



*Northern California Branch
American Society for Microbiology*



Verification of Antimicrobial Susceptibility Testing Methods

“A practical approach”

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Publication:



**Clinical
Microbiology**
NEWSLETTER

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Verification of Antimicrobial Susceptibility Testing Methods: a Practical Approach

[Jean B. Patel](#)  , [Susan Sharp](#), [Susan Novak-Weekley](#)

Preamble for Today's seminar



The process of verifying an antimicrobial susceptibility testing (AST) system can be very confusing.

- There are many different AST methods/instruments



- In addition, there are several different reasons why verification might be necessary
 - implementing a new AST method in the laboratory
 - adding a new antibiotic to an existing panel
 - implementing non-FDA breakpoints on an FDA-cleared AST system

Preamble for Today's seminar

The process of verifying an antimicrobial susceptibility testing (AST) system can be very confusing.

- The Clinical Laboratory Improvement Amendment (CLIA) provides some general guidance, but ultimately it is the responsibility of a laboratory director to decide on composition of a verification study protocol
- Variables to consider:
 - what methods should be compared
 - what isolates should be tested
 - how many isolates should be tested
 - how the results should be compared
 - what is an acceptable study outcome

We'll review some general guidelines for developing and conducting a verification study of an AST system.

Today's Agenda

- **Introduction**
- **What's involved in verification testing**
- **What is required under CLIA for laboratories**
- **Strategies for implementation of CLIA requirements**
 - Implementing a new AST method
 - Adding a new drug to the current AST method
 - Off-label use of a commercial AST device
- **Trouble-shooting**
- **Summary**

Introduction: Verification Testing

- **CLIA regulations require laboratories to verify the performance of a diagnostic test prior to its use for patient care.**

- FDA-approved or cleared
- Non FDA-approved or modified FDA-approved or cleared test



- **Verification of AST systems has gotten a lot of attention!**

- CLSI Subcommittee for Antimicrobial Susceptibility testing (AST Subcommittee) revised the Enterobacteriaceae breakpoints:
cephalosporins and carbapenems

NOTE The CLSI AST Subcommittee revised these breakpoints in order to improve the accuracy of detecting resistance and guiding therapeutic decisions.

- Implementation of the CLSI breakpoints on an FDA-approved AST system = **‘modification’** of the FDA-cleared test

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Introduction: Verification Testing

- **Laboratories that wish to implement the CLSI revised breakpoints:**
 - Significant obstacle = only FDA breakpoints can be used on FDA-approved devices
 - Use of CLSI alternative breakpoints:
 - requires an **in-house verification study** to establish performance specifications of the modified FDA-approved AST system
 - resource-intensive effort and most laboratories facing limited resources
 - we need a practical approach to meet these regulatory requirements

Verification vs. Validation

- **Validation is an on-going process of evaluating test performance over time and is part of a laboratory's quality assurance program**
 - quality control testing
 - internal and/or external proficiency testing
 - personnel competency assessments

Verification vs. Validation

- **Validation is an on-going process of evaluating test performance over time and are part of a laboratory's quality assurance program**
 - quality control testing
 - internal and external proficiency testing
 - personnel competency assessments
- **Verification is a one-time process of determining that a test performs correctly**
 - a verification study is conducted to determine the performance characteristics of an assay prior to use of the assay for patient care
 - performance characteristics are determined by comparing results from the method being evaluated (“new method”) to results from a gold standard/reference/existing method
 - disk diffusion testing \longleftrightarrow automated AST
 - relevant performance characteristics:
 - accuracy
 - precision (reproducibility)
 - essential agreement
 - category agreement
 - should include the testing of both patient and quality control organisms
 - data analysis

Verification Comparison Studies

1. MIC method to another MIC method
2. MIC method to a disk diffusion method

Verification - Comparison Study

1. MIC method to another MIC method
2. MIC method to a disk diffusion method

MIC vs. MIC Evaluation:

- **Essential agreement**
 - % of isolates producing MICs that are within ± 1 doubling dilution of the standard/reference/existing method
- **Category agreement**
 - % of isolates producing the same category result (S I R) as compared to the standard/reference/existing method

Comparison Study

MIC vs. MIC: EXAMPLE

- **100 isolates are tested by each method**
 - 92 of the isolates produced a MIC that was within 1 doubling dilution of the existing method
 - Essential agreement for the study is 92% (92/100)
 - 95 of the isolates fall within the same category as the existing method
 - Category agreement is 95% (95/100)
 - ERRORS: minor, major, very major errors

Comparison Study

MIC vs. MIC – Categorical Agreement:

•Minor errors

- susceptible vs. intermediate
- intermediate vs. resistant
- least detrimental effect on a therapeutic decision

•Major errors

- resistant results by the new method and susceptible results by the gold standard method
- this leads to an over-estimation of resistance by the new method
- this can result in a decision not to use a therapeutic agent which should have been effective
- these errors can have serious consequences if therapeutic options are very limited, but otherwise may not result in harm to the patient

•Very major error

- susceptible result by the new method and a resistant result by the gold standard method
- most serious error - the method failed to detect resistance which may result in the use of an ineffective therapeutic agent for treatment of an infection

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Comparison Study

MIC vs. MIC – Essential Agreement:

% of isolates producing MICs that are within ± 1 doubling dilution of the standard/reference method

100 isolates are tested

Comparison Study: Essential Agreement

GOLD STANDARD MIC METHOD

		GOLD STANDARD MIC METHOD									
		S					I		R		
		0.06	0.12	0.25	0.5	1	2	4	8	16	
NEW MIC METHOD	S	0.06									
		0.12		4	1						
		0.25		8	11	1					
		0.5		3	21	5					
		1		2	4	11	3		1		
	I	2			1	1	4		1		
		4					2	7	5		
		8					1	2	1		
		16									
	R										

Comparison Study: Essential Agreement

GOLD STANDARD MIC METHOD

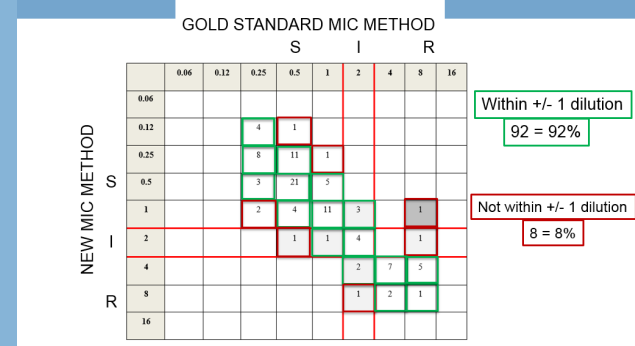
S I R

		0.06	0.12	0.25	0.5	1	2	4	8	16	
NEW MIC METHOD	S	0.06									
	0.12			4	1						
	0.25			8	11	1					
	0.5			3	21	5					
	1			2	4	11	3		1		
	I	2				1	1	4		1	
	4						2	7	5		
	R	8						1	2	1	
	16										

Within +/- 1 dilution
92/100 = 92%

Not within +/- 1 dilution
8/100 = 8%

Comparison Study



MIC vs. MIC – Essential Agreement:

% of isolates producing MICs that are within ± 1 doubling dilution of the standard method

92/100 are within ± 1 doubling dilution = 92% Essential Agreement (pass)

(Acceptability is $\geq 90\%$)

Comparison Study

MIC vs. MIC – Categorical Agreement:

% of isolates producing the same category result (S I R) as compared to the standard/reference method

Comparison Study: Categorical Agreement

GOLD STANDARD METHOD

		GOLD STANDARD METHOD								
		S			I			R		
		0.06	0.12	0.25	0.5	1	2	4	8	16
NEW METHOD	S	0.06								
	0.12			4	1					
	0.25			8	11	1				
	0.5			3	21	5				
	1			2	4	11	3		1	
	I	2			1	1	4		1	
	4						2	7	5	
	R	8					1	2	1	
	16									

Comparison Study: Categorical Agreement

GOLD STANDARD METHOD

S I R

NEW METHOD

S

I

R

	0.06	0.12	0.25	0.5	1	2	4	8	16
0.06									
0.12			4	1					
0.25			8	11	1				
0.5			3	21	5				
1			2	4	11	3		1	
2				1	1	4		1	
4						2	7	5	
8						1	2	1	
16									

Agreement

Comparison Study: Categorical Agreement

GOLD STANDARD METHOD

S I R

NEW METHOD

S

I

R

	0.06	0.12	0.25	0.5	1	2	4	8	16
0.06									
0.12			4	1					
0.25			8	11	1				
0.5			3	21	5				
1			2	4	11	3		1	
2				1	1	4		1	
4						2	7	5	
8						1	2	1	
16									

Agreement

Comparison Study: Categorical Agreement

GOLD STANDARD METHOD

		GOLD STANDARD METHOD								
		S			I		R			
		0.06	0.12	0.25	0.5	1	2	4	8	16
NEW METHOD	S	0.06								
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		0.5		3	21	5				
		1		2	4	11	3		1	
	I	2			1	1	4		1	
		4					2	7	5	
		8					1	2	1	
	R	16								

90 Agreement

Comparison Study: Categorical Agreement

GOLD STANDARD METHOD

		GOLD STANDARD METHOD								
			S	I	R					
		0.06	0.12	0.25	0.5	1	2	4	8	16
NEW METHOD	S	0.06								
	0.12			4	1					
	0.25			8	11	1				
	0.5			3	21	5				
	1			2	4	11	3		1	
	I	2			1	1	4		1	
	4						2	7	5	
	R	8					1	2	1	
	16									

9 Minor Errors

Comparison Study: Categorical Agreement

GOLD STANDARD METHOD

		GOLD STANDARD METHOD								
		S			I			R		
		0.06	0.12	0.25	0.5	1	2	4	8	16
NEW METHOD	S	0.06								
	0.12			4	1					
	0.25			8	11	1				
	0.5			3	21	5				
	1			2	4	11	3		1	
	I	2			1	1	4		1	
	4						2	7	5	
	R	8					1	2	1	
	16									
		S			I			R		

No Major Errors

Comparison Study: Categorical Agreement

GOLD STANDARD METHOD

		GOLD STANDARD METHOD									
			S	I	R						
		0.06	0.12	0.25	0.5	1	2	4	8	16	
NEW METHOD	S	0.06									
	0.12			4	1						
	0.25			8	11	1					
	0.5			3	21	5					
	1			2	4	11	3		1		
	I	2				1	1	4		1	
	4							2	7	5	
	R	8						1	2	1	
	16										

1 Very Major Error

Comparison Study: Categorical Agreement

GOLD STANDARD METHOD

		GOLD STANDARD METHOD								
			S	I	R					
		0.06	0.12	0.25	0.5	1	2	4	8	16
NEW METHOD	S	0.06								
	0.12			4	1					
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	0.5			3	21	5				
	1			2	4	11	3		1	
	I	2			1	1	4		1	
	4						2	7	5	
	R	8					1	2	1	
	16									

Total Errors
10/100

Comparison Study: Categorical Agreement

GOLD STANDARD METHOD

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		S		I		R				
		0.06	0.12	0.25	0.5	1	2	4	8	16
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	0.25		8	11	1					
	0.5		3	21	5					
	I	1		2	4	11	3			1
	2			1	1	4				1
	R	4					2	7	5	
	8						1	2	1	
16										

Total Errors = 10

MIC vs. MIC: EXAMPLE (100 isolates tested)

Results	New Method (MIC)	Total
Agreement	90	90%
Minor	9	10%
Major	0	
Very Major	1	

Categorical agreement is 90% (pass)

(Acceptability is $\geq 90\%$)

Comparison Study

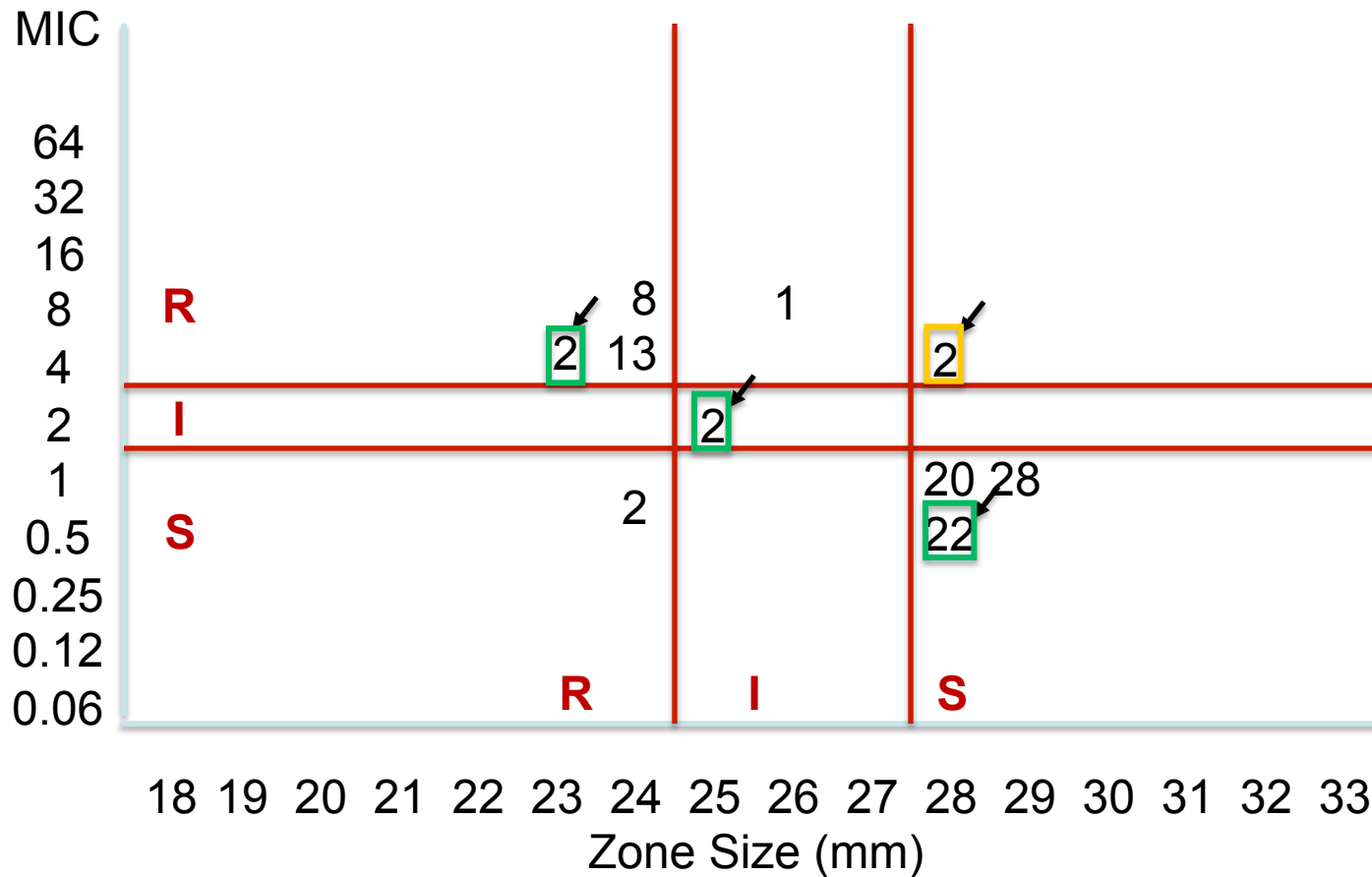
MIC vs. Disk Diffusion: Example (100 isolates tested)

Not possible to evaluate essential agreement ($\mu\text{g/mL}$ vs. zone size)

Only Category agreement is evaluated

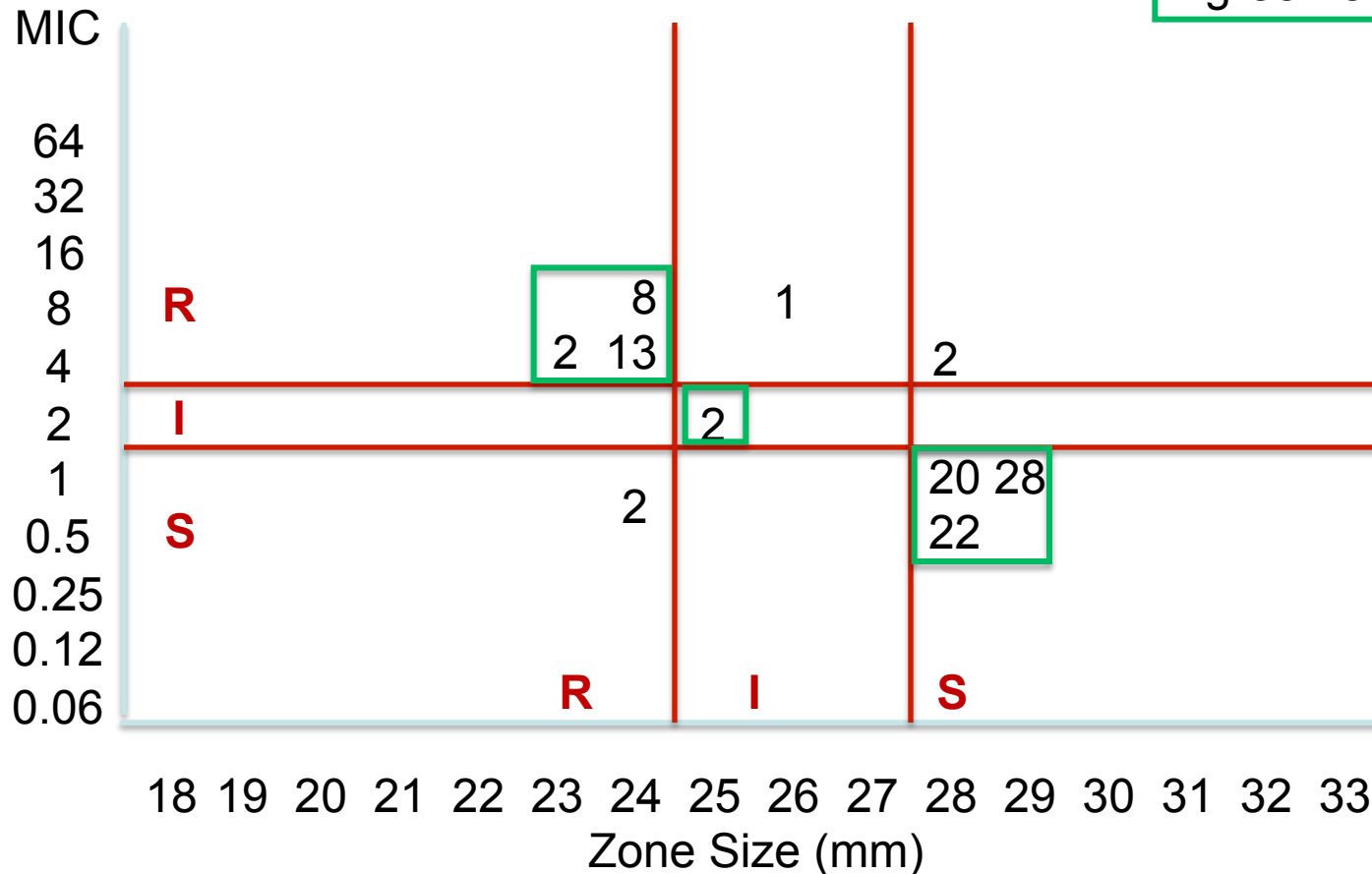
- % of isolates producing the same category result (S I R) as compared to the standard/reference method

Comparison Study: MIC to DD

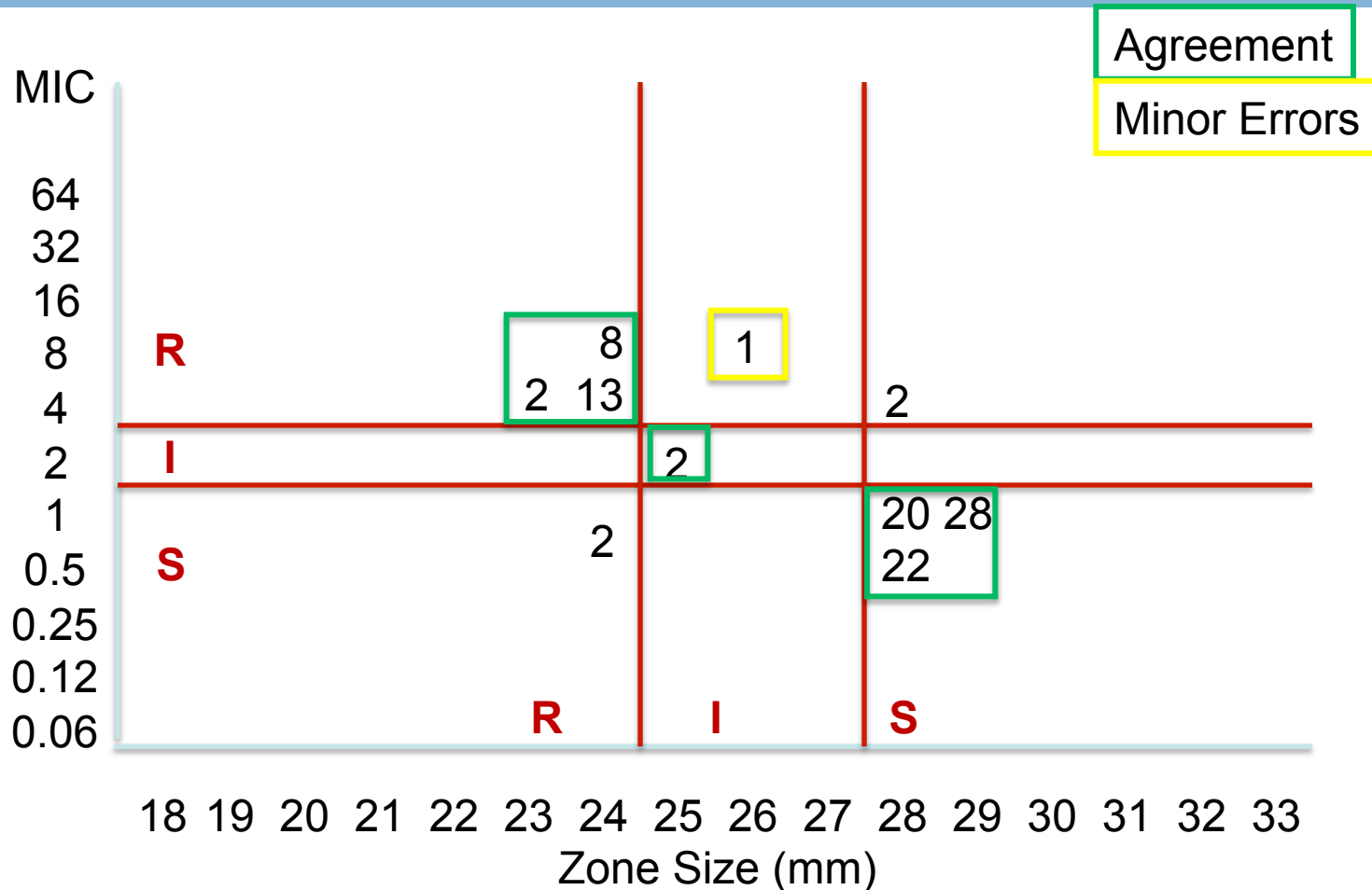


Comparison Study: MIC to DD

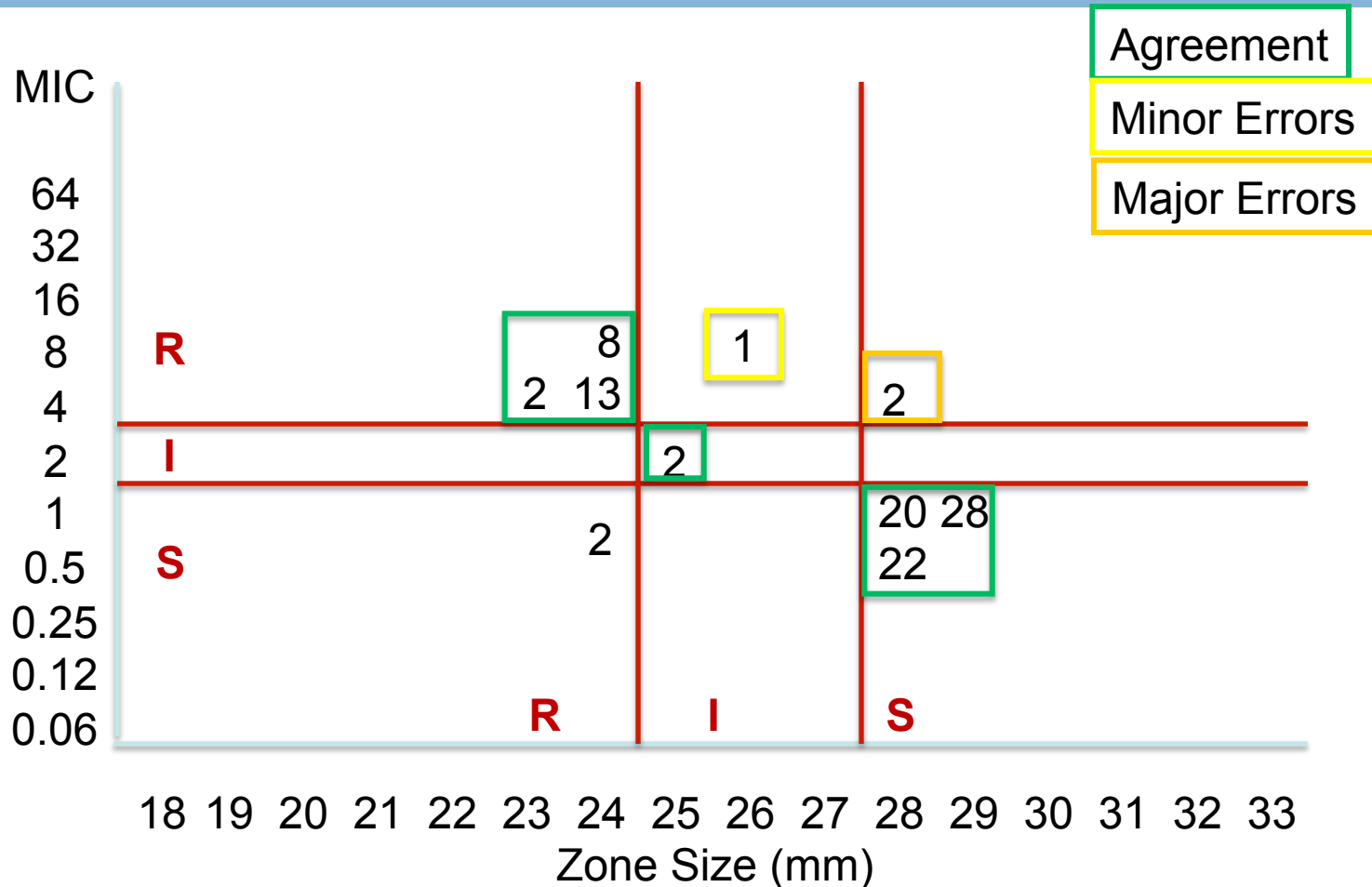
Agreement



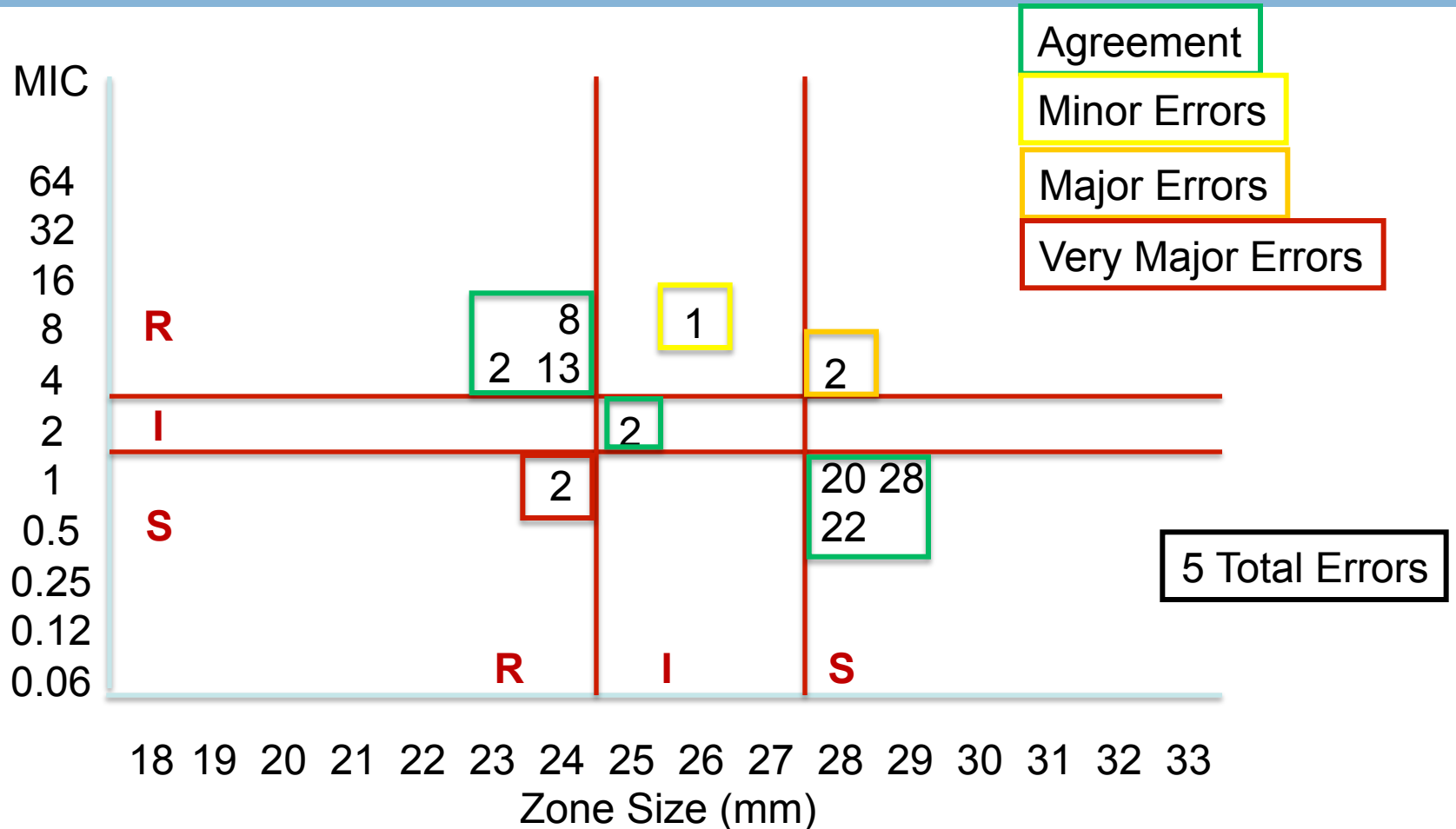
Comparison Study: MIC to DD



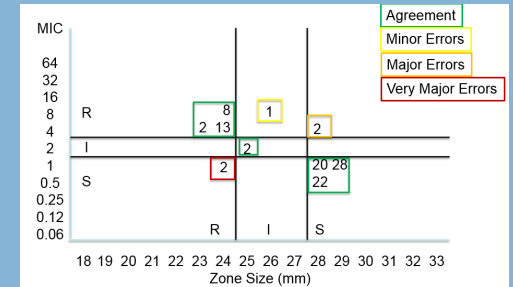
Comparison Study: MIC to DD



Comparison Study: MIC to DD



Comparison Study: MIC to DD



Results	New Method (MIC)	Total
Agreement	95	95%
Minor	1	5%
Major	2	
Very Major	2	

Categorical Agreement = 95% (pass)

(Acceptability is $\geq 90\%$)

Strategies for the implementation of CLIA requirements

The following methods are suggestions.

The laboratory director has the responsibility to identify the appropriate testing parameters and establish acceptability results for verification testing.

Perform verification testing:

- **1) Implementing a new AST method**
- **2) Adding a new drug to a laboratory's current AST method**
- **3) Off-label use of a commercial AST device**

What is required under CLIA

Requirement for verification studies of a unmodified FDA cleared test system:

- **CLIA standard 493.1253(b)(1),**
Verification of performance specifications
 - The requirement is to demonstrate that the test can obtain performance specifications comparable to those established by the manufacturer.
 - Accuracy
 - Precision (reproducibility)
 - Essential agreement
 - Categorical agreement
 - Clinical Isolates and QC organisms

1) Implementing a new AST method

Clinical isolates

- **Test clinical isolates in parallel by the new method and by the method that is being replaced**
 - Use same inoculum for both methods
 - If not possible, perform from same subculture plate at ~ the same time
 - # of isolates is not specified by CLIA
 - 100 - 200 randomly selected fresh clinical isolates representing various species
 - Supplemented with 5-10 resistant isolates (e.g., MRSA, VRE, ESBL or CRE) that commonly occur within the laboratory's patient population
 - Can also include resistant organisms from PT or reference strains with known resistance mechanisms
 - Testing a diverse collection of isolates would satisfy the CLIA requirement to evaluate the 'reportable range' of the test
- **Calculate accuracy with essential agreement and categorical agreement**

1) Implementing a new AST method

QC organisms

- **Specific recommendations for QC testing are outlined by CLSI**

- 20 to 30 day plan
- 3x5 replicate plan
 - Testing is performed in triplicate every day for 5 days.
 - The daily triplicate testing must be done using 3 different inoculum preparations
- Criteria for acceptability are described by CLSI
 - Generally ≤ 1 out of range result per quality control strain is needed for an acceptable result which allows for conversion from daily QC testing to weekly QC testing
- Unacceptable results require troubleshooting (described in the CLSI M100 document)

- **Precision or Reproducibility**

- Evaluated as part of the FDA clearance process
- Clinical laboratory = Precision/reproducibility can be assessed by testing the required QC organisms as described above using different personnel for the testing.

2) Adding a new drug to a current AST method

- **Reasons:**
 - Formulary change
 - an existing drug is needed to be added do to P&T requested changes
 - A brand new drug recently approved for sale
 - the methods by which the drug can be tested may be very limited
 - may not be on all manufacturer's antibiotic panels yet
 - may be impractical or impossible to compare results with another method
 - resistance strains may be rare or nonexistent

2) Adding a new drug to a current AST method

- **The new drug should have already been subjected to a verification study when it was implemented by the manufacturer**
- **Repeating an involved verification study should not be necessary in your laboratory**
 - Perform QC according to CLSI using the 20-30 day or the 3x5 replicate plan
 - May also want to use clinical isolates or reference strains with known resistance patterns if available

3) Off-label use of a commercial AST device

2010 - the CLSI revised breakpoints (now differed from FDA) for the *Enterobacteriaceae* with some cephalosporins and carbapenems

- the revised breakpoints more accurately predict therapeutic decisions
- eliminated the need to perform special phenotypic tests for ESBLs and CPE in the *Enterobacteriaceae* prior to reporting results

Implementation of the new CLSI breakpoints on FDA-approved devices

- Due to a new FDA policy stating that with FDA-approved AST system you must use FDA breakpoints
- The Joint Commission and the CAP allow the use of either FDA or CLSI breakpoints
- Per CLIA: if a clinical laboratory chooses to implement the revised CLSI breakpoints on their FDA-approved AST system they are implementing a ‘modification’ or ‘off-label use’ of their device - -
 - Therefore, the laboratory must verify the performance of the commercial AST system for use of the non-FDA breakpoints

3) Off-label use of a commercial AST device:

Implementing revised CLSI breakpoints on an AST system

Step 1

• Determine if the susceptibility panel contains the appropriate concentrations of antibiotic

- This has been an issue for several commercial manufactures as their systems do not have the full range of antimicrobial concentrations necessary to utilize the new, lowered CLSI breakpoints.
 - E.g.; the revised breakpoints for cefazolin with the *Enterobacteriaceae*
 - susceptible $\leq 2 \mu\text{g/ml}$ (8) {2009}
 - intermediate = $4 \mu\text{g/ml}$ (16)
 - resistant $\geq 8 \mu\text{g/ml}$ (32)
- Thus, the panel must contain at least a minimum of $2 \mu\text{g/ml}$ of cefazolin in order for this panel to be usable with the revised CLSI breakpoints.
- KP – NW: Vitek antimicrobial panels
 - susceptible $\leq 4 \mu\text{g/ml}$
 - resistant $\geq 8 \mu\text{g/ml}$

3) Off-label use of a commercial AST device:

Implementing revised CLSI breakpoints on an AST system

- If the susceptibility panels contain the correct drug concentrations = ALL concentrations have already been approved by the FDA. Essential agreement has already been done and found to be acceptable by the manufacturer.

Step 2

- In order to utilize the revised CLSI breakpoints the laboratory must determine if the AST panel will obtain the correct category agreement.
 - category agreement is assessed by comparing category results from the commercial AST system to a standard reference method:
 - disk diffusion

3) Off-label use of a commercial AST device: Implementing revised CLSI breakpoints on an AST system

Categorical Agreement

- **Test Enterobacteriaceae isolates against the cephalosporin(s) or carbapenem(s)**
 - Use isolates with MICs that span the new breakpoints
 - include isolates that are expected to be susceptible and resistant
 - Test a minimum of 20-30 clinical isolates from recent cultures in the laboratory
 - Should include isolates with and w/out ESBLs & CPE
 - If no ESBLs or CRE organisms are available, obtain from ATCC, proficiency testing challenges, or CAP/CDC →

3) Off-label use of a commercial AST device:

Implementing revised CLSI breakpoints on an AST system

CAP/CDC panel

- For implementing the new CLSI breakpoints a new organism panel was developed by CAP and CDC
- Panel of organisms = contains 31 well-characterized Enterobacteriaceae with reference (frozen broth microbroth dilution) MIC results
 - A laboratory can purchase and test these on their AST system, comparing their category AST results to the published results.
 - Comes with instructions and a worksheet to facilitate data analysis.
- After verification, if the laboratory has achieved categorical agreement (# isolates with the same interpretive result as the reference method / total # of isolates tested) of $\geq 90\%$ = it has verified the revised breakpoints

3) Off-label use of a commercial AST device:

Implementing revised CLSI breakpoints on an AST system

Procedural notes:

- Including organisms with resistance mechanisms will allow for a more robust challenge of the antimicrobial susceptibility testing method.
- Clinical isolates should be tested by both AST methods on the same day, using the same inoculum from the same subculture plate utilizing routine personnel.
- Compare the categorical (S-I-R) results obtained from your in-house MIC panels using the lowered CLSI breakpoints to those categorical results obtained from disk diffusion testing, frozen microbroth dilution MIC panels, or the CAP/CDC organisms = using the revised CLSI disk diffusion and MIC interpretive criteria.

3) Off-label use of a commercial AST device: Implementing revised CLSI breakpoints on an AST system

Categorical agreement

• # of isolates with the same interpretive result as the reference method _____ # of isolates tested

- Example: meropenem
 - 20 patient isolates + 31 CAP/CDC panel organisms (51)
 - Test 20 patient isolates by both DD and on the MIC panel (from the same inoculum)
 - Test 31 known organisms on the MIC panel
 - 48 had the same categorical interpretive result (using new guidelines)
 - 40 S/S; 4 I/I; 4 R/R
 - 2 S/I
 - 1 I/R
 - 48/51 = 94% categorical agreement ($\geq 90\%$ = acceptable)

• **The system has been ‘verified’ and the CLSI revised breakpoints for meropenem can now be used.**

3) Off-label use of a commercial AST device:

Implementing revised CLSI breakpoints on an AST system

- It should be noted that although the manufacturers of susceptibility testing products cannot provide software or alter their devices to provide non-FDA breakpoints, they can provide general instructions for how to customize the device's expert system to apply the revised breakpoints.
- As an alternative to this, the laboratory may choose to implement the revised, verified breakpoints through their laboratory information system.

Trouble-shooting and Documentation

If acceptability is not achieved (< 90% essential or categorical agreement):

Investigate the reason for this failure ...

- **Review technical errors/issues:**
 - **Random errors** (do not occur upon repeat testing)
 - **System errors** (will repeat if the problem is not corrected)
 - **Discordance b/w methods** (unlikely to resolve upon repeat testing)
- **Use a consistent approach to try to resolve errors**
 - For example, repeat acceptable 2 isolates for every 1 isolate with discrepant results.

Trouble-shooting and Documentation

Once corrective action has been put into place

- **Verification testing must be performed again**
 - with acceptable results ($\geq 90\%$ essential/categorical agreement) prior to implementing the new AST system or revised breakpoints.
- **If the re-verification study results meet the acceptability requirements ($\geq 90\%$ essential/categorical agreement)**
 - both original and repeat results must be documented including the likely source of error(s) seen in the first verification study.
- **If results are unacceptable on the second verification study**
 - agreement between methods cannot be achieved
 - consultation with the AST manufacturer and/or the vendor providing the challenge organisms may be necessary.

Trouble-shooting and Documentation

A verification study needs to be documented

- **Documents should include**
 - your study protocol
 - results of the study
 - data analysis
 - e.g., accuracy, reproducibility, essential & category agreement calculations
 - conclusion statement
- **Made available for review by regulatory officials**

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Summary

Verification studies take a lot of work !

- **Necessary for ensuring that a test is performing correctly in the laboratory.**
- **Fortunately, AST system performance characteristics are extensively verified in a pre-market evaluation by the manufacturer.**
 - Accuracy, precision, essential agreement & categorical agreement
- **Thus, it is incumbent upon all parties (government, industry, and standards setting organizations) to work together to minimize the need for in-house verification studies of AST systems.**

But in the mean time....

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