CRO Breakpoint Implementation Toolkit

VERIFICATION TEMPLATE

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

Laboratory Name: _____

Department: _____

Effective Date: _____

Verification Performed: _____ **to** _____

Test Implementation Date: _____

Antibiotics Verified: ☐ **Ertapenem** ☐ **Imipenem** ☐ **Meropenem** ☐ **Doripenem**

cAST System Used: _____

(Hereafter referred to simply as "the cAST system")

I. PURPOSE

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

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

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

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

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

II. RELEVANCE

The Clinical and Laboratory Standards Institute (CLSI) published revised breakpoints for *Enterobacterales* and carbapenems. Specifically CLSI revised breakpoints for ertapenem, meropenem, and imipenem and added breakpoints for doripenem in June 2010 (M100-S20-U; Supplement), and then revised ertapenem once more in 2012. The current breakpoints of our **cAST systems** for [ ] are not up to date.

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

III. VERIFICATION STUDY DESIGN

1. cAST System

Panel: [ ]

Software Version: [ ]

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

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

2. Range Criteria

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

3. Accuracy

Strategy

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

Sample Size

Thirty-one select isolates of *Enterobacterales* from the CDC & FDA AR Isolate Bank (*Enterobacterales* Carbapenem Breakpoint Panel) representing a variety of species, carbapenem MICs and carbapenem resistance mechanisms were tested on the **cAST System** and results compared to those provided by CDC with the isolates.

4. Reproducibility (Precision)

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

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

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

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

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

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

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

5. Quality Control

QC Isolate 1:

QC Isolate 2:

☐ QC will be performed each day during verification testing.

☐ QC will be performed weekly, as acceptability for performance of weekly QC for the **cAST System** was previously established and documented. **cAST System**'s IQCP number:

6. Documentation

See **Appendix B1** for worksheets with recorded results of this verification.

7. Analysis

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

IV. PROCEDURE

1. Isolates

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

2. Materials and testing procedure

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

3. Quality Control

_____ and _____ were tested each day of verification testing.

4. Discrepancy resolution

Discrepancies were resolved by:

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

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

☐ At least one discrepancy was found. Number of discrepancies:

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

☐ No further VMEs or MEs were found in any of the replicates.

☐ All or some of the three replicates continued to show VMEs or MEs, therefore additional testing by a comparator method was used to confirm the reference results provided by CDC & FDA.
Additional testing included:

☐ Disk diffusion

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

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

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

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

Drug B: _____

Calculations

Table 3-B. 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

☐ At least one discrepancy was found. Number of discrepancies: ____

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

☐ No further VMEs or MEs were found in any of the replicates.

☐ All or some of the three replicates continued to show VMEs or MEs, therefore additional testing by a comparator method was used to confirm the reference results provided by CDC & FDA.
Additional testing included:

☐ Disk diffusion

☐ Another method available in the laboratory or a referral laboratory that has been verified for current breakpoints: _____

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

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

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

Drug C: _____

Calculations

Table 3-C. 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

☐ At least one discrepancy was found. Number of discrepancies: ____

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

☐ No further VMEs or MEs were found in any of the replicates.

☐ All or some of the three replicates continued to show VMEs or MEs, therefore additional testing by a comparator method was used to confirm the reference results provided by CDC & FDA.
Additional testing included:

☐ Disk diffusion

☐ Another method available in the laboratory or a referral laboratory that has been verified for current breakpoints: _____

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

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

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

Drug D: _____

Calculations

Table 3-D. 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

☐ At least one discrepancy was found. Number of discrepancies: ____

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

☐ No further VMEs or MEs were found in any of the replicates.

☐ All or some of the three replicates continued to show VMEs or MEs, therefore additional testing by a comparator method was used to confirm the reference results provided by CDC & FDA.
Additional testing included:

☐ Disk diffusion

☐ Another method available in the laboratory or a referral laboratory that has been verified for current breakpoints: _____

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

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

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

2. Reproducibility/Precision

☐ Reproducibility was not assessed.

☐ Reproducibility was assessed. For each drug, a total of ____ isolates were tested in triplicate over ____ day(s).

Number of isolates used: Susceptible: ____ Intermediate: ____ Resistant: ____

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

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

☐ No discrepancies were identified.

☐ At least one discrepancy was found. Number of discrepancies: ____

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

☐ No further VMEs or MEs were found in any of the replicates.

☐ All or some of the three replicates continued to show VMEs or MEs, therefore additional testing by a comparator method was used to confirm the reference results provided by CDC & FDA.
Additional testing included:

☐ Disk diffusion

☐ Another method available in the laboratory or a referral laboratory that has been verified for current breakpoints: _____

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

VI. SUMMARY OF RESULTS OBTAINED

1. Accuracy

Table 6. Summary of Accuracy Results

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

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

2. Reproducibility

Reproducibility of each agent was analyzed separately at the time the cAST System was initially verified with _____ on _____, which found _____% of results were reproducible.

The following antibiotics required additional reproducibility assessment:

☐ Ertapenem ☐ Imipenem ☐ Meropenem ☐ Doripenem

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

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

VII. CONCLUSION

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

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

Reviewed by: _____ Date: _____

Signature: _____

VIII. REFERENCES

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