouTube videos!

How You Ask?

- Currently, breakpoints are not being updated quickly enough by commercial AST device manufacturers: Vitek, Sensitre, Microscan, Phoenix etc, and the FDA. This may result in ineffective treatment when currently in use higher than effective concentrations (MICs) of an antibiotic are reported as sensitive.
- CAP Regulations to update breakpoints can save lives.

What Tools Does This BIT Living (regularly updating) software have in it?

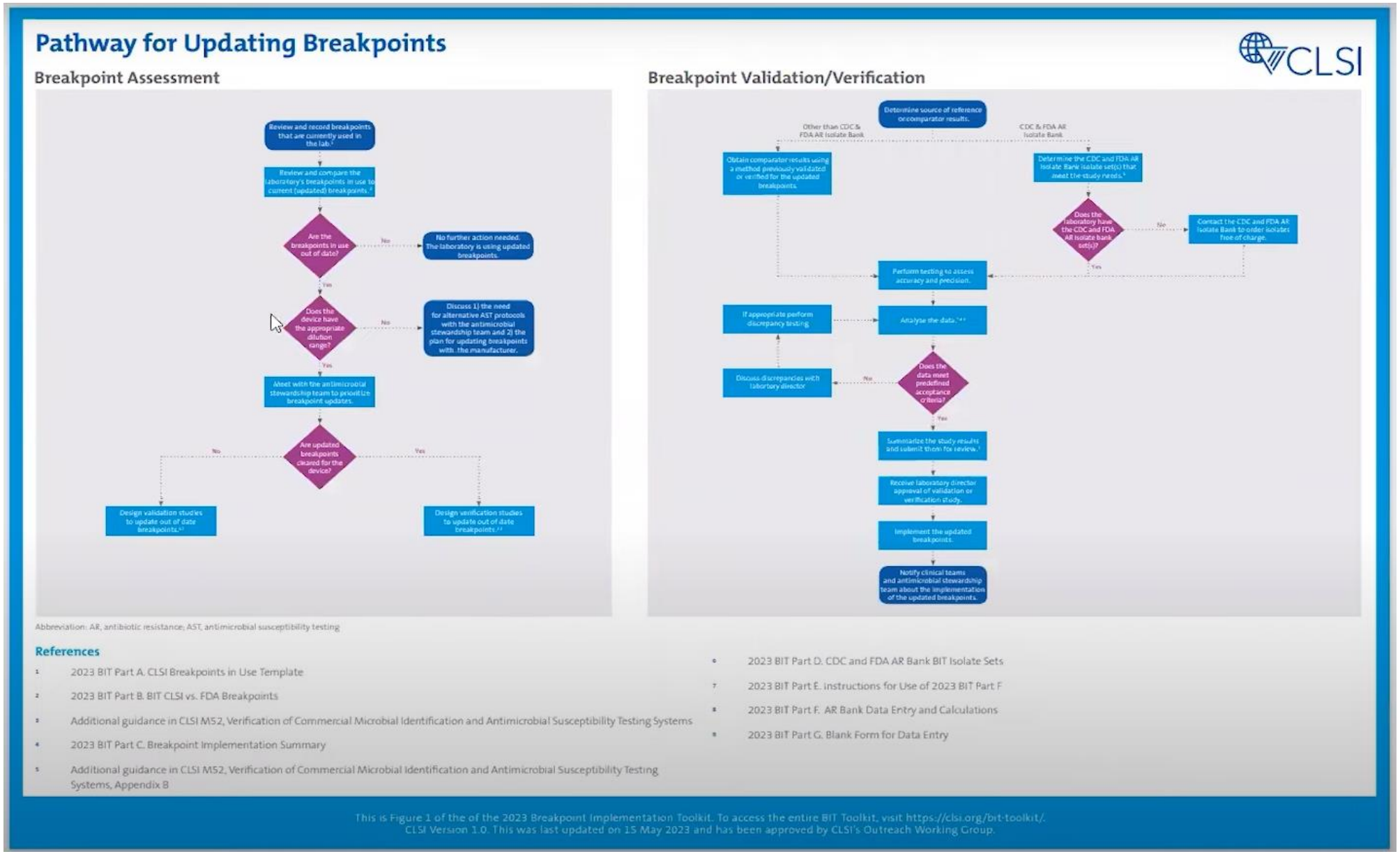
Effective January 2024, clinical laboratories performing antimicrobial susceptibility testing (AST) will be required to use breakpoints currently recognized by Clinical and Laboratory Standards Institute (CLSI) or US Food and Drug Administration (FDA).

Together CLSI, Association of Public Health Laboratories (APHL), American Society for Microbiology (ASM), College of American Pathologists (CAP), and Centers for Disease Control and Prevention (CDC), have jointly developed this Breakpoint Implementation Toolkit (BIT) to assist clinical laboratories in updating minimal inhibitory concentration (MIC) breakpoints.

The kit is provided in a streamlined format and designed to guide performance of a verification or validation study required to update breakpoints. Also included are links to other resources that explain the rationale behind breakpoint updates, regulatory requirements for updating breakpoints, and detailed instructions for performing an AST breakpoint validation or verification. Manufacturers of AST systems can provide guidance on breakpoints used and clearance status with their systems.

- Introduction: 2023 Breakpoint Implementation Toolkit
  - How to Use 2023 Breakpoint Implementation Toolkit
  - Definitions/References/Resources
  - Pathway for Updating Breakpoints (Figure 1)
- Part A. Breakpoints in Use
  - Template for Documenting Breakpoints in Use
- Part B. CLSI vs FDA Breakpoints
  - Listing of all current CLSI breakpoints (M100-Ed33) and corresponding FDA breakpoints
- Part C. Breakpoint Implementation Summary
  - Template for documenting results of a validation study to update breakpoints
- Part D. CDC and FDA AR Bank BIT Isolate Sets
  - CDC and FDA Antibiotic Resistance Isolate Bank isolate sets available for use in breakpoint verification and validation studies
- Part E. Instructions for Use of 2023 BIT Part F
  - Instructions for using the prefilled Excel workbook
- Part F. AR Bank Data Entry and Calculations
  - Excel workbook prefilled with the AR Bank BIT set isolates and their results
- Part G. Blank Form for Data Entry
  - Template for validations or verifications using isolates other than those listed in the AR Bank BIT sets included in this toolkit

Algorithm Guide included (Pathway for Updating Breakpoints)!



Synopsis and Definitions

How to Use 2023 Breakpoint Implementation Toolkit

It is assumed that those using this toolkit have some knowledge of the need to update AST breakpoints. Please check references in the list below for more detailed information about updating breakpoints.

The APHL-ASM CRO Breakpoint Implementation Toolkit published in 2022 and accessible here contains detailed instructions, as well as worksheets and forms for validating updated carbapenem breakpoints, and is based on the CAP Breakpoint Implementation Toolkit (2012, no longer available). These instructions can be adapted to verification or validation of other breakpoints when following guidance included in the 2023 Breakpoint Implementation Toolkit.

In brief, it is suggested that you proceed as follows:

1. Determine what breakpoints are in use in your laboratory.
2. Determine which of these breakpoints are old or out of date (eg, no longer recognized by CLSI or FDA) and would require updating for continued reporting.
3. Develop a priority list and a plan for updating breakpoints.

Refer to the flowchart in Figure 1 that highlights a detailed pathway for updating breakpoints.

Refer to Part A here to assist you in documenting breakpoints in use.

IMPORTANT NOTES:

1. Laboratories are encouraged to implement updated CLSI breakpoints as listed in M100, 33rd ed.
2. If CLSI breakpoints differ from FDA breakpoints, (see Part B) a laboratory can elect to use current CLSI or FDA breakpoints.
3. Manufacturers of commercial AST must use FDA breakpoints that are current at the time they submit a test for FDA clearance.
4. A laboratory should NOT use breakpoints that are no longer recognized by CLSI or FDA.
5. As of January 2024, CAP-accredited laboratories may be penalized if they use breakpoints that are no longer recognized by CLSI or FDA. US laboratories must at least adopt breakpoints within three years of the date of official publication by FDA accepting the revised breakpoints.

Definitions/References/Resources

In the context of updating to current CLSI breakpoints:

Verification is used to evaluate the performance of breakpoints which have been FDA cleared for use on a device manufacturer's AST system (ie, FDA recognizes the CLSI breakpoints, and the manufacturer has obtained clearance by FDA for the current CLSI/FDA breakpoints on their AST system).

Validation refers to any other scenario not covered by verification and when the laboratory is modifying an FDA-cleared test (eg, using breakpoints that are different from those that are FDA cleared for use on the device manufacturer's AST system).

NOTE: Details regarding how AST device manufacturers implement updated breakpoints for their system can be found in Table 3.

CLSI Version 1.0. This was last updated on 15 May 2023 and has been approved by CLSI's Outreach Working Group.

When To Use Verification or Validation

CLSI

Table 1. Situations Where Breakpoint Verification or Validation Is Required to Use Updated CLSI Breakpoints

Updated Breakpoint Status	Commercial Antimicrobial Susceptibility Testing System Status	Performance Assessment Required[a]
CLSI=FDA	CLSI breakpoints are FDA cleared and available on panel/software.	• Verification[b] • 10 to 15 isolates/drug
CLSI=FDA	Device manufacturer has notified customers that the device has received FDA clearance with updated CLSI/FDA breakpoints and has advised customers how to implement breakpoints with their panels/software.	• Verification[b] • 10 to 15 isolates/drug
CLSI=FDA	Device manufacturer has not received FDA clearance of the device with updated CLSI/FDA breakpoints.	• Validation (if desire to use CLSI breakpoints) • 30 isolates/drug
CLSI≠FDA	Manufacturer must provide FDA breakpoints; use of CLSI breakpoints would be off label.	• Validation (if desire to use CLSI breakpoints) • 30 isolates/drug

[a] Consensus suggestions from authors of 2023 Breakpoint Implementation Toolkit

[b] If no change to the test has been made by the AST manufacturer (eg, no reformulation of drug dilutions), a verification of reporting may be sufficient. This would involve ensuring MIC results are interpreted correctly on patient reports.

Resources

CLSI

Table 2. Resources for Updating Breakpoints

Resource	Location	Comments
Current CLSI Breakpoints	Performance Standards for Antimicrobial Susceptibility Testing, 33rd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2023.	Updated annually, usually in February
Current FDA Breakpoints	Susceptibility Test Interpretive Criteria (STIC)	Updated as new information is obtained from CLSI and/or pharmaceutical company and reviewed by FDA
CLIA regulations for verification and performance specifications	CLIA §493.1253(b)(1);	No specific details for verification of AST systems
CAP requirements for updating Breakpoints	Contact CAP to obtain complete checklist; see below for specific breakpoint requirements	(Available to CAP-accredited laboratories)
CLSI. Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems. 1st ed. CLSI guideline M52. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.	Purchase from clsi.org	1st edition is currently under revision; describes verification in detail; validation is discussed briefly in Appendix B
APHL-ASM CRO Breakpoint Implementation Toolkit	APHL Implementation Toolkit	Contains extensive instructions, worksheets, and forms for validating updated carbapenem breakpoints

Table 3. Considerations for Updated Breakpoint Implementation in Commercial AST Devices

- FDA clearance status of a test for an AST device may not be synonymous with up to date FDA breakpoints (ie, clearance may have been granted with previously published FDA breakpoints).
- If a test was FDA cleared, and breakpoints were subsequently updated by CLSI, the manufacturer must wait for FDA to review rationale documentation[a] and update the FDA Susceptibility Test Interpretive Criteria (STIC) website to reflect FDA acceptance of the updated CLSI breakpoints.
- The AST manufacturer can re-submit performance data to FDA for authorization to update the breakpoints for the test for that device only when the FDA STIC website update has occurred.

[a] Rationale documents prepared by CLSI when breakpoints are updated can be found here with a companion webinar here.

Hyperlinks Galore including to FDA STIC site

Antibacterial Susceptibility Test Interpretive Criteria

Development Resources

Advancing Real-World Evidence Program

Antibacterial Drug Development Task Force

BEST Resource Taxonomy

Clinical Outcome Assessment Compendium

Complex Innovative Trial Design Meeting Program

Division of Pediatric and Maternal Health

FDA-Recognized Antimicrobial Susceptibility Test Interpretive Criteria

This web page provides information about the *in vitro* susceptibility of bacteria to certain drugs.

The safety and efficacy of these drugs in treating clinical infections due to such bacteria may or may not have been established in adequate and well-controlled clinical trials and the clinical significance of such susceptibility information in those instances is unknown.

The approved product labeling for specific drugs provides the uses for which the product is approved.

Labeling for these products can be found at Drugs@FDA or FDA Online Label Repository.

Recognized Standards

Performance Methods and Quality Control

FDA recognizes consensus standards for performance standards, methods standards, and quality control parameter standards including ranges for antimicrobial susceptibility testing.

Susceptibility Test Interpretive Criteria

The table below lists antibacterial drugs and indicates which, if any, susceptibility test interpretive criteria, also known as "breakpoints" (abbreviated as STIC), are recognized or identified by FDA for that drug.

With certain exceptions and additions, identified in the table, FDA recognizes the standard

Also To Current CLSI Breakpoints

References

References

References Addressing Potential Negative Impact of Using Obsolete Breakpoints

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*. 2020;7(7). doi: https://doi.org/10.1093/ofid/ofaa084.

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

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

Yarbrough ML, Wallace MA, Potter RF, et al. 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;39(1):187-195. doi: https://doi.org/10.1007/s10096-019-03711-y.

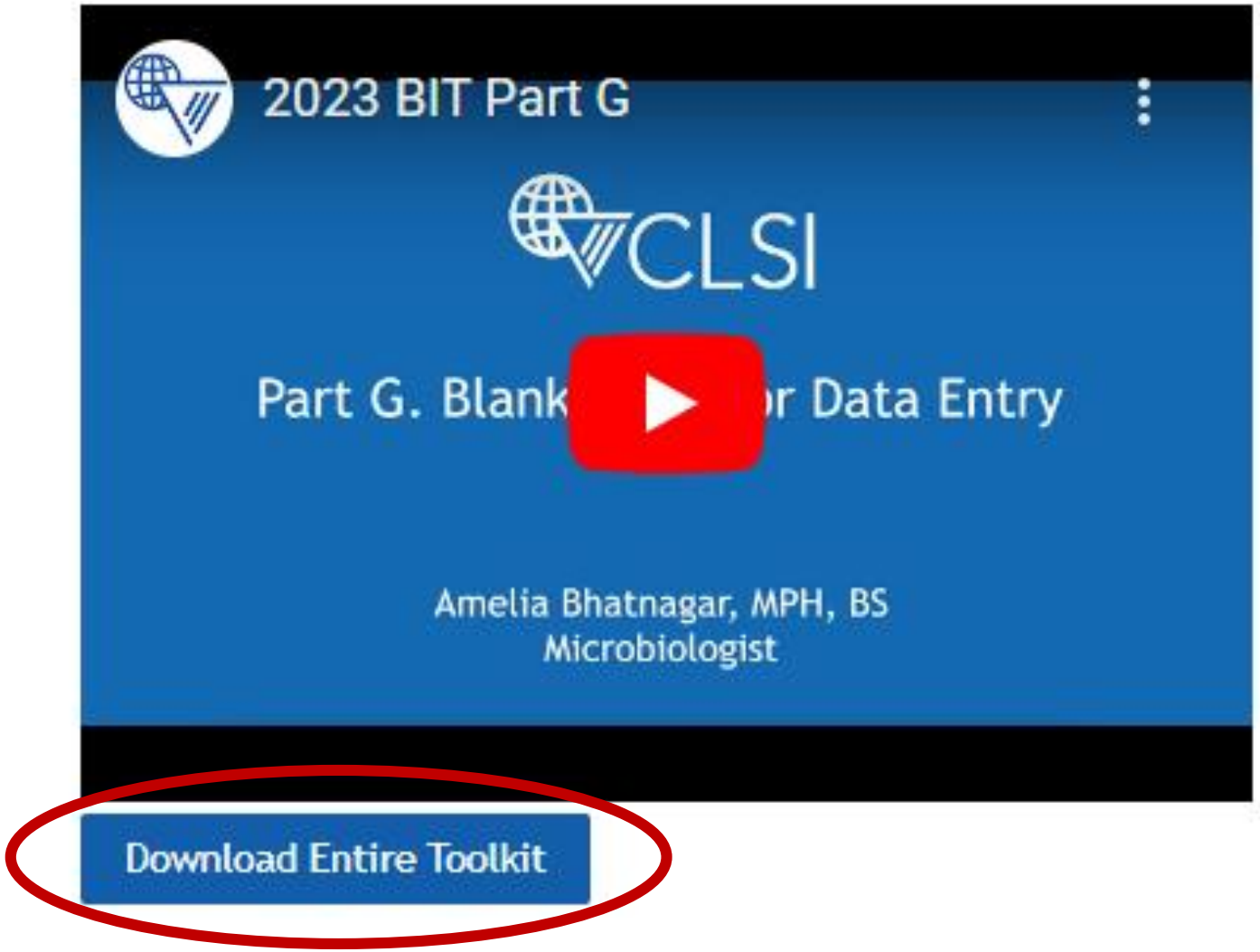
References Addressing Understanding Breakpoint Updating

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

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 March 16, 2023.

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

To Download Entire Toolkit First Click That Button On the Bottom of the 1st Page



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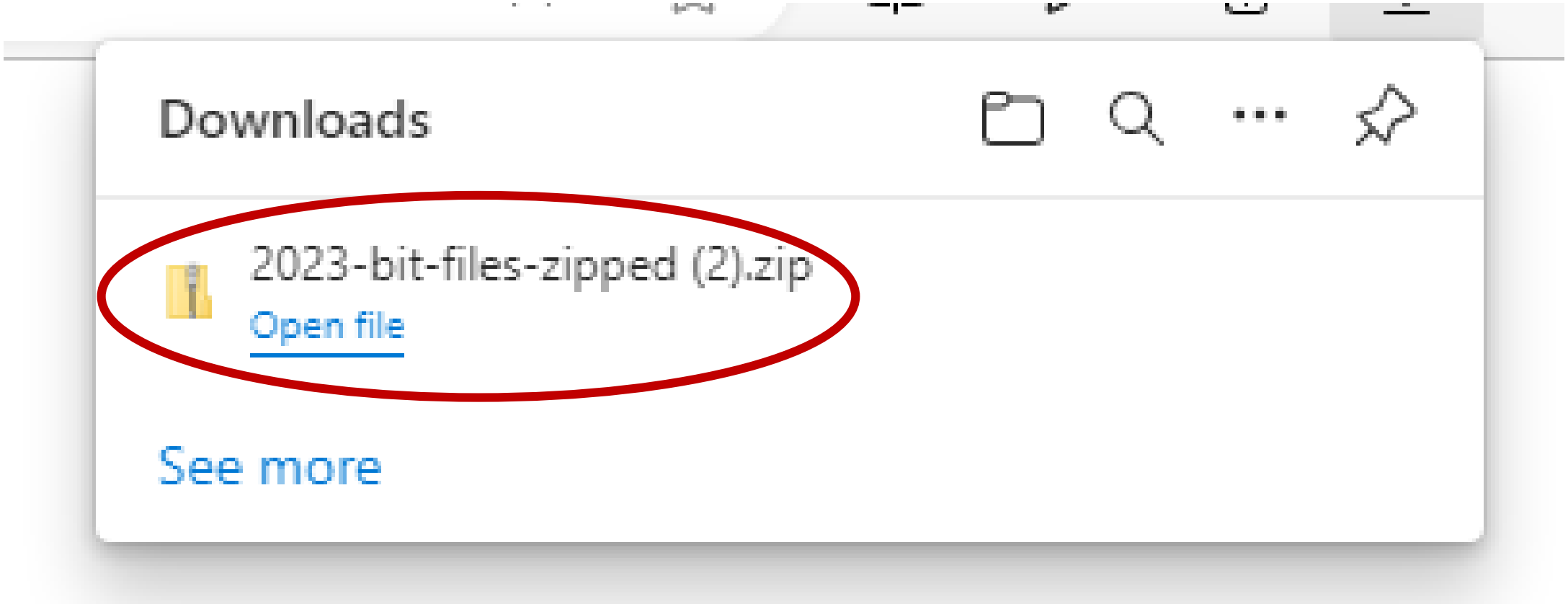

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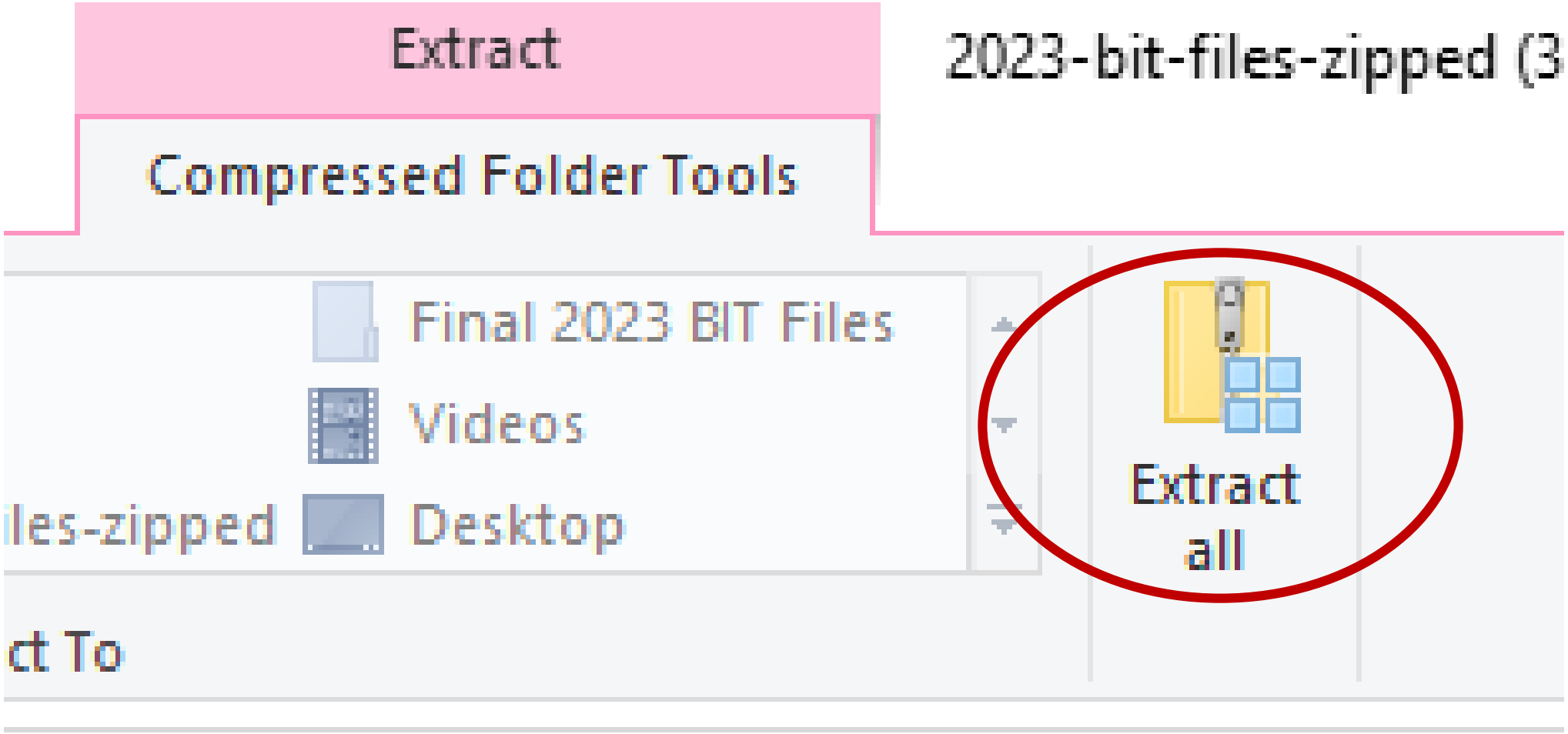

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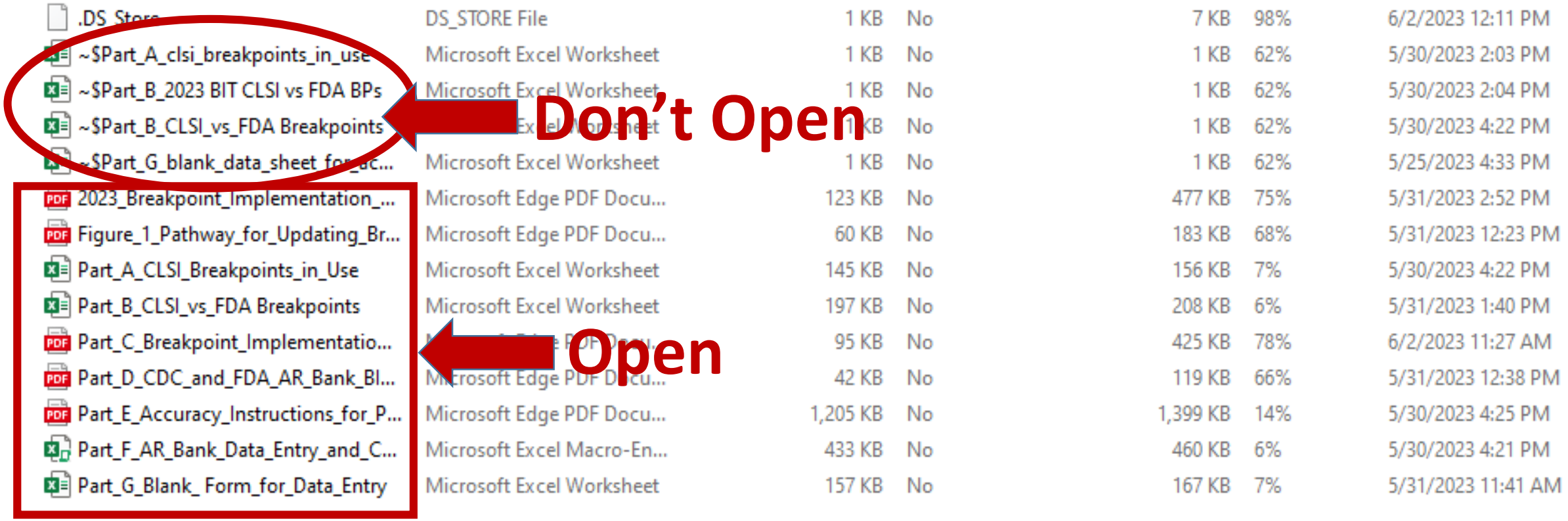

2023 BIT Parts A and B

Raymond Podzorski

COLLEGE of AMERICAN PATHOLOGISTS

Microbiology Checklist

CAP Accreditation Program

REVISED *10/24/2022*

MIC.11380 Antimicrobial Susceptibility Test Interpretation Criteria **Phase II**

For antimicrobial susceptibility testing (AST) 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.

NOTE: This checklist item applies to all antibacterial, antifungal, and antimycobacterial agents tested in the laboratory. The same criteria applied to clinical test results must be used for proficiency testing results.

The laboratory may use interpretive criteria from standards development organizations such as Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST), the FDA, or in rare instances, validated institution-specific criteria.

The source of the breakpoints applied to interpret AST results must be documented for both manual and automated antimicrobial susceptibility testing methods, including the reference with the year it was published (eg, CLSI M100-S32, 2022). For automated susceptibility testing systems, laboratories may contact the manufacturer to understand the breakpoints applied by the automated expert rules programmed into the system for the test panels in use, if not already known.

Criteria must be reviewed by the laboratory and with the antimicrobial stewardship program in the institution (if applicable) annually. The records of the review must be available.

Evidence of Compliance:

- ✓ Listing of antimicrobial susceptibility test interpretive criteria applied to test results and the specific source document for these **AND**
- ✓ Patient reports with reporting of antimicrobial agents following written protocol **AND**
- ✓ Records of annual breakpoint review **AND**
- ✓ Proficiency testing susceptibility results following written policy

COLLEGE of AMERICAN PATHOLOGISTS

Microbiology Checklist

CAP Accreditation Program

***NEW/REVISED** 10/24/2022

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. New breakpoints are implemented within three years of the date of publication by the FDA for laboratories subject to US regulations, or within three years of publication by CLSI, EUCAST or other standards development organization (SDO) for laboratories not subject to US regulations.

NOTE 1: For laboratories subject to US regulations, a breakpoint is considered obsolete three years after publication of an update by the FDA, though the laboratory may use currently accepted breakpoints from other SDOs with validation to support use. SDOs that develop breakpoints include the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Whether using breakpoints from the FDA or other SDOs, US laboratories must, at a minimum, adopt the change within three years of the official publication date of the updated breakpoint by the FDA.

NOTE 2: For laboratories not subject to US regulations, a breakpoint is considered obsolete three years after publication of an update by the SDO used by the laboratory. Laboratories must, at minimum, adopt the change within three years of the official publication date of the updated breakpoint by the SDO.

NOTE 3: Not all FDA-cleared susceptibility test systems apply current FDA-recognized breakpoints. Laboratories must determine if the breakpoints applied by their system are current and if they are not, validate changes to breakpoints as needed prior to use in patient result interpretation. Laboratories may also validate susceptibility test systems for use with alternative breakpoints (eg, those from SDOs or, more rarely, those that are institution-specific).

COLLEGE of AMERICAN PATHOLOGISTS

Microbiology Checklist

CAP Accreditation Program

***NEW/REVISED** 10/24/2022*

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. New breakpoints are implemented within three years of the date of publication by the FDA for laboratories subject to US regulations, or within three years of publication by CLSI, EUCAST or other standards development organization (SDO) for laboratories not subject to US regulations.

NOTE 4: Laboratories may choose to use CLSI, EUCAST, or FDA breakpoints. In rare instances, hospital-based laboratories may choose to use alternative breakpoints (eg, institution-derived breakpoints not recognized by SDOs or the FDA) that address unique patient and/or antimicrobial stewardship needs. In this case, the laboratory must have written documentation (eg, minutes from a pharmacy and therapeutic committee meeting, or a letter of approval signed by stakeholders) for the following:

- *Scientific and medical reasoning and institutional review/approval of institution-specific breakpoints*
- *Review and agreement to use alternative breakpoints by stakeholders (eg, chief medical officer, pharmacy, infectious diseases, and/or antimicrobial stewardship partners).*

Evidence of Compliance:

✓ Records of validation reports for breakpoints that differ from those included in the FDA-clearance of an instrument **AND**

✓ Records of the interpretive criteria used for antimicrobial susceptibility testing **AND**

✓ Source document (including year of publication) from which the interpretive criteria were derived **AND**

✓ Patient or LIS reports with interpretations matching the source document

FDA-Recognized Antimicrobial Susceptibility Test Interpretive Criteria

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

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Content current as of:
06/21/2023

Regulated Product(s)
Drugs

Antibacterial Susceptibility Test Interpretive Criteria

Development Resources

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BEST Resource Taxonomy

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Complex Innovative Trial Design Meeting Program

This web page provides information about the *in vitro* susceptibility of bacteria to certain drugs.

The safety and efficacy of these drugs in treating clinical infections due to such bacteria may or may not have been established in adequate and well-controlled clinical trials and the clinical significance of such susceptibility information in those instances is unknown.

The approved product labeling for specific drugs provides the uses for which the product is approved.

Labeling for these products can be found at Drugs@FDA or FDA Online Label Repository.

Recognized Standards

Performance Methods and Quality Control

FDA recognizes consensus standards for performance standards, methods standards, and quality control parameter standards including ranges for antimicrobial susceptibility testing.

Content current as of:
05/25/2023

Regulated Product(s)
Drugs

Antibacterial Susceptibility Test Interpretive Criteria

Drug	Route of Administration	STIC for Drug Included in the Recognized CLSI Standard	Exceptions or Additions to the Recognized CLSI Standard
Amikacin	Injection	Yes	Yes
Amoxicillin	Oral	Yes	No
Amoxicillin and clavulanate	Oral	Yes	Yes
Ampicillin	Injection, Oral	Yes	No
Ampicillin and sulbactam	Injection	Yes	Yes
Azithromycin	Injection, Oral	Yes	Yes
Aztreonam	Injection	Yes	Yes

Part A. Breakpoints in Use

The instructions, Breakpoints in Use template, and examples (ie, "Demo Data") provided herein are suggestions for documenting breakpoints in use.

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

Suggested procedure for completing "Breakpoints 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) breakpoints may be currently stored and applied at your institution. At many institutions, breakpoints 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 breakpoints being used by your lab to those in the current edition of CLSI's document M100. Flag the breakpoints being used in your laboraory that differ from the current breakpoints (found in CLSI document M100).
4. Cross-check breakpoints that are flagged in #3 with breakpoints provided on the FDA susceptibility test interpretive category (STIC [BPs]) website listed below to see if CLSI breakpoints = FDA breakpoints.

   https://www.fda.gov/drugs/development-resources/antibacterial-susceptibility-test-interpretive-criteri

   a. If CLSI breakpoints = FDA breakpoints but are different from those in use in your laboratory: Develop a plan for implementing updated breakpoints. This might involve meeting with your antimicrobial stewardship program (ASP) team to prioritize updates (if multiple breakpoint updates for different drugs are required) and review reporting needs for the drug(s).
   b. If CLSI breakpoints ≠ FDA breakpoints: Meet with your ASP to discuss which breakpoints are appropriate for your facility.
5. Develop a plan (including timeline) to update any breakpoints in use that do not coincide with CLSI M100 (current version) and/or FDA STIC (breakpoints).

Part A. Breakpoints in Use

The instructions, Breakpoints in Use template, and examples (ie, "Demo Data") provided herein are suggestions for documenting breakpoints in use.

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

Antimicrobial Agent	Organism/Group	Test System	Test System Reportable Dilutions (µg/mL)	Interpretive Categories and MIC BPs (µg/mL) or Zone Diameter BPs (nearest whole mm)				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
				Susceptible, MIC ≤ or ZD ≥	Susceptible Dose-Dependent	Intermediate	Resistant, MIC ≥ or ZD ≤					
Amikacin	Enterobacterales	Vitek 2	≤ 2 to ≥ 64	16	n/a	32	64	Instrument	No	Yes	pre-2015	5/11/2023
Amikacin	Enterobacterales	MicroScan	≤ 16 to ≥ 32	16	n/a	32	64	LIS	No	Yes	pre-2015	6/2/2023
Ampicillin	Enterobacterales	Vitek 2	≤ 2 to ≥ 64	8	n/a	16	32	Instrument	Yes	Yes	pre-2015	5/12/2023
Ampicillin	Enterobacterales	MicroScan	≤ 8 to ≥ 16	8	n/a	16	32	LIS	Yes	Yes	pre-2015	6/2/2023
Ampicillin	*Enterococcus spp.*	Vitek 2	≤ 2 to ≥ 32	8	n/a	n/a	16	Instrument	Yes	Yes	pre-2015	5/12/2023
Ampicillin	Enterobacterales	Disk diffusion	n/a	17	n/a	14-16	13	LIS	Yes	Yes	pre-2015	5/12/2023

Part B. CLSI vs FDA Breakpoints

Introduction

This spreadsheet contains a listing of all disk diffusion and MIC breakpoints published in CLSI document M100-Ed33. The FDA breakpoints are presented in the table if the CLSI breakpoints do not match the FDA breakpoints published on the FDA Susceptibility Test Interpretive Criteria (STIC) website as of May 22, 2023:
fda.gov/drugs/development-resources/antibacterial-susceptibility-test-interpretive-criteria

Part B. CLSI vs FDA Breakpoints

Provided below are suggestions for using this tool as a reference to determine if breakpoints used in your laboratory are current.

1. Prepare a spreadsheet containing all breakpoints used in your laboratory. Use the "Part A. CLSI Breakpoints In Use" template found at clsi.org/bit-toolkit, if desired. You may wish to contact the manufacturer of your AST device for assistance in accessing current breakpoints in your AST system.
2. Sort and filter the attached spreadsheet to identify the antimicrobials and reporting groups tested in your laboratory. For the MIC breakpoint Table, breakpoint values are displayed in µg/mL. For the Disk Diffusion Breakpoint Table, breakpoint values are displayed as zone diameters read to the nearest whole mm.
3. Compare the two lists and note any breakpoints that are not current on your AST system.
4. Proceed to develop a plan to prioritize updating the out-of-date breakpoints.

Note that CLSI breakpoints are published in M100 annually, and may include revised/updated breakpoints for some drugs. FDA STIC may change breakpoints at variable times. Please check STIC website page here:
https://www.fda.gov/drugs/development-resources/notice-updates

Part B. CLSI vs FDA Breakpoints

DRUG NAME	Organism/Organism Grou	CLSI <= S	CLSI = I/SDD	CLSI >= R	FDA STIC <=S	FDA STIC =I	FDA STIC >=R	CLSI & FDA match?
Amikacin	Enterobacterales	4	8	16	16	32	64	No
Amoxicillin-Clavulanate	Enterobacterales	8/4	16/8	32/16	same	same	same	Yes
Ampicillin	Enterobacterales	8	16	32	same	same	same	Yes
Ampicillin-Sulbactam	Enterobacterales	8/4	16/8	32/16	same	same	same	Yes
Aztreonam	Enterobacterales	4	8	16	same	same	same	Yes
Cefaclor	Enterobacterales	8	16	32	-	-	-	No
Cefamandole	Enterobacterales	8	16	32	-	-	-	No
Cefazolin - infections other tha	Enterobacterales	2	4	8	same	same	same	Yes
Cefazolin - uncomplicated UTIs	Enterobacterales	16	-	32	-	-	-	No
Cefdinir	Enterobacterales	1	2	4	-	-	-	No

Part B. CLSI vs FDA Breakpoints

Introduction

This spreadsheet contains a listing of all disk diffusion and MIC breakpoints published in CLSI document M100-Ed33. The FDA breakpoints are presented in the table if the CLSI breakpoints do not match the FDA breakpoints published on the FDA Susceptibility Test Interpretive Criteria (STIC) website as of May 22, 2023:
fda.gov/drugs/development-resources/antibacterial-susceptibility-test-interpretive-criteria

FDA-Recognized Antimicrobial Susceptibility Test Interpretive Criteria

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Antibacterial Susceptibility Test Interpretive Criteria

Antifungal Susceptibility Test Interpretive Criteria

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Content current as of:
06/21/2023

Regulated Product(s)
Drugs

Notice of Updates

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For information regarding prior changes to the STIC webpages, see https://www.govinfo.gov/content/pkg/FR-2019-05-03/pdf/2019-09007.pdf

https://www.federalregister.gov/documents/2020/10/22/2020-23439/21st-century-cures-act-annual-compilation-of-notices-of-updates-from-the-susceptibility-test

Content current as of:
05/25/2023

Regulated Product(s)
Drugs

Updates by Drug:

Drug	Route of Administration	Action Taken	Therapeutic Category	Date
Amikacin	Injection	FDA does not recognize MI00 standard (MIC and disk diffusion) for Enterobacterales and *Pseudomonas aeruginosa*	Antibacterial	4/21/2023
Sulbactam and Durlobactam	Injection	FDA identified STIC for *Acinetobacter baumannii-calcoaceticus* complex.	Antibacterial	05/25/2023

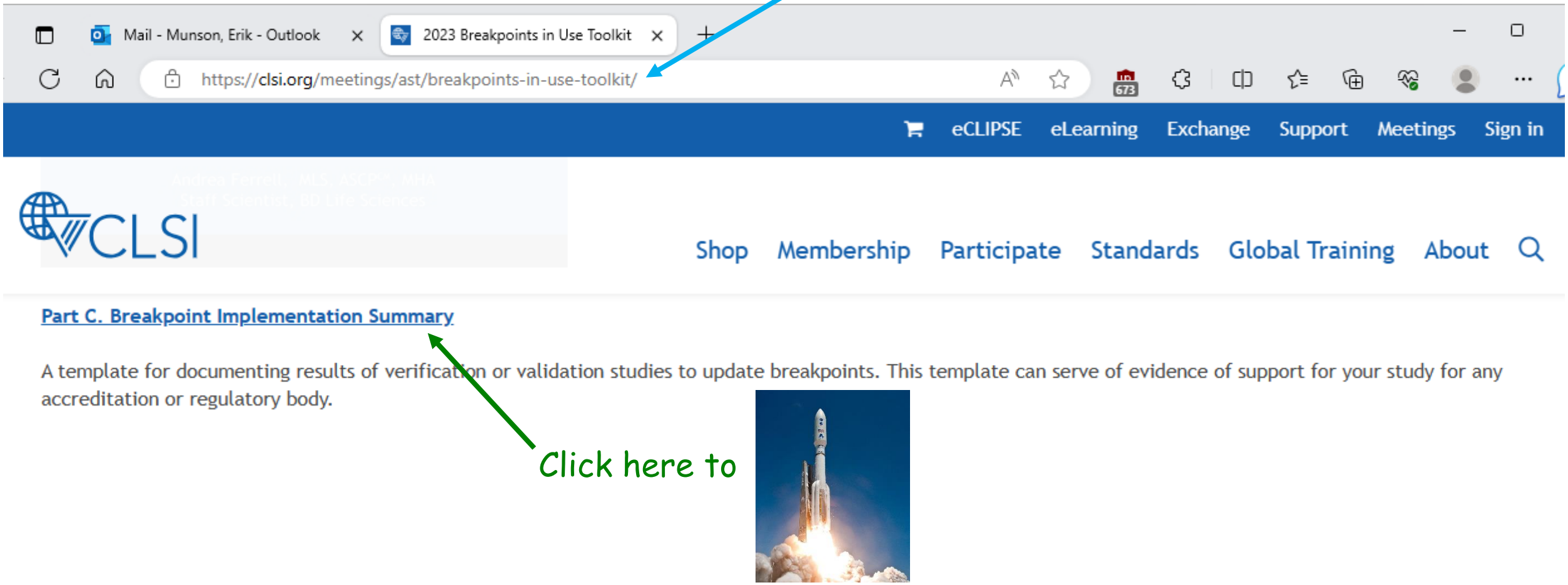
2023 BIT Parts C and D

Erik Munson

Breakpoint Implementation Toolkit (2023 BIT)
Part C. Breakpoint Implementation Summary

DOCUMENTATION

Here's the web address; easy to find



Click here to

Breakpoint Implementation Toolkit (2023 BIT)
Part C. BIT Summary Template

Part C. BIT Summary Template

CLSI

☐ Verification of ☐ Validation of _____ Breakpoints for (organism/organism group) _____

tested by (AST Method) _____

Studies performed (dates): _____

I. Purpose

☐ Verify or ☐ Validate performance of (Name of Method or Commercial AST Device) _____

For ☐ organism or ☐ organism group _____

Reference/Comparator results from (see NOTE below, II.B.) _____

For Antimicrobial(s) and Breakpoint Values

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

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible; SDD, susceptible dose dependent.

II. Verification/Validation Study

A. AST System

Panel/Card _____ Software version _____

B. Accuracy

Number of isolates _____

Isolate source(s) _____
(eg, CDC & FDA Antibiotic Resistance (AR) Isolate Bank, clinical isolates)

Reference result source(s) _____
(eg, 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 breakpoints or preestablished using a reference (eg, CDC & FDA AR Isolate Bank) or verified/validated comparator method.

CLSI

CLSI Version 1.0. This was last updated on 15 May 2023 and has been approved by CLSI's Outreach Working Group.

Toll Free (US): 877.447.1888 | P: +1.610.688.0100 | F: +1.610.688.0700 | E: customerservice@clsi.org

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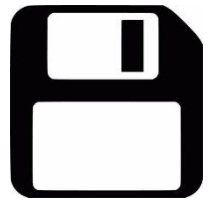
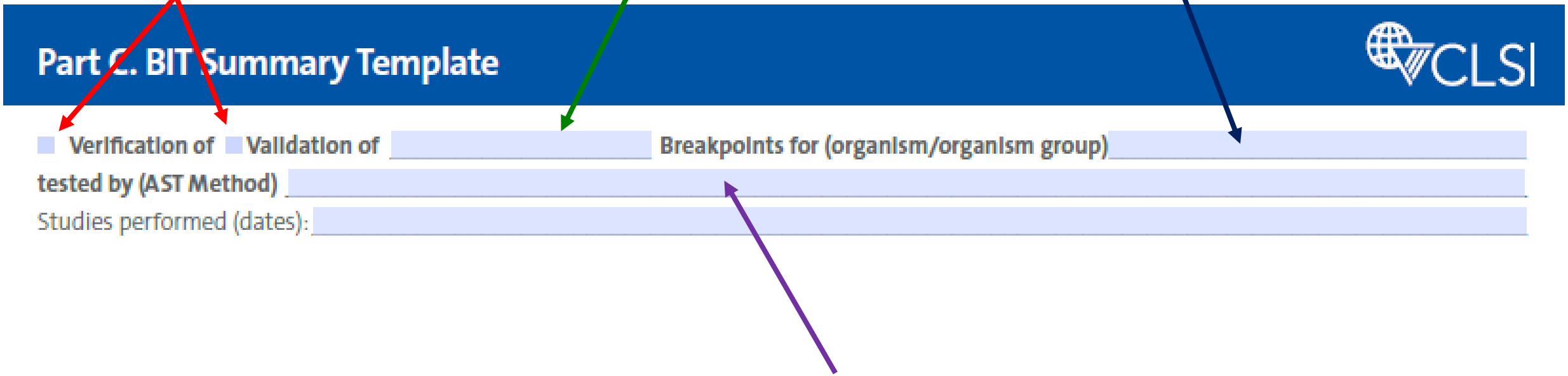


Breakpoint Implementation Toolkit (2023 BIT)
Part C. BIT Summary Template

depends if organism/drug combination is FDA-cleared on your panel

antimicrobial agent

likely the Table(s) 2 in CLSI M100 supplement



your automated antimicrobial susceptibility testing instrument (better yet, your specific card/panel) of choice

Part C: BIT Summary Template Purpose

First two lines: rinse and repeat from previous slide

Third line: consider source(s) of isolates and reference testing method
[e.g., AR bank (will discuss later) tested by broth microdilution]

I. Purpose

☐ Verify or ☐ Validate performance of (Name of Method or Commercial AST Device) _____
For ☐ organism or ☐ organism group _____
Reference/Comparator results from (see NOTE below, II.B.) _____

For Antimicrobial(s) and Breakpoint Values

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

Abbreviations: I, intermediate; MIC, minimal inhibit[illegible] concentration; R, resistant; S, susceptible; SDD, susceptible dose dependent.

You may not need this section if "bringing on a new drug"

DOCUMENTATION

Potential example of “New Drug” or “Breakpoint Shift”

Enterobacteriaceae

Method	Meropenem-vaborbactam New		
	S	I	R
BMD	≤ 4/8	8/8	≥ 16/8

CLSI M100-Ed29, 2019

Enterobacterales

Method	Piperacillin-tazobactam Previous			Piperacillin-tazobactam New		
	S	I	R	S	SDD	R
BMD	≤ 16	32-64	≥ 128	≤ 8	16	≥ 32

CLSI M100-Ed31, 2021; -Ed32, 2022

Part C: BIT Summary Template Verification/validation Study (Planning)

DOCUMENTATION

Lots of rinse and repeat

II. Verification/Validation Study

A. AST System

Panel/Card _____ Software version _____

B. Accuracy

Number of isolates _____

Isolate source(s) _____
(eg, CDC & FDA Antibiotic Resistance (AR) Isolate Bank, clinical isolates)

Reference result source(s) _____
(eg, CDC & FDA AR Isolate Bank MICs, in-house reference broth microdilution, reference laboratory)

*

C. Reproducibility (precision)

Number of isolates _____

Isolate source(s) _____
(eg, CDC & FDA AR Isolate Bank, clinical isolates quality control strains)

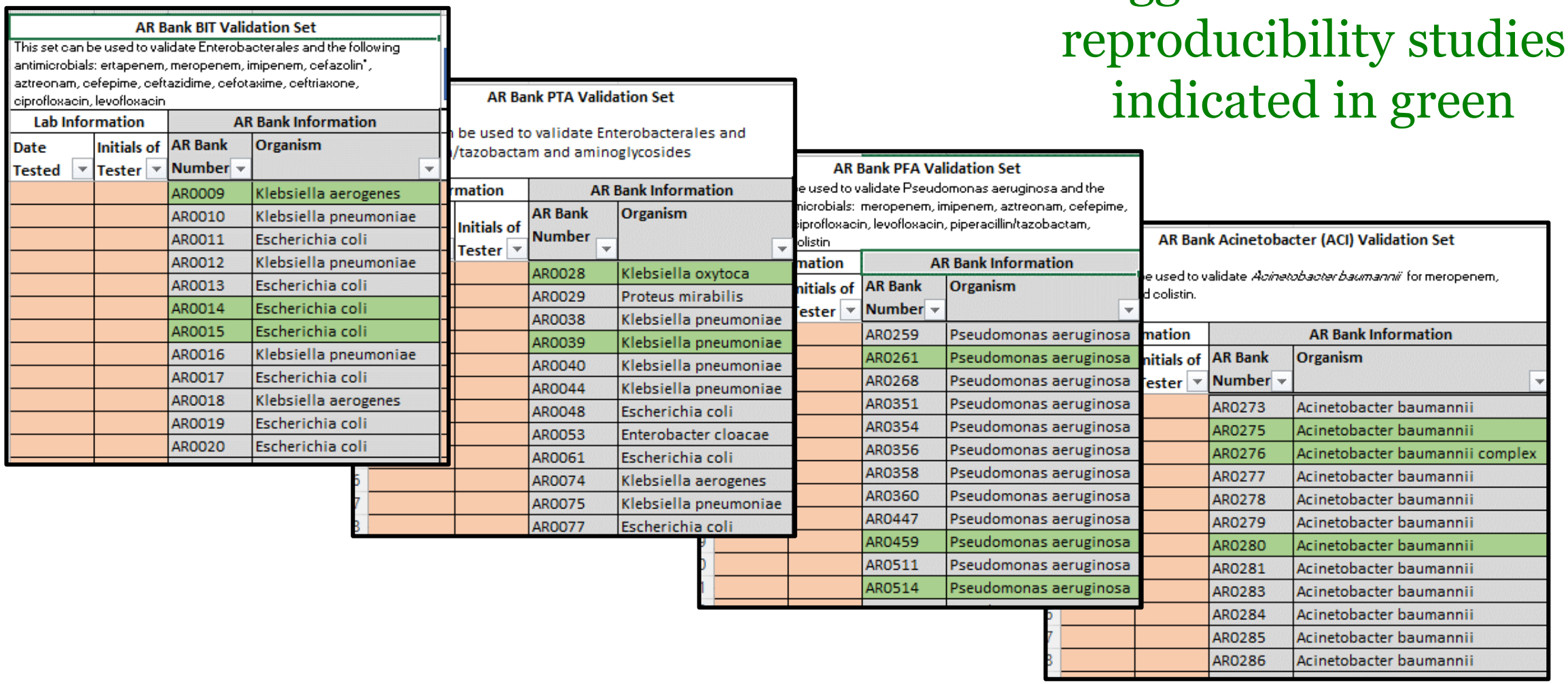
Number of replicates _____

D. Quality Control

Isolate(s) _____ Testing frequency _____
(ie, name/strain number) (eg, per run)

Reproducibility

Suggested isolates for reproducibility studies indicated in green



AR Bank BIT Validation Set

This set can be used to validate Enterobacterales and the following antimicrobials: ertapenem, meropenem, imipenem, cefazolin*, aztreonam, cefepime, ceftazidime, cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin

Lab Information		AR Bank Information	
Date Tested	Initials of Tester	AR Bank Number	Organism
		AR0009	Klebsiella aerogenes
		AR0010	Klebsiella pneumoniae
		AR0011	Escherichia coli
		AR0012	Klebsiella pneumoniae
		AR0013	Escherichia coli
		AR0014	Escherichia coli
		AR0015	Escherichia coli
		AR0016	Klebsiella pneumoniae
		AR0017	Escherichia coli
		AR0018	Klebsiella aerogenes
		AR0019	Escherichia coli
		AR0020	Escherichia coli

AR Bank PTA Validation Set

be used to validate Enterobacterales and /tazobactam and aminoglycosides

rmation		AR Bank Information	
	Initials of Tester	AR Bank Number	Organism
		AR0028	Klebsiella oxytoca
		AR0029	Proteus mirabilis
		AR0038	Klebsiella pneumoniae
		AR0039	Klebsiella pneumoniae
		AR0040	Klebsiella pneumoniae
		AR0044	Klebsiella pneumoniae
		AR0048	Escherichia coli
		AR0053	Enterobacter cloacae
		AR0061	Escherichia coli
		AR0074	Klebsiella aerogenes
		AR0075	Klebsiella pneumoniae
		AR0077	Escherichia coli

AR Bank PFA Validation Set

e used to validate Pseudomonas aeruginosa and the microbials: meropenem, imipenem, aztreonam, cefepime, iprofloxacin, levofloxacin, piperacillin/tazobactam, olistin

mation		AR Bank Information	
nitials of ester		AR Bank Number	Organism
		AR0259	Pseudomonas aeruginosa
		AR0261	Pseudomonas aeruginosa
		AR0268	Pseudomonas aeruginosa
		AR0351	Pseudomonas aeruginosa
		AR0354	Pseudomonas aeruginosa
		AR0356	Pseudomonas aeruginosa
		AR0358	Pseudomonas aeruginosa
		AR0360	Pseudomonas aeruginosa
		AR0447	Pseudomonas aeruginosa
		AR0459	Pseudomonas aeruginosa
		AR0511	Pseudomonas aeruginosa
		AR0514	Pseudomonas aeruginosa

AR Bank Acinetobacter (ACI) Validation Set

e used to validate *Acinetobacter baumannii* for meropenem, d colistin.

mation		AR Bank Information	
nitials of ester		AR Bank Number	Organism
		AR0273	Acinetobacter baumannii
		AR0275	Acinetobacter baumannii
		AR0276	Acinetobacter baumannii complex
		AR0277	Acinetobacter baumannii
		AR0278	Acinetobacter baumannii
		AR0279	Acinetobacter baumannii
		AR0280	Acinetobacter baumannii
		AR0281	Acinetobacter baumannii
		AR0283	Acinetobacter baumannii
		AR0284	Acinetobacter baumannii
		AR0285	Acinetobacter baumannii
		AR0286	Acinetobacter baumannii

Part C: BIT Summary Template Verification/validation Study (Criteria)

- Accuracy versus reference standard

Categoric Agreement (CA)	≥ 90%
Very Major Error (VME) rate	< 3%
Major Error (ME) rate	< 3%
Minor Error (MiE) rate	determined by laboratory director

CA can be <90% if majority of errors are minor

and MIC values fall within ± 1 doubling dilution

- Reproducibility

95% of replicate results for given antimicrobial agent/organism combination fall into appropriate S, SDD, I, or R category

Part C: BIT Summary Template Calculations

- CA = same category results/total x 100

  VME = susceptible (test system)/resistant (reference) x 100

  ME = resistant (test system)/susceptible (reference) x 100

  MiE = I or SDD (test system) when other result is S or R (reference)/

  all isolates x 100

- Reproducibility

  reproducible results per agent-organism combination (test system)/

  x 100

Part C: BIT Summary Template Procedural

- Discrepancy resolution (outside of obvious human or clerical error)
  - Retesting in triplicate
  - Adjudication by disk diffusion
  - Forward to a reference laboratory
- Update data tables (discussed in later slides); prepare for data analysis

“They do this for you” Examples (prelude to part F)

AR Bank Information		Cefazolin AR Bank Data			Cefazolin Test Data			Cefazolin Calculations			
AR Bank Number	Organism	Sign	MIC	Interp	Test Sign	Test MIC	Test Interp	EA	Dilutions	CA	Error
AR0010	Klebsiella pneumoniae	>	8	R		8	R	YES	-1	YES	
AR0011	Escherichia coli	>	8	R		8	R	YES	-1	YES	
AR0012	Klebsiella pneumoniae	>	8	R		8	R	YES	-1	YES	
AR0013	Escherichia coli	>	8	R		2	S	NO	-3	NO	VME
AR0014	Escherichia coli	>	8	R		8	R	YES	-1	YES	
AR0015	Escherichia coli	>	8	R		8	R	YES	-1	YES	
AR0016	Klebsiella pneumoniae		4	I		8	R	YES	1	NO	minE
AR0017	Escherichia coli		2	S		8	R	NO	2	NO	ME
AR0018	Klebsiella aerogenes										
AR0019	Escherichia coli		8	R		8	R	YES	0	YES	
AR0020	Escherichia coli	>	8	R		8	R	YES	-1	YES	
AR0021	Citrobacter freundii										

You only have to play with these two columns

Excel does the rest

“They do this for you” Examples (prelude to part F)

AR Bank Information		Cefazolin AR Bank Data		Cefazolin Test Data		Cefazolin Calculations		
AR Bank Number	Organism					Dilutions	CA	Error
AR0010	Klebsiella pneumor					-1	YES	
AR0011	Escherichia coli					-1	YES	
AR0012	Klebsiella pneumor					-1	YES	
AR0013	Escherichia coli					-3	NO	VME
AR0014	Escherichia coli					-1	YES	
AR0015	Escherichia coli					-1	YES	
AR0016	Klebsiella pneumor					1	NO	minE
AR0017	Escherichia coli					2	NO	ME
AR0018	Klebsiella aerogene							
AR0019	Escherichia coli					0	YES	
AR0020	Escherichia coli					-1	YES	
AR0021	Citrobacter freundii							

Summary Data			
	%	N	Total N
EA	89.5%	17	19
CA	84.2%	16	19
minE	5.3%	1	19
ME	50.0%	1	2
VME	6.3%	1	16

Part C: BIT Summary Template Summary (Your/Excel Calculations)

V. Summary of Results Obtained

A. Accuracy

Agent(s)	# of Isolates*					CA	VME	ME	MIE
	Total	S	SDD	I	R	# (%)	# (%)	# (%)	# (%)

*Numbers represent a summary of the reference results.
Abbreviations: CA, categorical agreement; I, Intermediate; ME, major errors; MIE, minor errors; S, susceptible; SDD, susceptible dose dependent; R, resistant; VME, very major errors.

B. Reproducibility

_____% of _____ (agent/organism) results were reproducible.

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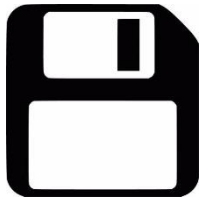

Dot the I's, Cross the T's

I have reviewed the _____ (verification/validation) data for accuracy and precision, for the (AST test method) _____, and the performance of the method is considered acceptable for patient testing.

Reviewed by: _____ Date: _____

Signature: _____

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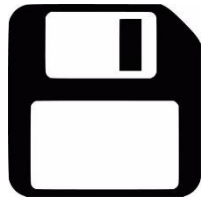
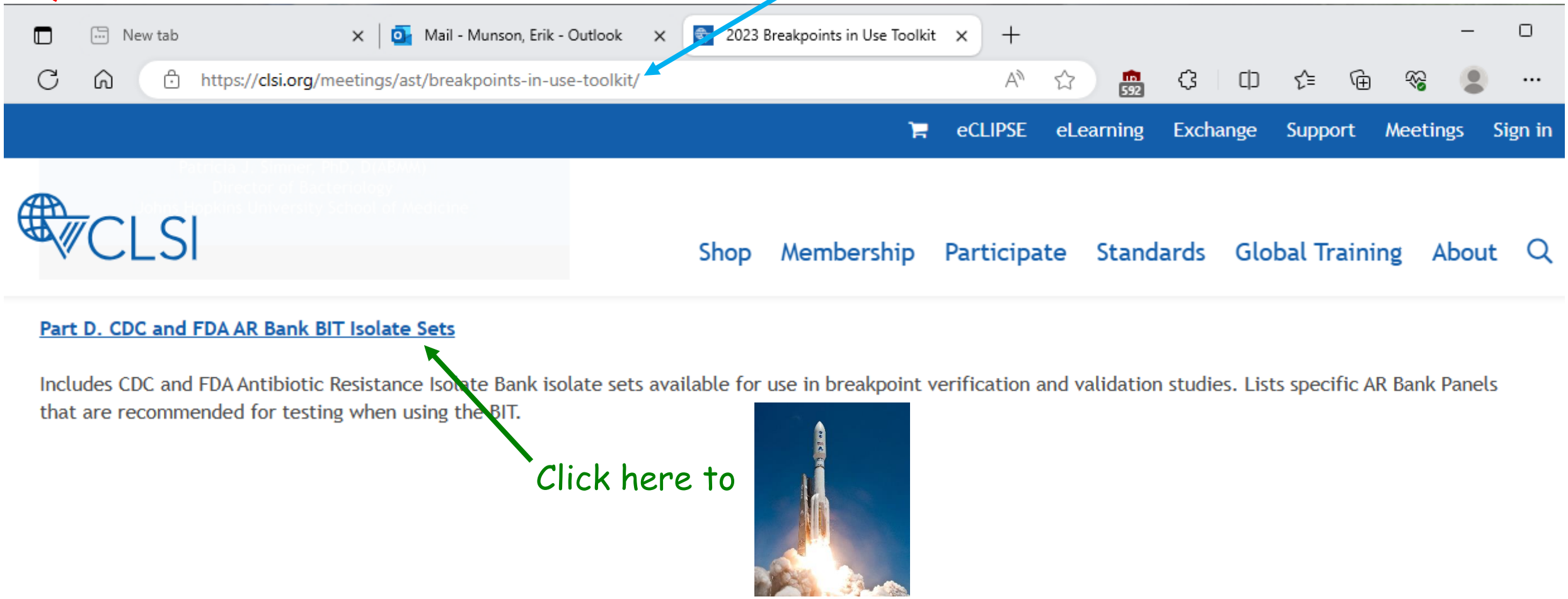


Breakpoint Implementation Toolkit (2023 BIT)
Part D. CDC and FDA AR Bank BIT Isolate Sets

WHAT TO USE

Here's the web address; easy to find



Click here to

Part D. CDC and FDA AR Bank BIT Isolate Sets

2023 Breakpoint Implementation Toolkit Set #	Organism Group	Antimicrobial Breakpoints to Validate	Isolate Selection AR Bank Panel(s)	Notes
1	Enterobacterales	Carbapenems Cephalosporins Fluoroquinolones	Enterobacterales Carbapenem Breakpoint Implementation Toolkit (BIT)	For cefazolin, 2 susceptible (S) isolates are in this set. Cefazolin-S isolates from other panels can be added, if desired: Carbapenemase Detection panel (n=3) or Delafloxacin panel (n=9)
2	Enterobacterales	Aminoglycosides Colistin Piperacillin-tazobactam	Piperacillin-tazobactam and aminoglycosides for Enterobacterales (PTA)	–
3	*Pseudomonas aeruginosa*	Aminoglycosides Carbapenems Cephalosporins Colistin Fluoroquinolones Piperacillin-tazobactam	Fluoroquinolones and aminoglycosides *Pseudomonas aeruginosa* verification (PFA)	The Fluoroquinolones and aminoglycosides *Pseudomonas aeruginosa* verification set is also suitable for evaluation of other *P. aeruginosa* breakpoints
4	*Acinetobacter baumannii* complex	Carbapenems Colistin	*Acinetobacter baumannii* (ACI)	–

We'll look at one

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
0002	*Enterobacter cloacae*	aac(6')-Ib, aadA1, ACT-45, aph(3')-Ia, aph(3')-Ib, aph(6)-Id, dfrA14, **KPC-3**, OmpF2, OXA-9, sul2, TEM-1A	SAMN04014843
0003	*Klebsiella pneumoniae*	aac(6')-Ib, aadA1, aph(3')-Ib, aph(6)-Id, dfrA14, EMRD, KDEA, **KPC-3**, Omp35, OmpK35, oqxA, oqxB, OXA-9, SHV-12, sul2, TEM-1A	SAMN04014844
0004	*Klebsiella pneumoniae*	aac(6')-Ib, aadA1, aadA2, catA1, dfrA12, EMRD, KDEA, **KPC-3**, Omp35, OmpK35, oqxB, OXA-9, SHV-11, sul1, TEM-1A	SAMN04014845
0006	*Escherichia coli*	aac(3)-IIa, aac(6')-IB3, aadA5, ACRF, aph(3')-Ib, aph(6)-Id, cmlA1, CMY-2, CTX-M-14, dfrA17, EMRD, MDF(A), mph(A), OmpC, OmpC2, OmpF, sul1, sul2, TEM-1, tet(A)	SAMN04014847
0009	*Klebsiella aerogenes*	CMY2-MIR-ACT-EC, Omp35, Omp36, OmpK35, OmpK36	SAMN04014850

OR

AR Bank # 0001 *Escherichia coli*

Biosample Accession #: SAMN04014842

MLST: 43(Pasteur), 131(Achtman)

Can also be downloaded to an all-in-one PDF

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).

Panel: Piperacillin-tazobactam +aminoglycosides for Enterobacterales (PTA) (Custom) | GN7F ARLN (Custom) | Piperacillin-tazobactam +aminoglycosides for Enterobacterales (PTA) (Custom)

"instructions for use"

genotype

MIC | MMR | Propagation | Temp & Biosafety | Isolate History

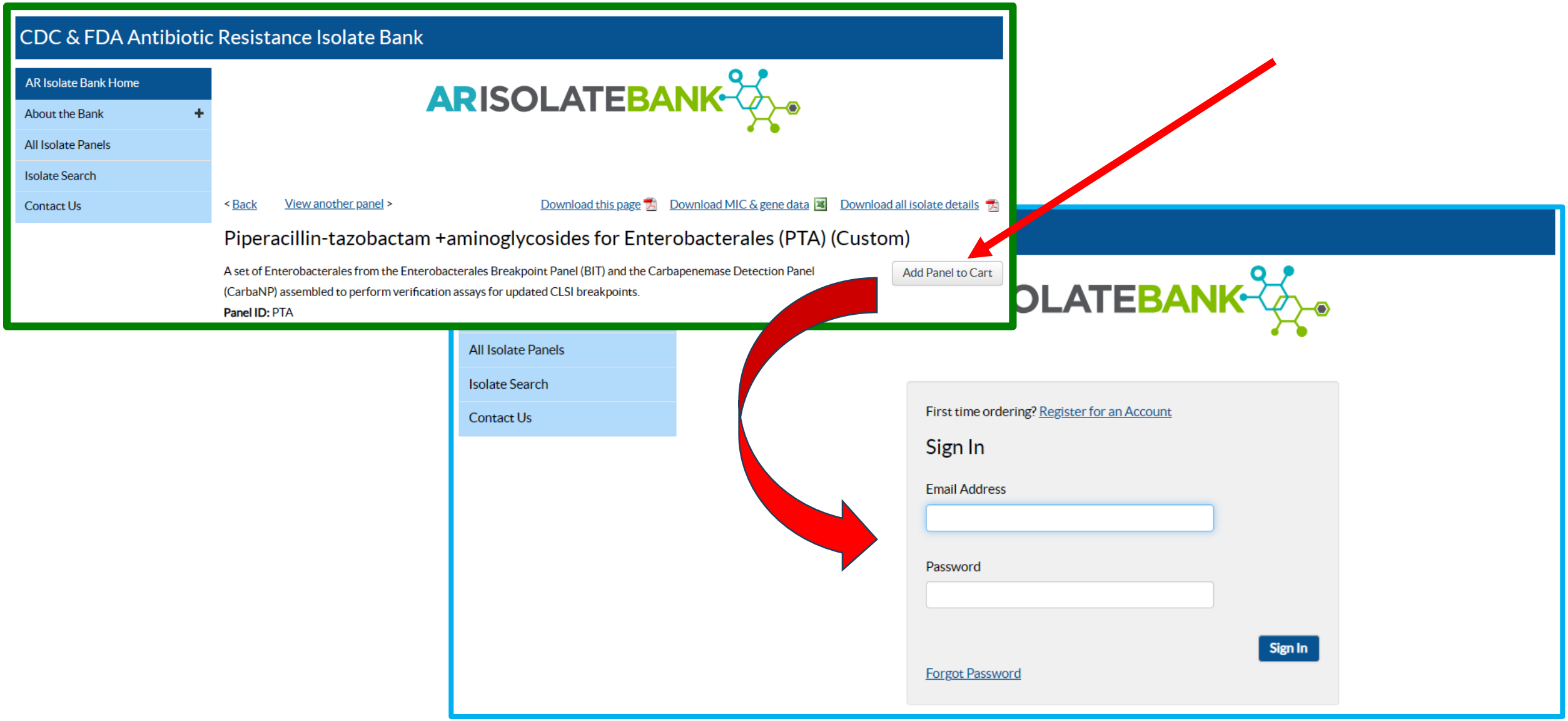
MIC (µg/ml) Results and Interpretation		
Drug	**MIC (µg/ml)**	**INT**
Amikacin	16	R
Ampicillin	>32	R

phenotype

General Comments

- 30-34 isolates per panel
  - Variety of MIC values (i.e., mix of interpretations)
  - Variety of species within *Enterobacterales*
  - CDC goal is to assist in verification/validation of multiple agents
- Some panels may be a bit short on “susceptible” isolates
  - Use own clinical isolates
  - Partner/share with local, regional laboratories
  - Surveillance collections with raw MIC data (SWOTARE)

How Do You Get These?



After Registration…

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

CDC & FDA Antibiotic Resistance Isolate Bank

CDC > Antibiotic / Antimicrobial Resistance

AR ISOLATE BANK

Choose an Isolate Panel

Go

Choose a Custom Isolate Panel

Custom panels are sets of AR Bank isolates that belong to other established panels. These sets are assembled for a specific intended use, e.g., Fluoroquinolone verification.

Go

AR Isolate Bank By the Numbers

33	panels
1031	isolates
4369	orders
2811	registered users

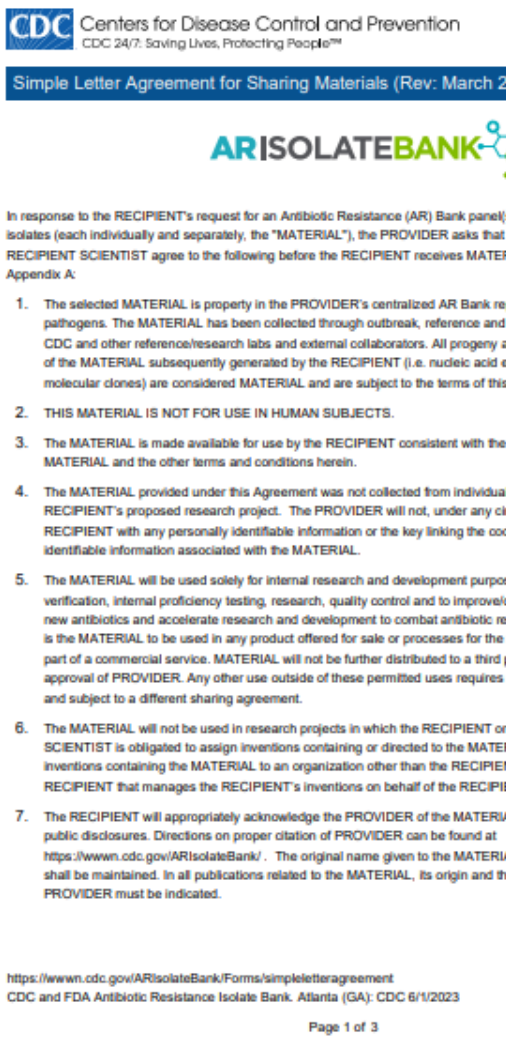
Order Processing

CDC provides email notifications on the status of your order. Below are the different statuses an order will undergo:

- **Awaiting Required Documents:** Order has been submitted, but CDC has not yet received your required forms to process the order.
- **Documents Verified:** CDC has received and verified that all required documents are complete and signed.
- **Order Verified:** Information on the forms match the order request.
- **Approved for Shipping:** Order has been sent to laboratory to be fulfilled.
- **Shipped:** Order has been shipped, tracking number will be added to order information under your account.
- **Delivered:** Courier has marked this order as delivered.

...shipped within ten business days after approval

Simple Letter Agreement for Sharing Materials



CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Simple Letter Agreement for Sharing Materials (Rev: March 2

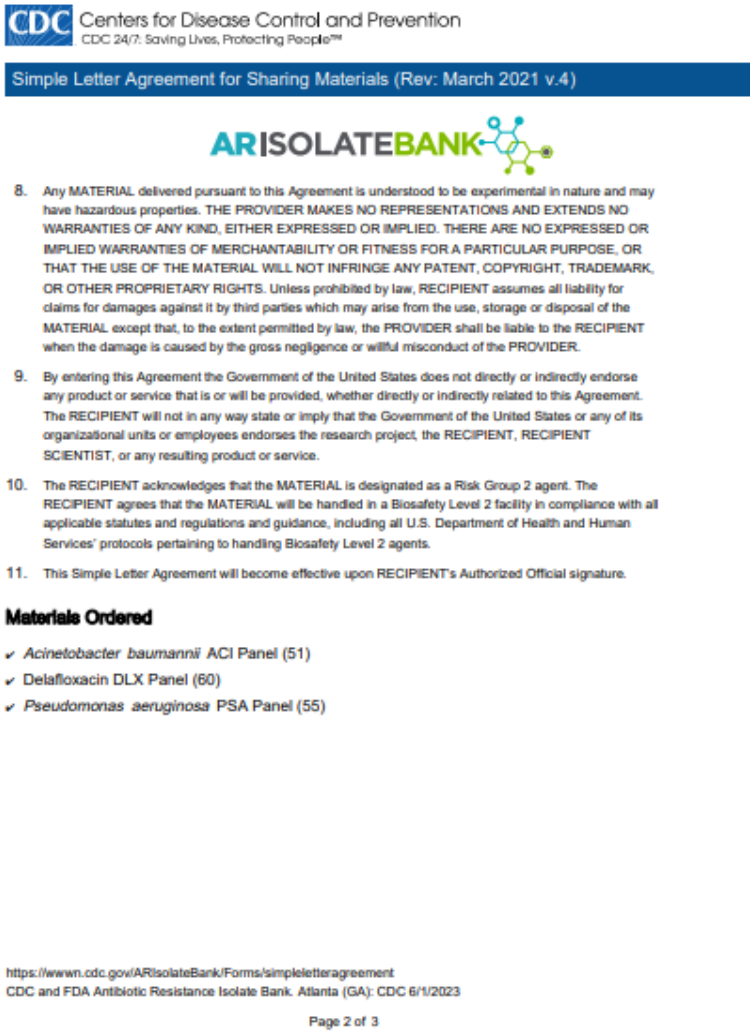
ARISOLATEBANK

In response to the RECIPIENT's request for an Antibiotic Resistance (AR) Bank panel(
isolates (each individually and separately, the "MATERIAL"), the PROVIDER asks that
RECIPIENT SCIENTIST agree to the following before the RECIPIENT receives MATE
Appendix A:

1. The selected MATERIAL is property in the PROVIDER's centralized AR Bank re
pathogens. The MATERIAL has been collected through outbreak, reference and
CDC and other reference/research labs and external collaborators. All progeny
of the MATERIAL subsequently generated by the RECIPIENT (i.e. nucleic acid
molecular clones) are considered MATERIAL and are subject to the terms of this
2. THIS MATERIAL IS NOT FOR USE IN HUMAN SUBJECTS.
3. The MATERIAL is made available for use by the RECIPIENT consistent with the
MATERIAL and the other terms and conditions herein.
4. The MATERIAL provided under this Agreement was not collected from individua
RECIPIENT's proposed research project. The PROVIDER will not, under any ci
RECIPIENT with any personally identifiable information or the key linking the co
identifiable information associated with the MATERIAL.
5. The MATERIAL will be used solely for internal research and development purpo
verification, internal proficiency testing, research, quality control and to improve
new antibiotics and accelerate research and development to combat antibiotic re
is the MATERIAL to be used in any product offered for sale or processes for the
part of a commercial service. MATERIAL will not be further distributed to a third
approval of PROVIDER. Any other use outside of these permitted uses requires
and subject to a different sharing agreement.
6. The MATERIAL will not be used in research projects in which the RECIPIENT or
SCIENTIST is obligated to assign inventions containing or directed to the MATE
inventions containing the MATERIAL to an organization other than the RECIPIE
RECIPIENT that manages the RECIPIENT's inventions on behalf of the RECIPI
7. The RECIPIENT will appropriately acknowledge the PROVIDER of the MATERI
public disclosures. Directions on proper citation of PROVIDER can be found at
https://www.cdc.gov/ARIsolateBank/ . The original name given to the MATERI
shall be maintained. In all publications related to the MATERIAL, its origin and th
PROVIDER must be indicated.

https://wwwn.cdc.gov/ARIsolateBank/Forms/simpleletteragreement
CDC and FDA Antibiotic Resistance Isolate Bank. Atlanta (GA): CDC 6/1/2023

Page 1 of 3



CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Simple Letter Agreement for Sharing Materials (Rev: March 2021 v.4)

ARISOLATEBANK

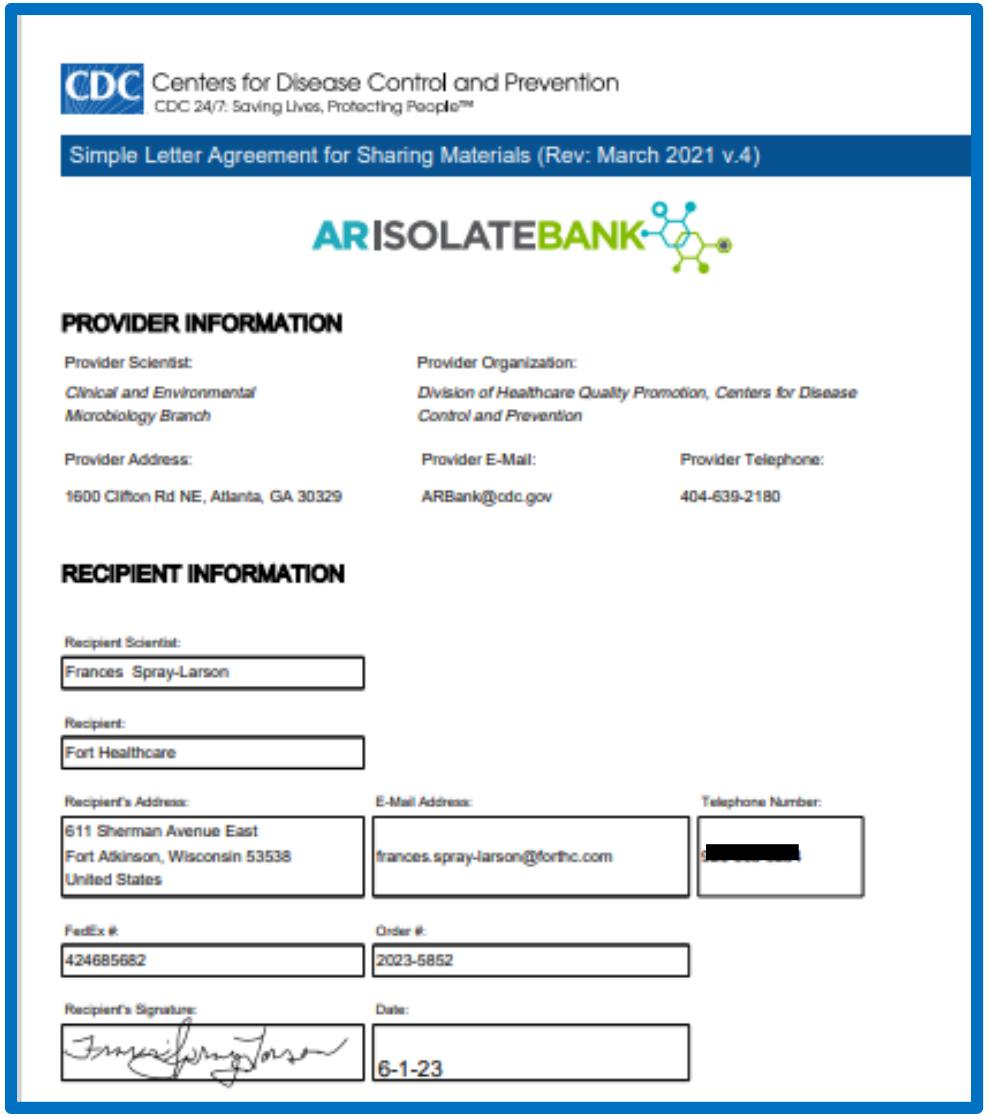
8. Any MATERIAL delivered pursuant to this Agreement is understood to be experimental in nature and may have hazardous properties. THE PROVIDER MAKES NO REPRESENTATIONS AND EXTENDS NO WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED. THERE ARE NO EXPRESSED OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR THAT THE USE OF THE MATERIAL WILL NOT INFRINGE ANY PATENT, COPYRIGHT, TRADEMARK, OR OTHER PROPRIETARY RIGHTS. Unless prohibited by law, RECIPIENT assumes all liability for claims for damages against it by third parties which may arise from the use, storage or disposal of the MATERIAL except that, to the extent permitted by law, the PROVIDER shall be liable to the RECIPIENT when the damage is caused by the gross negligence or willful misconduct of the PROVIDER.
9. By entering this Agreement the Government of the United States does not directly or indirectly endorse any product or service that is or will be provided, whether directly or indirectly related to this Agreement. The RECIPIENT will not in any way state or imply that the Government of the United States or any of its organizational units or employees endorses the research project, the RECIPIENT, RECIPIENT SCIENTIST, or any resulting product or service.
10. The RECIPIENT acknowledges that the MATERIAL is designated as a Risk Group 2 agent. The RECIPIENT agrees that the MATERIAL will be handled in a Biosafety Level 2 facility in compliance with all applicable statutes and regulations and guidance, including all U.S. Department of Health and Human Services' protocols pertaining to handling Biosafety Level 2 agents.
11. This Simple Letter Agreement will become effective upon RECIPIENT's Authorized Official signature.

Materials Ordered

- ✔ *Acinetobacter baumannii* ACI Panel (51)
- ✔ Delafloxacin DLX Panel (60)
- ✔ *Pseudomonas aeruginosa* PSA Panel (55)

https://wwwn.cdc.gov/ARIsolateBank/Forms/simpleletteragreement
CDC and FDA Antibiotic Resistance Isolate Bank. Atlanta (GA): CDC 6/1/2023

Page 2 of 3



CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Simple Letter Agreement for Sharing Materials (Rev: March 2021 v.4)

ARISOLATEBANK

PROVIDER INFORMATION

Provider Scientist:	Provider Organization:	
Clinical and Environmental Microbiology Branch	*Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention*	
Provider Address:	Provider E-Mail:	Provider Telephone:
1600 Clifton Rd NE, Atlanta, GA 30329	ARBank@cdc.gov	404-639-2180

RECIPIENT INFORMATION

Recipient Scientist: Frances Spray-Larson

Recipient: Fort Healthcare

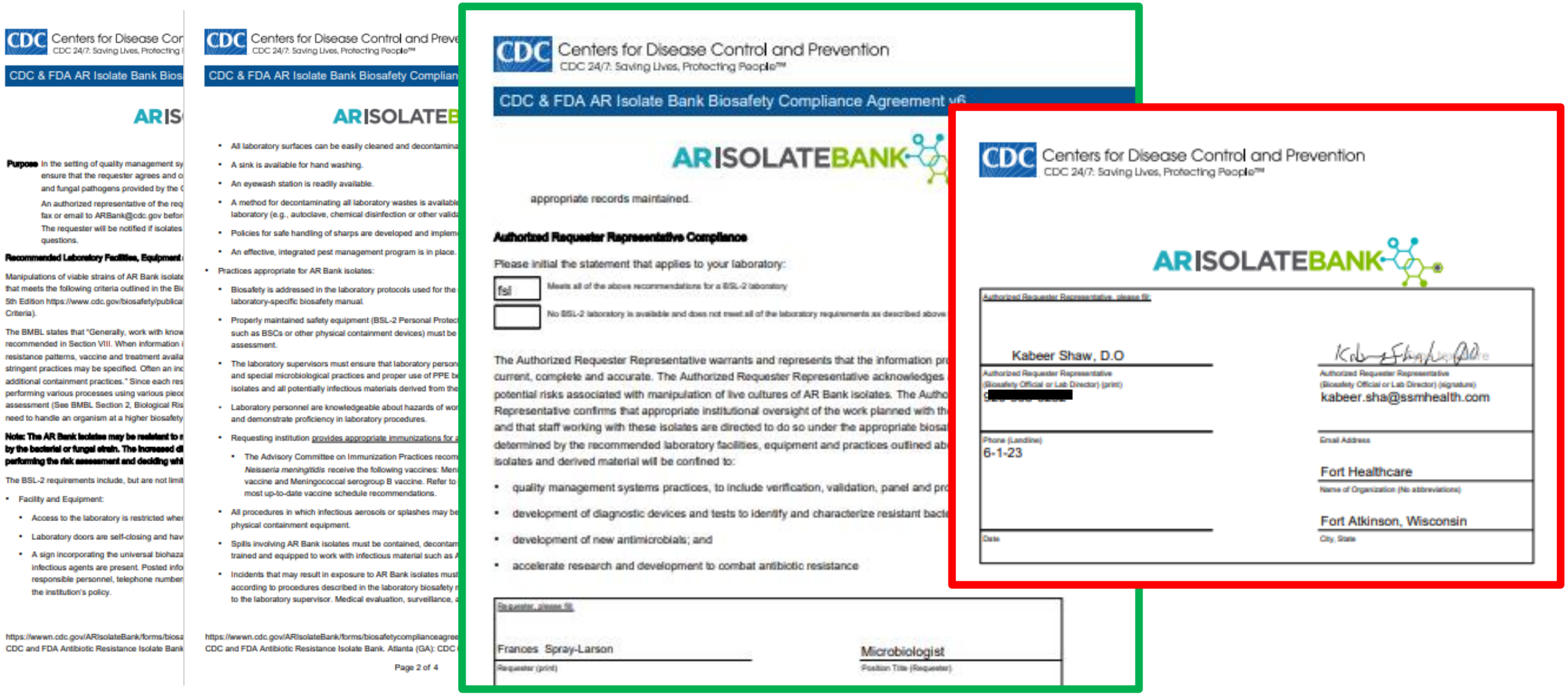
Recipient's Address:	E-Mail Address:	Telephone Number:
611 Sherman Avenue East Fort Atkinson, Wisconsin 53538 United States	frances.spray-larson@forthc.com	[redacted]

FedEx #:	Order #:
424685682	2023-5852

Recipient's Signature:	Date:
[signature]	6-1-23

Courtesy Frances Spray-Larson, Ph.D.

Biosafety Compliance Agreement



Courtesy Frances Spray-Larson, Ph.D.

2023 BIT Parts E and F

Thomas Novicki

The Magic Begins

Part E

CLSI

WORKBOOK

Instructions for Use of 2023 BIT Part F

Accuracy Instructions for Prefilled Excel

Table of Contents

Bottom Line Up Front: Part F

- File: Part F_AR_Bank_Data_Entry_and_Calculations.xlsx

- Multiple tabs:
  - Breakpoint Overview
  - Summary
  - Accuracy and Precision tabs for each of the 4 BIT libraries

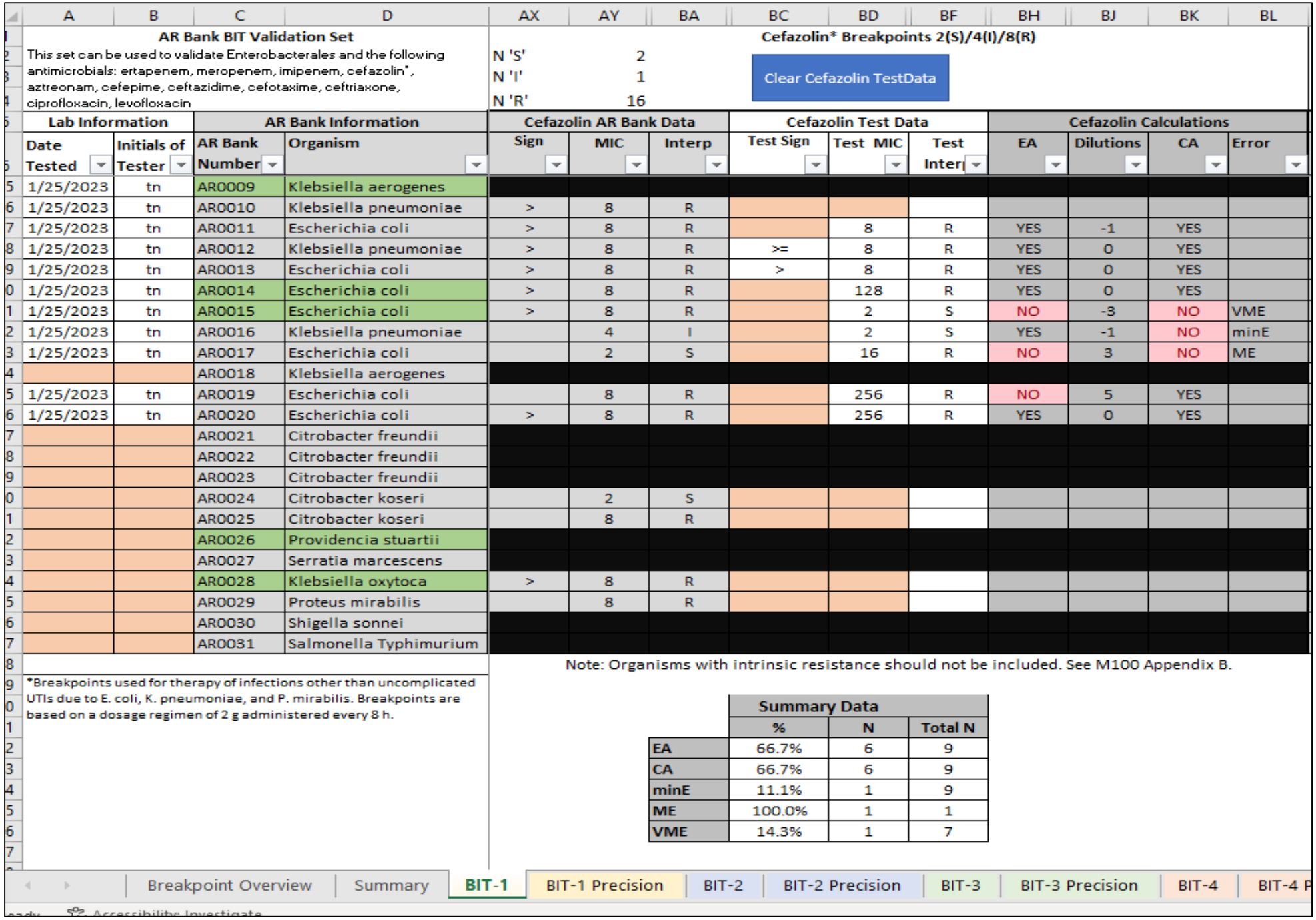
- Contains a macro to clear all date entered in the Accuracy tabs
  - Not necessary for the table to operate; fields can be manually cleared using “Clear Contents”.

Bottom Line Up Front: Part F

- Embedded formulae perform calculations and summarizes data.
- Each column may be sorted and filtered for further data analysis.
- Is locked to prevent accidental keystrokes: only the orange sections are editable with predefined entries.
- Isolates in green: suggestions for precision studies.
- **Limitation: Table F is MIC only. BIT sets may be used for disk diffusion, with data-keeping in table G. (Next section.)**

Part F:

A Multi-Tab Excel Spread-sheet



AR Bank BIT Validation Set

This set can be used to validate Enterobacterales and the following antimicrobials: ertapenem, meropenem, imipenem, cefazolin*, aztreonam, cefepime, ceftazidime, cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin

Cefazolin* Breakpoints 2(S)/4(I)/8(R)

N 'S' 2
N 'I' 1
N 'R' 16

Clear Cefazolin TestData

Lab Information		AR Bank Information		Cefazolin AR Bank Data			Cefazolin Test Data			Cefazolin Calculations			
Date Tested	Initials of Tester	AR Bank Number	Organism	Sign	MIC	Interp	Test Sign	Test MIC	Test Interp	EA	Dilutions	CA	Error
1/25/2023	tn	AR0009	Klebsiella aerogenes										
1/25/2023	tn	AR0010	Klebsiella pneumoniae	>	8	R							
1/25/2023	tn	AR0011	Escherichia coli	>	8	R		8	R	YES	-1	YES	
1/25/2023	tn	AR0012	Klebsiella pneumoniae	>	8	R	>=	8	R	YES	0	YES	
1/25/2023	tn	AR0013	Escherichia coli	>	8	R	>	8	R	YES	0	YES	
1/25/2023	tn	AR0014	Escherichia coli	>	8	R		128	R	YES	0	YES	
1/25/2023	tn	AR0015	Escherichia coli	>	8	R		2	S	NO	-3	NO	VME
1/25/2023	tn	AR0016	Klebsiella pneumoniae		4	I		2	S	YES	-1	NO	minE
1/25/2023	tn	AR0017	Escherichia coli		2	S		16	R	NO	3	NO	ME
		AR0018	Klebsiella aerogenes										
1/25/2023	tn	AR0019	Escherichia coli		8	R		256	R	NO	5	YES	
1/25/2023	tn	AR0020	Escherichia coli	>	8	R		256	R	YES	0	YES	
		AR0021	Citrobacter freundii										
		AR0022	Citrobacter freundii										
		AR0023	Citrobacter freundii										
		AR0024	Citrobacter koseri		2	S							
		AR0025	Citrobacter koseri		8	R							
		AR0026	Providencia stuartii										
		AR0027	Serratia marcescens										
		AR0028	Klebsiella oxytoca	>	8	R							
		AR0029	Proteus mirabilis		8	R							
		AR0030	Shigella sonnei										
		AR0031	Salmonella Typhimurium										

*Breakpoints used for therapy of infections other than uncomplicated UTIs due to E. coli, K. pneumoniae, and P. mirabilis. Breakpoints are based on a dosage regimen of 2 g administered every 8 h.

Note: Organisms with intrinsic resistance should not be included. See M100 Appendix B.

Summary Data			
	%	N	Total N
EA	66.7%	6	9
CA	66.7%	6	9
minE	11.1%	1	9
ME	100.0%	1	1
VME	14.3%	1	7

Breakpoint Overview | Summary | BIT-1 | BIT-1 Precision | BIT-2 | BIT-2 Precision | BIT-3 | BIT-3 Precision | BIT-4 | BIT-4 P

Accuracy Examples

					Cefazolin* Breakpoints 2(S)/4(I)/8(R)							
					N 'S' 2							
					N 'I' 1							
					N 'R' 16							
					Clear Cefazolin TestData							
	AR Bank Information		Cefazolin AR Bank Data			Cefazolin Test Data			Cefazolin Calculations			
	AR Bank Number	Organism	Sign	MIC	Interp	Test Sign	Test MIC	Test Interp	EA	Dilutions	CA	Error
1.	AR0011	Escherichia coli	>	8	R		8	R	YES	-1	YES	
	AR0012	Klebsiella pneumoniae	>	8	R	>=	8	R	YES	0	YES	
	AR0013	Escherichia coli	>	8	R	>	8	R	YES	0	YES	
	AR0014	Escherichia coli	>	8	R		128	R	YES	0	YES	
2.	AR0015	Escherichia coli	>	8	R		2	S	NO	-3	NO	VME
	AR0016	Klebsiella pneumoniae		4	I		2	S	YES	-1	NO	minE
	AR0017	Escherichia coli		2	S		16	R	NO	3	NO	ME
3.	AR0019	Escherichia coli		8	R		256	R	NO	5	YES	
4.	AR0020	Escherichia coli	>	8	R		128	R	YES	0	YES	

(Adapted from Part F_AR_Bank_Data_Entry_and_Calculations.xlsx)

Precision

	A	B	C	D	E	F	G	H	I	J	K	L
14	Cefazolin (systemic)											
15	Isolate	Organism	Result 1		Result 2		Result 3		Interpretation Mode or Median	Result 1	Result 2	Result 3
16			Intials, Date Tested	Interp	Intials, Date Tested	Interp	Intials, Date Tested	Interp		Equivalency	Equivalency	Equivalency
17	AR0011	Escherichia coli	TN, 1/28/23	R	MS, 1/29/23	S	DK, 1/30/23	S	S	No	Yes	Yes
18	AR0012	Klebsiella pneumoniae	TN, 1/28/23	S	MS, 1/29/23	S	DK, 1/30/23	I	S	Yes	Yes	No
19	AR0013	Escherichia coli	TN, 1/28/23	S	MS, 1/29/23	S	DK, 1/30/23	S	S	Yes	Yes	Yes
20	ATCC 25922	Escherichia coli	TN, 1/28/23	S	MS, 1/29/23	S	DK, 1/30/23	S	S	Yes	Yes	Yes
21	Lab 5-2021	K. aerogenes	TN, 1/28/23	R	MS, 1/29/23	R	DK, 1/30/23	R	R	Yes	Yes	Yes

R	S	T	U
	N	%	N Results Total
Category Equivalency	13	87%	15

AR0012 Result 3: This discrepancy = 1 dilution difference. Acceptable variation per CLSI M52 1st Ed. Corrected result 14/15= 93%.

(Adapted from Part F_AR_Bank_Data_Entry_and_Calculations.xlsx)

Summary Tab

	A	B	C	E	F	I	K	M	O	Q	R	S
1						Accuracy: Agreement and Errors				Precision/Reproducibility		
2	Set	Reporting Group	Antimicrobial	N 'S'	N 'R'	% CA	Errors			N	% Equivalent	N Equivalent
3							%Minor	%Major	% Very Major			
4	BIT-1	Enterobacterales	Aztreonam	0	0					0		0
5	BIT-1	Enterobacterales	Cefazolin	1	8	60.0%	20.0%	100.0%	12.5%	15	87%	13

Scroll Down

	A	B	C	D	E	F	I	K	M	O	Q	R	S
1							Accuracy: Agreement and Errors				Precision/Reproducibility		
2	Set	Reporting Group	Antimicrobial	N	N 'S'	N 'R'	% CA	Errors			N	% Equivalent	N Equivalent
3								%Minor	%Major	% Very Major			
33	NA	Combined	Aztreonam	0	0	0					0		0
34	NA	Combined	Cefepime	0	0	0					0		0
35	NA	Combined	Ceftazidime	0	0	0					0		0
36	NA	Combined	Ciprofloxacin	0	0	0					0		0
37	NA	Combined	Colistin	0	0	0					0		0
38	NA	Combined	Imipenem	0	0	0					0		0
39	NA	Combined	Levofloxacin	0	0	0					0		0
40	NA	Combined	Meropenem	0	0	0					0		0
41	NA	Combined	Piperacillin/tazobactam	1	1	0					0		0
42	NA	Combined	Tobramycin	0	0	0					0		0

(Adapted from Part F_AR_Bank_Data_Entry_and_Calculations.xlsx)

Discrepancy Resolution: Considerations

- Error was random? Retest x3
  - A strain may be inherently imprecise for a given drug. BIT sets shouldn't have these, but….
- Working strain went bad? Fresh sub from master stock, passed ≥2X. Use CLSI M2, M7 storage protocol
- Multiple drugs failed for a given strain? Strain may be mutated or contaminated
  - Consider KB or agar dilution. (At least one major reference lab offers the latter)
- Problems continue? Consult the instrument's tech service

Discrepancy Resolution: A few more considerations…

Precision (reproducibility) is defined as the closeness of agreement between the results of successive measurements of the same analyte. For ASTS, precision (reproducibility) should take the normal variation of ± 1 doubling dilution for antibacterial agents (± 2 doubling dilutions for antifungal agents) for testing into account. For example, MIC readings of 1 µg/mL or 2 µg/mL would be considered equivalent results. *

- Evaluate precision failures; consider accepting ± 1 dilution deviations.
- FYI CLSI M52 1st Ed. has a section on the validation of alternative breakpoints.

* CLSI M52 1st Ed: Verification of Commercial Microbial Identification and Antimicrobial Ssceptibility Testing Systems. Section 3.8.4

2023 BIT Part G Summary

Megan Selle

The Triwizard Cup- Part G: CLSI Toolkit

- The validation is nearly finished, the Triwizard cup is in sight.
- Part G of the CLSI toolkit includes a spreadsheet to fill out the data summary of the following:
  - Categorical agreement
  - Percentages of minor, major and very major errors
  - Precision results and percent agreement

Part G: Accuracy and Precision Worksheet

Lab Information		Comparator Isolate Information		Comparator Results		Test System Results		EA (Y/N)	CA(Y/N)	CA Error Type (VME, ME, minE)	Comments
Date Tested	Initials of Tester	ID Number	Organism	MIC (µg/mL)	Interpretation	MIC (µg/mL)	Interpretation				
6/5/2023	MES	123456	Kleb. Oxytoca	<=0.25	S	0.5	I	Y	N	minE	

EA: Essential Agreement; CA: category agreement; VME: very major error; ME: Major Error; minE: minor errors

Test System	Vitek 2, AST-GN79			
Antimicrobial	Ciprofloxacin			
Record Breakpoints	S	SDD	I	R
Old Breakpoints (µg/mL)	<=1		2	>=4
New Breakpoints (µg/mL)	<=0.25		0.5	>=1

Accuracy after discrepancy testing				
Analysis	%	N	Total Tested	
Category Agreement	93.4		30	
Minor Error	6.7	2	30	
Major Error	0	0	30	
Very Major	0	0	30	

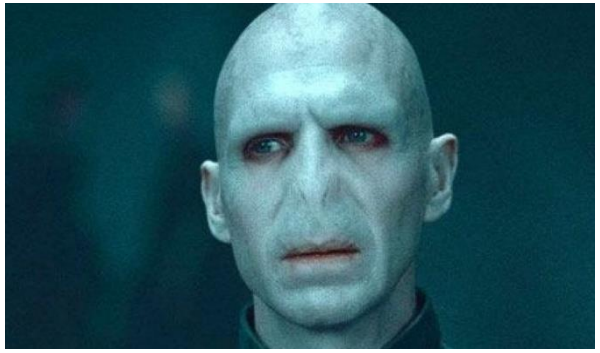
Perform calculations for precision here				
Analysis	%	N	Total Tested	
Equivalency	100	0	9	

Precision

Isolate	Organism	Result 1		Result 2		Result 3	
		Initials, Date Tested	Interp	Initials, Date Tested	Interp	Initials, Date Tested	Interp
ATCC25922	E. coli	6/5/2023, MES	S	6/6/23, CK	S	6/7/23, LK	S
AR-0160	K. pneumoniae	6/5/2023, MES	R	6/6/23, CK	R	6/7/23, LK	R
AR-0156	P. mirabilis	6/5/2023, MES	R	6/6/23, CK	R	6/7/23, LK	R

Part G: CLSI Toolkit

- The summary data can now be placed into the Breakpoint Implementation Summary for a medical director to sign.
- This wraps up the validation data until the next CLSI M100 update.

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Questions