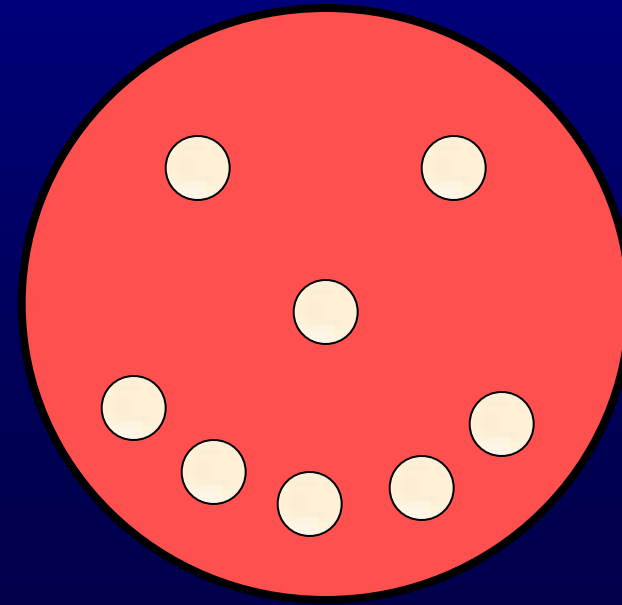
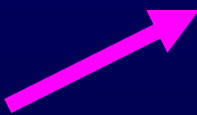


# Ongoing Evaluation of AST Systems to Ensure Quality

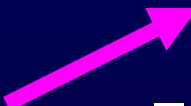
janet hindler



# CLIA 493.1253 Establishment and Verification of Performance Specifications

- ◆ Each lab that introduces an unmodified, FDA-cleared or approved test system must do the following before reporting patient test results:
    - Demonstrate that it can obtain **performance specifications** comparable to those established by the manufacturer for the following performance characteristics:
      - Accuracy
      - Precision (reproducibility)
      - Reportable range of test results for the test system. Verify that the manufacturer's reference intervals (normal values) are appropriate for the laboratory's patient population.
- 

# CLIA 493.1253 Establishment and Verification of Performance Specifications

- ◆ Each lab that modifies an FDA-cleared or approved test system, or introduces a test system not subject to FDA clearance or approval (including methods developed in-house), or uses a test system in which performance specifications are not provided by the manufacturer must, before reporting patient test results:
    - Establish for each test system the performance specifications for the following performance characteristics, as applicable:
      - Accuracy
      - Precision
      - Analytical sensitivity
      - Analytical specificity to include interfering substances
    - Reportable range of test results for the test system. Reference intervals (normal values).
      - Any other performance characteristic required for test performance.
- 

# Usage of Terms

	<b>Initial in-house assessment of NEW test</b>	<b>Ongoing evaluation of IN-USE test</b>
<b>CLIA</b>	<b>Verification</b>	<b>Validation</b>
<b>CAP</b>	<b>Verification</b>	<b>Ongoing evaluation</b>
<b>JCAHO</b>	<b>Validation</b>	<b>Verification</b>

**Verification  
(initial assessment)**

**Accuracy**

**Reproducibility  
(Precision)**

**Compare NEW w/ REF  
or CURRENT method**

**Run to run -  
ATCC strains\***

**Within run -  
5 bugs x 3 x 3d\***

**Manufacturer's  
performance data**

**In-house  
studies**

**Literature**

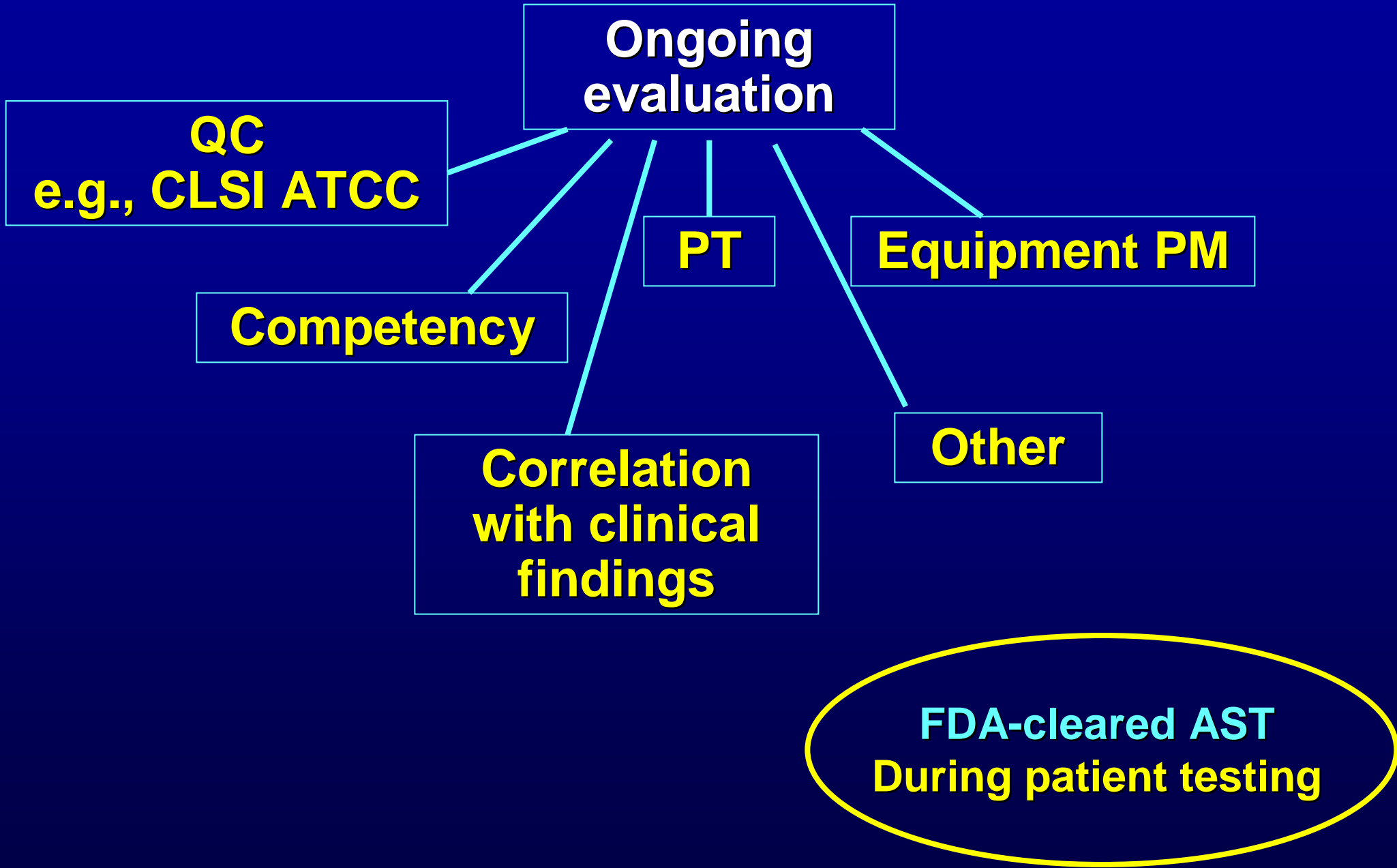
**Test minimum of  
100 isolates???**

**Anecdotal  
information**

**FDA-cleared AST  
...Prior to patient testing**

# Ongoing Evaluation

- ◆ Documentation that a **test which has been verified** is repeatedly giving the expected results
- ◆ Most commonly involves following **CLSI QC procedures**
  - Specified by CLIA, CAP, JCAHO
- ◆ Other components.....



# *Where do we find information on legal requirements for “verification” and “ongoing evaluation”?*

- ◆ **CLIA** – sections 493.1253
- ◆ **CAP** - MIC.21040, GEN.42020 – GEN.42160
- ◆ **JCAHO** – Quality control sections QC.1.20 – QC.1.150
- ◆ **Forthcoming**
  - Update of ASM’s Cumitech 31 (verification/validation)
  - CLSI M52-P

# CLSI M52-P

## Validation/Verification of Microbial Identification and Antimicrobial Susceptibility Testing Systems; Proposed Guideline

- ◆ For commercial systems
- ◆ **Verification:**
  - Rely much on manufacturer's in-house quality measures
  - Limited testing by user's lab
- ◆ **AST verification issues:**
  - Number/types of isolates (30??)
  - 20-30 days of daily QC for precision?

**Forthcoming 2010**

# What QC strain(s) should we use?

Table 2A. Zone Diameter and MIC Interpretive Standards for *Enterobacteriaceae*

<p><b>Testing Conditions</b></p> <p><b>Medium:</b> Disk diffusion: MHA Broth dilution: CAMHB Agar dilution: MHA</p> <p><b>Inoculum:</b> Growth method or direct colony suspension, equivalent to a 0.5 McFarland standard</p> <p><b>Incubation:</b> 35 ± 2 °C; ambient air; Disk diffusion: 16 to 18 hours Dilution methods: 16 to 20 hours</p>	<p><b>Minimal QC Recommendations</b> (See Table 3 for acceptable QC ranges.)</p> <p><i>Escherichia coli</i> ATCC® 25922 <i>Escherichia coli</i> ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)</p> <p>Refer to Appendixes A and G for additional testing and reporting suggestions.</p>
---	--

### General Comments

- (1) For disk diffusion, measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the petri plate a few inches above a black, nonreflecting background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., ignore the thin veil of swarming growth in an otherwise obvious zone of growth inhibition. With trimethoprim

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Breakpoints, nearest whole mm			MIC Interpretive Standard (µg/mL)		Comments
			S	I	R	S	R	
<b>PENICILLINS</b>								
A	Ampicillin	10 µg	≥ 17	14-16	≤ 13	≤ 8		
B	Piperacillin	100 µg	≥ 21	18-20	≤ 17	≤ 16		
C	Mecillinam	10 µg	≥ 15	12-14	≤ 11	< 8		

**CLSI Table 2A  
Interpretive Criteria  
Enterobacteriaceae**

## Appendix F. Quality Control Strains for Antimicrobial Susceptibility Tests

### Appendix F. (Continued)

### QC Strains

Quality Control Strain	Characteristics	Disk Diffusion Tests	MIC Tests	Screening Tests	Other
<i>Staphylococcus aureus</i> ATCC <sup>®</sup> 29213	<ul style="list-style-type: none"> <li>Weak <math>\beta</math>-lactamase producing strain</li> <li><i>mecA</i> negative</li> </ul>		<ul style="list-style-type: none"> <li>Nonfastidious gram-positives</li> <li>Potential agents of bioterrorism</li> </ul>	<ul style="list-style-type: none"> <li>Oxacillin agar</li> </ul>	<ul style="list-style-type: none"> <li>Assess suitability of cation content in each batch/lot of Mueller-Hinton for daptomycin disk diffusion.</li> </ul>
<i>Staphylococcus aureus</i> ATCC <sup>®</sup> 43300	<ul style="list-style-type: none"> <li>Oxacillin-resistant, <i>mecA</i> positive</li> </ul>	<ul style="list-style-type: none"> <li>Cefoxitin disk testing</li> </ul>	<ul style="list-style-type: none"> <li>Cefoxitin MIC testing</li> </ul>	<ul style="list-style-type: none"> <li>Oxacillin agar</li> </ul>	
<i>Staphylococcus aureus</i> ATCC <sup>®</sup> BAA-1708	<ul style="list-style-type: none"> <li>High-level mupirocin resistance mediated by the <i>mupA</i> gene</li> </ul>	<ul style="list-style-type: none"> <li>Screening test for high-level mupirocin resistance</li> </ul>	<ul style="list-style-type: none"> <li>Screening test for high-level mupirocin resistance</li> </ul>		
<i>Streptococcus pneumoniae</i> ATCC <sup>®</sup> 49619	<ul style="list-style-type: none"> <li>Penicillin intermediate by altered penicillin binding protein</li> </ul>	<ul style="list-style-type: none"> <li><i>Streptococcus pneumoniae</i></li> <li><i>Streptococcus</i> spp.</li> <li><i>Neisseria meningitidis</i></li> </ul>	<ul style="list-style-type: none"> <li><i>Streptococcus pneumoniae</i></li> <li><i>Streptococcus</i> spp.</li> <li><i>Neisseria meningitidis</i></li> <li>Potential agents of bioterrorism</li> </ul>		
<b>Supplemental QC<sup>§</sup></b>					
<b>Supplemental QC Strains</b>					
<i>Enterococcus faecalis</i> ATCC <sup>®</sup> 33186					<ul style="list-style-type: none"> <li>Alternative to <i>E. faecalis</i> ATCC<sup>®</sup> 29212 to assess suitability of medium for sulfonamide or trimethoprim MIC tests.<sup>§</sup></li> </ul>
<i>Haemophilus influenzae</i> ATCC <sup>®</sup> 10211					<ul style="list-style-type: none"> <li>Assess each batch/lot for growth capabilities of HTM.</li> </ul>
<i>Klebsiella pneumoniae</i> ATCC <sup>®</sup> BAA-1705	<ul style="list-style-type: none"> <li>KPC-producing strain<sup>§</sup></li> <li>Modified Hodge test positive</li> </ul>	<ul style="list-style-type: none"> <li>Phenotypic confirmatory test for carbapenemase production (modified Hodge test)</li> </ul>			
<i>Klebsiella pneumoniae</i> ATCC <sup>®</sup> BAA-1706	<ul style="list-style-type: none"> <li>Resistant to carbapenems by mechanisms other than carbapenemase</li> <li>Modified Hodge test negative</li> </ul>	<ul style="list-style-type: none"> <li>Phenotypic confirmatory test for carbapenemase production (modified Hodge test)</li> </ul>			

CLSI M100-S19.

# “QC Strain” vs. “Supplemental QC Strain”

- ◆ “QC Strain”
  - Test regularly (daily or weekly)
- ◆ “Supplemental QC Strain”
  - May have “S” or “R” characteristic specific for one or more “special AST”
    - Example: ATCC BAA-977 has inducible clindamycin resistance
  - Use to assess new test, training, competency
  - NOT necessary to test regularly (daily or weekly)

# QC of Antimicrobial Susceptibility Tests

- ◆ Test **QC strains** like patient isolates
- ◆ QC testing frequency:
  - Daily **OR**
  - Concurrent with testing patient isolates **OR**
  - Weekly (after 20-30 days of satisfactory daily QC)
- ◆ If **QC out-of-control**, do not release patient results for the out-of-control drug prior to investigation of the problem

# ***When is 20-30 day QC required before going to weekly QC?***

- ◆ **New test method used (also validation needed)**
- ◆ **New drug added**
- ◆ **When weekly QC demonstrates out-of-control result**
  - **Not identifiable AND**
  - **Not resolved with “immediate corrective action”**
    - **5 days of daily QC testing and all results in control**
- ◆ **Other**

# ***If QC is out-of-control because of an “identifiable error”....***

## **Then**

- ◆ Repeat QC same day;
  - If in control, go back to weekly QC
- ◆ No need to retest patient isolates

## **Identifiable errors are:**

- contamination
- wrong QC strain used
- wrong disk / panel tested
- wrong test conditions used
- other

**CLSI M02-A10.  
CLSI M07-A8.**

***If QC is out-of-control and reason for error cannot be identified (probable system error).....***

**Then**

- ◆ Perform “Immediate Corrective Action” (CA)
- ◆ Continue daily QC until problem is resolved
- ◆ Once problem is resolved, **do 5 days daily QC** before returning to weekly QC

**Out-of-control QC results suggesting a “system problem”:**

- Same drug off for more than 1 QC organism
- Same drug off on more than 1 day
- Several drugs off

**CLSI M02-A10.  
CLSI M07-A8.**

## ***What should we do with patient results if we identify a “system problem”?***

- ◆ **If one drug out-of-control, do not report the drug**
- ◆ **If >1 drug out-of-control, do not report patient results until problem is resolved**
  - use different lots of materials
  - use different test method
  - patient results are often “atypical” for the problem drug(s)

# *When a weekly QC result is out-of-control, what should we consider when determining CA strategy?*

- ◆ **Extent and direction** of QC error
  - 1 dilution (MIC)?
  - Would error contribute to false “S” or false “R”?
- ◆ Are patient results close to **breakpoint**?
- ◆ Results for **other QC strains**?
- ◆ Results for **other drugs** with QC strains?
- ◆ **Previous QC** results?
- ◆ **Patient results** since last in-control QC results?
- ◆ Is QC strain/agent **indicator** for procedural or storage issue (e.g., temperature stability, inoculum)

# Example 1: Disk Diffusion QC Weekly Results

QC Strain	Drug	Acceptable Range	Result	Comment
E. coli ATCC 25922	gentamicin	19-26	22	
	tobramycin	18-26	22	
P. aeruginosa ATCC 27853	gentamicin	16-21	14	Reason for error identifiable
	tobramycin	19-25	23	

**Identifiable error = disk not flat on agar surface**

**Corrective action:**

**Repeat QC test; OK to report patient results**

# Example 2: Disk Diffusion QC Weekly Results

QC Strain	Drug	Acceptable Range	Result	Comment
E. coli ATCC 25922	gentamicin	19-26	22	
	tobramycin	18-26	22	
PSA ATCC 27853	gentamicin	16-21	14	Reason for error <u>not</u> identifiable
	tobramycin	19-25	23	

## Corrective action:

Do 5 days daily QC; if all in control go back to weekly QC

If 5 daily QC results not all in, do 20-30 days before going back to weekly QC

# Example 3: Disk Diffusion QC Weekly Results

QC Strain	Drug	Acceptable Range	Result	Comment
E. coli ATCC 25922	gentamicin	19-26	22	
	tobramycin	18-26	22	
PSA ATCC 27853	gentamicin	16-21	14	Reason for error <u>not</u> identifiable but used new lot of media (lot A)
	tobramycin	19-25	15	Reason for error <u>not</u> identifiable but used new lot of media (lot A)

## Corrective action:

Suppress aminoglycoside results for patient PSA isolates tested on lot A; Only those that would be interpreted as "R"? Use new lot of media (lot B); Once problem identified and corrected, do 5 days daily QC; if all in control go back to weekly QC. **Note:** media should be QC'd before patient testing.

# Table 3C. Disk Diffusion QC Troubleshooting Guide

**Table 3C. Disk Diffusion Quality Control Troubleshooting Guide**

This table provides guidance for troubleshooting and corrective action for out-of-range QC, primarily using antimicrobial susceptibility tests with MHA. Refer to M02-A10, Section 15, Quality Control and Quality Assurance Procedures for additional information. Out-of-range QC tests should first be repeated. If the issue is unresolved, this troubleshooting guide provides additional suggestions for troubleshooting out-of-range QC results. In addition, if unresolved, manufacturers should be notified of potential product problems.

### General Comments

- (1) Quality control organism maintenance: avoid repeated subcultures. Retrieve new QC strain from stock. If using lyophilized strains, follow the maintenance recommendations of the manufacturer. Store *E. coli* ATCC<sup>®</sup> 35218 and *K. pneumoniae* ATCC<sup>®</sup> 700603 stock cultures at -60 °C or below and prepare working stock cultures weekly.

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Action
Aminoglycosides	Any	Zone too small	pH of media too low	Acceptable pH range = 7.2-7.4 Avoid CO <sub>2</sub> incubation, which lowers pH.
Aminoglycosides	Any	Zone too large	pH of media too high	Acceptable pH range = 7.2-7.4
Aminoglycosides	<i>P. aeruginosa</i> ATCC <sup>®</sup> 27853	Zone too small	Ca <sup>++</sup> and/or Mg <sup>++</sup> content too high	Use alternative lot of media.
Aminoglycosides	<i>P. aeruginosa</i> ATCC <sup>®</sup> 27853	Zone too large	Ca <sup>++</sup> and/or Mg <sup>++</sup> content too low	Use alternative lot of media.
Amoxicillin-clavulanic acid	<i>E. coli</i> ATCC <sup>®</sup> 35218	Zone too small	Clavulanic acid is labile. Disk has lost potency.	Use alternative lot of disks. Check storage conditions and package integrity.
Ampicillin	<i>E. coli</i> ATCC <sup>®</sup> 35218	Zone too large (should be no zone — resistant)	Spontaneous loss of the plasmid encoding the β-lactamase	See comment (1) on QC organism maintenance.

# Reference Guide to QC Testing Frequency (MIC example)

## CLSI M100-S19 Table 4E

**Table 4E. MIC Testing – Reference Guide to Quality Control Testing Frequency**

This table summarizes the suggested frequency of testing CLSI-recommended ATCC<sup>®</sup> QC strains to be performed by the user of antimicrobial susceptibility tests (AST). It applies only to antimicrobial agents for which 20 or 30 consecutive test days of QC testing produced satisfactory results.

Test Modification	Number of days of consecutive QC testing required <sup>b</sup>			Comments
	1	5	20 or 30	
<b>MIC Tests(s)</b>				
Use new shipment or lot number	X			
Expand dilution range	X			Example: Convert from breakpoint to expanded range MIC panels.
Reduce dilution range	X			Example: Convert from expanded dilution range to breakpoint panels.
Use new method (same company)			X	Examples: Convert from visual to instrument reading of panel.  Convert from overnight to rapid MIC test.  In addition, perform in-house validation studies.
Use new manufacturer of MIC test			X	In addition, perform in-house validation studies.
<b>Use new manufacturer of broth or agar</b>		X		
<b>Inoculum Preparation</b>				
Convert inoculum preparation/standardization to use of a device that has its own QC protocol		X		Example: Convert from visual adjustment of turbidity to use of a photometric device for which a QC procedure is provided.
Convert inoculum preparation/standardization to a method that is dependent on user technique			X	Example: Convert from visual adjustment of turbidity to another method that is not based on a photometric device.
<b>Instrument/Software</b>				
Software update that affects AST results		X		Monitor all drugs, not just those implicated in software modification.
Repair of instrument that affects AST results	X			Depending on extent of repair (eg, critical component such as the optics), additional testing may be appropriate (eg, five days).

**NOTE 1:** Addition of any new antimicrobial agent requires 20 or 30 consecutive days of satisfactory testing (see M07-A8, Section 16.7) prior to use of this guide.

**NOTE 2:** Quality control can be performed prior to or concurrent with testing patient isolates. Patient results can be reported for that day if QC results are within the acceptable limits.

**NOTE 3:** Manufacturers of commercial or in-house prepared tests should follow their own internal procedures and applicable regulations.

**NOTE 4:** Acceptable MIC QC limits for FDA-cleared antimicrobial susceptibility tests may differ slightly from acceptable CLSI QC limits. Users of each device should utilize the manufacturer's procedures and QC limits as indicated in the instructions for use.

# Excerpt from: “Reference Guide to QC Testing Frequency”

(for ATCC QC strains after 20-30 consecutive days of satisfactory daily testing)

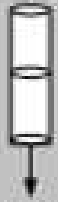
MIC test modification	No. of days of consecutive QC testing required <sup>a</sup>			Comments
	1	5	20 or 30	
Use new shipment or lot number	X			
Use new manufacturer of MIC test			X	In addition, perform in-house validation studies
Software update that affects AST results		X		Monitor all drugs not just those implicated in software modification

<sup>a</sup>Does not eliminate the need for routine weekly or daily QC testing.

CLSI M100-S19 Table 4E

Appendix E. Quality Control Strain Maintenance (also refer to Section 16.4)

1. Rehydrate one stock culture or obtain strain from frozen stock.

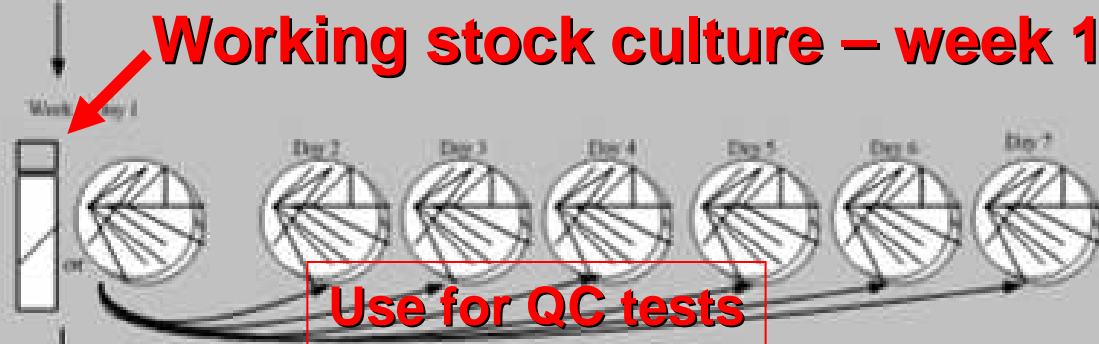


**Stock culture**

2. Subculture to appropriate media and incubate (primary subculture).



3. Subculture, incubate, and store as appropriate for the organism type. Use isolated colonies from Days 1 to 7 as working cultures for testing.



**Working stock culture – week 1**

4. Prepare new subculture every seven days (from slant in Day 1 working culture). Store at appropriate temperature for organism type. Use fresh working cultures each day.



**Working stock culture – week 2**

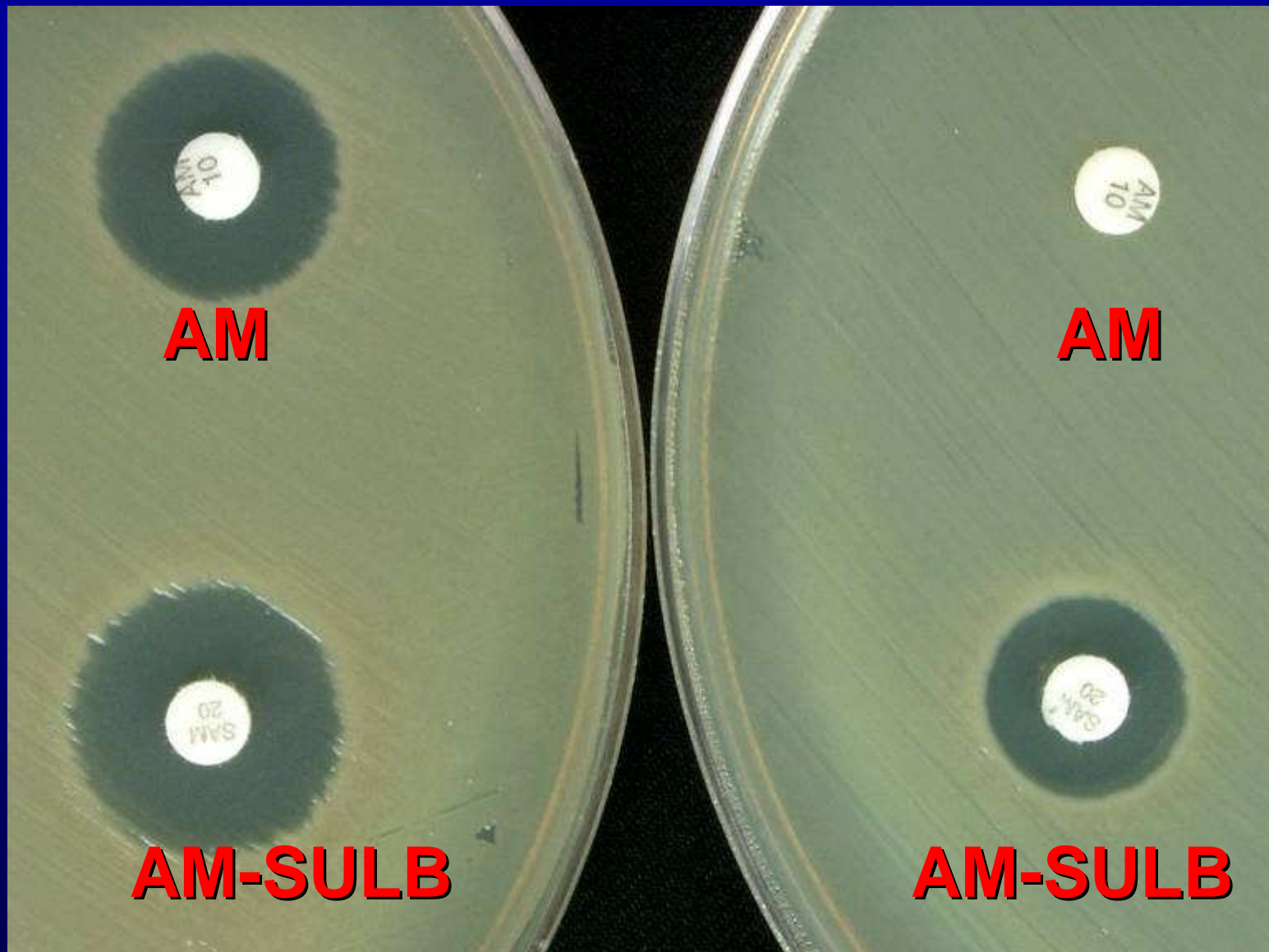
5. Repeat for Week 3 and Week 4. After four weeks, discard subculture and pull strain from frozen stock or rehydrate new stock culture.

# Maintaining Quality Control Strains (1)

CLSI M02-A10.  
CLSI M07-A8.

# Maintaining Quality Control Strains (2)

- ◆ *E. coli* ATCC 35218; *K. pneumoniae* ATCC 700603
  - Beta-lactamase producers
  - Beta-lactamase is plasmid-mediated
  - Store at -60°C or lower to prevent loss of plasmid; if plasmid is lost, strain would appear susceptible to beta-lactams



*E. coli* ATCC 25922  
 $\beta$ -lactamase neg

*E. coli* ATCC 35218  
 $\beta$ -lactamase pos

# Some Additional Notes Re: QC Testing

- ◆ Keep 20-30 day QC records indefinitely
- ◆ Use manufacturer's QC ranges if different from CLSI ranges (see CLSI M100-S19 Table 4E; Note 4)
- ◆ For screen tests:
  - If perform at least once/week, weekly QC OK
  - Exception oxacillin-salt MRSA – QC daily

# ***Why do we review” results of patient’s isolates?***

- ◆ **Testing routine QC strains doesn’t ensure every result on a patient’s isolate is accurate**
- ◆ **Patient results may be erroneous due to:**
  - **mixed culture, misidentification**
  - **individual drug/bug problem**
  - **other technical error**

# ***Enterobacter aerogenes***

ampicillin	R
cefazolin	R
cefoxitin	R
ceftriaxone	S
ciprofloxacin	S
gentamicin	S
pip-tazo	S
trim-sulfa	S

**“Combination therapy (e.g.,  $\beta$ -lactam + aminoglycoside or fluoroquinolone) should be considered for this *Enterobacter aerogenes*”**

## **Review:**

- ◆ **Do ID and AST results correlate?**
- ◆ **Were appropriate *drugs reported?***
- ◆ **Are results from *similar drugs (drug class) OK?***
- ◆ **Were appropriate *comments added?***

# ***What do we mean by “intrinsic” or “acquired” resistance?***

- ◆ **Intrinsic R** – natural or innate R a bacterium possess to some antimicrobial agents
  - Vancomycin resistance in *E. coli*; vancomycin too large to get through cell surface of gram-negative bacteria
- ◆ **Acquired R** – R in a bacterium that was previously susceptible that occurs as a result of:
  - Chance gene mutation
  - Acquisition of R genes from another bacterium



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## The European Committee on Antimicrobial Susceptibility Testing – EUCAST

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**ESCMID** EUROPEAN SOCIETY  
OF CLINICAL MICROBIOLOGY  
AND INFECTIOUS DISEASES

## The European Committee on Antimicrobial Susceptibility Testing - EUCAST

EUCAST is a standing committee jointly organized by ESCMID, ECDC and European national breakpoint committees. The Steering Committee is the decision making body. It is supported by a General Committee with representatives from European countries, FESCI and ISC. The Steering Committee also consults experts within the fields of Infectious Diseases and Microbiology, pharmaceutical companies and susceptibility testing device manufacturers on EUCAST proposals.

EUCAST has subcommittees on antifungal susceptibility testing, expert rules for antimicrobial susceptibility testing, and antimicrobial susceptibility testing of anaerobes.

EUCAST has harmonized most antimicrobial MIC breakpoints in Europe. Breakpoints for new agents are set as part of the licensing process for new agents through EMEA. EUCAST breakpoints will be available in device manufacturers' software for automated susceptibility testing during 2009. A disk diffusion test calibrated to EUCAST MIC breakpoints is being developed for launch around the end of 2009.

EUCAST invites anyone with an interest in antimicrobial agents in general and antimicrobial breakpoints in particular to contact EUCAST, ESCMID or one of the National Breakpoint Committees.

<http://www.eucast.org/>



EUROPEAN CENTRE FOR  
DISEASE PREVENTION  
AND CONTROL

**Table 1: Intrinsic resistance (R) in Enterobacteriaceae**

Enterobacteriaceae are also intrinsically resistant to penicillin G, glycopeptides, fusidic acid, macrolides (with some exceptions<sup>1</sup>), lincosamides, streptogramins, rifampicin, daptomycin and linezolid.

Rule no.	Organisms	Ampicillin	Amoxicillin-clavulanate	Ticarcillin	Piperacillin	Cefazolin	Cefoxitin	Cefamandole	Cefuroxime	Aminoglycosides	Tetracyclines/tigecycline	Polymyxin B/Colistin	Nitrofurantoin
1.1	<i>Citrobacter koseri</i>	R		R	R								
1.2	<i>Citrobacter freundii</i>	R	R			R	R						
1.3	<i>Enterobacter cloacae</i>	R	R			R	R						
1.4	<i>Enterobacter aerogenes</i>	R	R			R	R						
1.5	<i>Escherichia hermannii</i>	R		R	R								
1.6	<i>Hafnia alvei</i>	R	R			R	R						
1.7	<i>Klebsiella spp.</i>	R		R	R								
1.8	<i>Morganella morganii</i>	R	R			R			R		R	R	R
1.9	<i>Proteus mirabilis</i>										R	R	R
1.10	<i>Proteus vulgaris</i>	R				R		R	R		R	R	R
1.11	<i>Proteus penneri</i>	R				R		R	R		R	R	R
1.12	<i>Providencia rettgeri</i>	R	R			R				R <sup>2</sup>		R	R
1.13	<i>Providencia stuartii</i>	R	R			R				R <sup>2</sup>		R	R
1.14	<i>Serratia marcescens</i>	R	R			R		R	R	Note <sup>3</sup>		R	
1.15	<i>Yersinia enterocolitica</i>	R	R	R	R	R	R	R					
1.16	<i>Yersinia pseudotuberculosis</i>											R	

<sup>1</sup> Azithromycin is effective in vivo for the treatment of typhoid fever and erythromycin may be used to treat travellers' diarrhoea.

<sup>2</sup> All *Providencia* spp. produce a chromosomal AAC(2'')-Ia enzyme. *Providencia* spp. should be considered resistant to all aminoglycosides except amikacin and streptomycin. Some isolates express the enzyme poorly and can appear susceptible to netilmicin *in vitro*, but should be reported as resistant as mutation can result in overproduction of this enzyme.

<sup>3</sup> All *Serratia marcescens* produce a chromosomal AAC(6'')-Ic enzyme that may affect moderate the activity of all aminoglycosides except streptomycin and gentamicin.

**Intrinsic resistance**

Table 5: Exceptional phenotypes of Gram-negative bacteria.

Rule no.	Organisms	Exceptional phenotypes
5.1	Any Enterobacteriaceae	Resistant to ertapenem, meropenem, imipenem (except <i>Proteus</i> spp.).
5.2	<i>Pseudomonas aeruginosa</i> and <i>Acinetobacter</i> spp.	Resistant to colistin.
5.3	<i>Haemophilus influenzae</i>	Resistant to any third-generation cephalosporin, carbapenems, fluoroquinolones.
5.4	<i>Moraxella catarrhalis</i>	Resistant to ciprofloxacin, any third-generation cephalosporin.
5.5	<i>Neisseria meningitidis</i>	Resistant to penicillin (MIC >1 mg/L), third generation cephalosporins, ciprofloxacin.
5.6	<i>Neisseria gonorrhoeae</i>	Resistant to third-generation cephalosporins, spectinomycin.

**Exceptional phenotypes  
(similar to CLSI “Verification Table”)**

Table 8: Interpretive rules for  $\beta$ -lactam agents and Gram-positive cocci

Rule no.	Organisms	Agent	Rule	Exceptions	Scientific basis	Evidence grade	References
8.1	<i>Staphylococcus</i> spp.	Isoxazoly-penicillins	If resistant to isoxazoly-penicillins (as determined with oxacillin, cefoxitin, or by detection of <i>mecA</i> -gene or of PBP2a) report as resistant to all $\beta$ -lactams.	Developmental anti-MRSA cephalosporins, e.g. ceftobiprole and ceftaroline.	Production of PBP2a (encoded by <i>mecA</i> ) leads to cross resistance to $\beta$ -lactams except ceftobiprole and ceftaroline.	A	Chambers HF <i>et al.</i> , 1990 Page MG <i>et al.</i> , 2006
8.2	<i>Staphylococcus</i> spp.	Penicillins	If penicillinase is detected, report as resistant to all penicillins, regardless of MIC, except the isoxazoly-penicillins and combinations with $\beta$ -lactamase inhibitors.	Testing of penicillinase production may be discouraged in certain countries due to high prevalence of penicillinase producers (>90%) and technical problems. In this case it may be considered appropriate to report all isolates resistant to benzylpenicillin, ampicillin and amoxicillin.	Production of penicillinase leads to resistance to all penicillins except the isoxazoly-analogues.	C	Nathwani D <i>et al.</i> Drugs. 1993
8.3	$\beta$ -Haemolytic streptococci (Group A, B, C, G)	Benzylpenicillin	If susceptible to penicillin report susceptible to aminopenicillins, cephalosporins and carbapenems. If resistant to penicillin check identification and susceptibility.	Rare isolates of group B streptococci may have diminished susceptibility to penicillins.	Susceptibility to penicillins is currently the rule. No resistance to $\beta$ -lactams reported so far except in Group	C	Karłowsky JA <i>et al.</i> , 2002 Casey JR <i>et al.</i> , Clin Infect Dis 2004
8.4	<i>Streptococcus pneumoniae</i>	$\beta$ -lactams	If resistant by the oxaci screening test, perform				

**Interpretive Rules  
(similar to CLSI “Comments”)**

**Table 1. Suggested Groupings of Antimicrobial Agents With FDA Clinical Indications That Should Be Considered for Routine Testing and Reporting on Nonfastidious Organisms by Clinical Microbiology Laboratories in the United States**

GROUP A PRIMARY TEST AND REPORT	Enterobacteriaceae <sup>a</sup>	<i>Pseudomonas aeruginosa</i>	
	Ampicillin <sup>b</sup>	Ceftazidime	
			Clindamycin <sup>d</sup>
			Oxacillin (cefoxitin) <sup>k,l</sup>
	Cefazolin <sup>a</sup> Cephalothin <sup>a</sup>	Gentamicin Tobramycin	Penicillin <sup>k</sup>
	Gentamicin	Piperacillin	Trimethoprim-

**CLSI Table 1  
Drugs to Test/Report**

**VI. Warning**

Some of the comments in the tables relate to dangerously misleading results that can occur when certain antimicrobial agents are tested and reported as susceptible against specific organisms. These are denoted with the word **“Warning.”**

**“Warning”:** The following antimicrobial agent/organism combinations may appear active *in vitro*, but are not effective clinically and should not be reported as susceptible.

Location	Organism	Antimicrobial agents that must not be reported as susceptible
Table 2A	ESBL- producing <i>K. pneumoniae</i> , <i>K. oxytoca</i> , <i>E. coli</i> , and <i>P. mirabilis</i>	penicillins, cephalosporins, and carbapenems
Table 2A	<i>Salmonella</i> spp., <i>Shigella</i> spp.	1st- and 2nd-generation cephalosporins, cephamycins, and aminoglycosides
Table 2C	oxacillin-resistant <i>Staphylococcus</i> spp.	penicillins, β-lactam/β-lactamase inhibitor combinations, cephems, and carbapenems
Table 2D	<i>Enterococcus</i> spp.	aminoglycosides (except high concentrations), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole
Table 2K	<i>Yersinia pestis</i>	β-lactam antimicrobial agents

**CLSI Introduction  
“Warning Comments”**

## Appendix E. Suggestions for Verification of Antimicrobial Susceptibility Test Results and Confirmation of Organism Identification

This table reflects the drugs listed for testing against the respective organisms in Tables 2A through 2J and gives some examples to consider for verification protocols at a given institution. The list includes phenotypes that: 1) have never been documented; 2) are uncommon in some geographic areas; and/or 3) represent results that could easily occur from technical errors and that may have significant clinical consequences.

NOTE: For critical results, communicate preliminary findings to the laboratory director or supervisor.

Organism or Group	Category I <sup>a</sup> Phenotypes that have not been reported, are uncommon, and/or result from technical errors	Category II <sup>b</sup> Phenotypes that may be uncommon at a given institution and/or result from technical errors
<b>Gram-negative organisms</b>		
<i>Enterobacteriaceae</i> (any)	carbapenem - I or R	amikacin - R fluoroquinolone - R
<i>Citrobacter freundii</i> <i>Enterobacter</i> spp. <i>Serratia marcescens</i>	ampicillin, cefazolin, or cephalothin - S	
<i>Escherichia coli</i>		ESBL confirmed positive
<i>Klebsiella</i> spp.	ampicillin - S	ESBL confirmed positive
<i>Proteus vulgaris</i> <i>Providencia</i> spp.	ampicillin - S	
<i>Salmonella</i> spp.		
<i>Pseudomonas aeruginosa</i>		
<i>Stenotrophomonas maltophilia</i>	carbapenem - S	
<i>Haemophilus</i>	aztreonam - NS	ampicillin - R and $\beta$ -lactamase-

**CLSI M100-S19 Appendix E  
Verification Table  
Includes "Exceptional Phenotypes"**

# Excerpt from Appendix E: Results that should be verified

Organism or Group	Category I Confirm at all labs	Category II Confirm – institution specific
<i>Klebsiella</i> spp.	ampicillin – S	ESBL confirmed positive
.....	.....	.....
<i>Staphylococcus aureus</i>	daptomycin - NS linezolid – NS quin-dalfo – I or R vancomycin – I or R	oxacillin – R

**NS, not susceptible**

**CLSI M100-S19 Appendix E.**

# CLSI M100-S19

## Verification Tables

- ◆ **Category I – ALL SHOULD VERIFY**
  - Not been reported
  - Uncommon
  - Prone to technical errors
- ◆ **Category II – each laboratory to decide if they will verify**
  - Uncommon at **some institutions**
  - Prone to technical errors

# *How do we “confirm” or “verify” results on patient’s isolates?*

- ◆ Check transcription
- ◆ Reexamine plate/tray, purity plate, etc.  
...make sure no contamination
- ◆ Check previous isolates on patient

**IF the above are not revealing**

**THEN .....**

- ◆ Confirm ID **and/or**
- ◆ Repeat AST (sometimes different method)
- ◆ Get assistance from referral lab

## ***More on the “verification process”....***

### **◆ Repeat test (same method)**

- If suspect isolate problem, e.g.,
  - Occult contamination?
  - Inadequate inoculum?

### **◆ Repeat test (alternate method)**

- If suspect problem with test system
  - Had same or similar problem with drug/bug previously
- If strain grows poorly in test system
- Use CLSI reference method (DD or MIC) or commercial test that is FDA cleared for drug/bug

# CAP Checklist

## MIC.21950

- ◆ Does the **procedure manual** address unusual or inconsistent antimicrobial susceptibility testing results?
  - Testing QC strains doesn't guarantee accurate patient results
  - Unusual or inconsistent results should be investigated



# Examples: “Verify” AST Results (1)

Antimicrobial Agent	<i>E. coli</i>	<i>Stenotrophomonas maltophilia</i>	<i>Klebsiella pneumoniae</i>
Ampicillin	S	R	R
Cefazolin	S	R	S
Cefoxitin	S	R	S
Ceftriaxone	S	R	S
Gentamicin	R*	R	S
Imipenem	S	S	R**
Tobramycin	S	R	S
Trimeth-sulfa	S	R**	S

\* Atypical results; probable error

\*\*Uncommon results in many facilities; confirm

# Examples: “Verify” AST Results (2)

Antimicrobial Agent	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Streptococcus pneumoniae</i>
Ampicillin	R	R**	S
Cefazolin	S*	NA	NA
Clindamycin	R	NA	S
Erythromycin	R	NA	S
Oxacillin	R	NA	NA
Penicillin	R	S	S
Vancomycin	S	S	R***

\* Oxacillin-R staphylococci should be reported as “R” to all  $\beta$ -lactams

\*\* Atypical results; possible identification error (*E. faecium* are ampicillin-R)

\*\*\* Vancomycin-R *S. pneumoniae* never reported; probable error

# Examples: “Verify” AST Results (3)

Antimicrobial Agent	<i>Klebsiella pneumoniae</i>	<i>Enterobacter cloacae</i>	<i>Pseudomonas aeruginosa</i>
Ampicillin	S*	R	S*
Cefazolin	S	S*	R
Cefoxitin	S	R	R
Ceftriaxone	S	R	R
Gentamicin	S	S	S
Tobramycin	S	S	S
Trimeth-sulfa	S	S	R

\*Species “intrinsically” R to agent highlighted; edit “S” to “R” ??

# ***Klebsiella pneumoniae***

ampicillin	<del>S</del> R
cefazolin	S
cefoxitin	S
ceftriaxone	S
ciprofloxacin	S
gentamicin	S
pip-tazo	S
trim-sulfa	S

## **Checklist for editing ampicillin “S” to “R”**

- ◆ *Is K. pneumoniae intrinsically “R” to ampicillin?*
- ◆ *Are ID test results OK?*
- ◆ *Did tech performing AST follow the procedure? (inoculum preparation critical)*
- ◆ *Was AST growth satisfactory?*
- ◆ *Was AST QC in control?*

# UCLA Additional “S” to “R” Editing Rules (based on intrinsic R)

Organisms	Edit “S” to “R”
<i>Citrobacter freundii</i> <i>Enterobacter aerogenes</i> <i>Enterobacter cloacae</i> <i>Morganella morganii</i> <i>Proteus vulgaris</i> <i>Providencia stuartii</i> <i>Serratia marcescens</i>	ampicillin ampicillin-sulbactam cefazolin
<i>Klebsiella oxytoca</i> <i>Klebsiella pneumoniae</i>	ampicillin

# CLSI “S” to “R” Editing Rules

Organisms	Criteria	Edit any “S” to ”R”
<i>E. coli</i> <i>Klebsiella spp.</i> <i>Proteus mirabilis</i>	ESBL positive*	Cephalosporins (not cephameycins), penicillins, (NOT $\beta$ -lactam- $\beta$ -lactamase inhibitor combinations), aztreonam
<i>Staphylococcus spp.</i>	Oxacillin “R”*	All $\beta$ -lactams
<i>Staphylococcus spp.</i> Beta streptococci	Inducible clindamycin resistance	Clindamycin

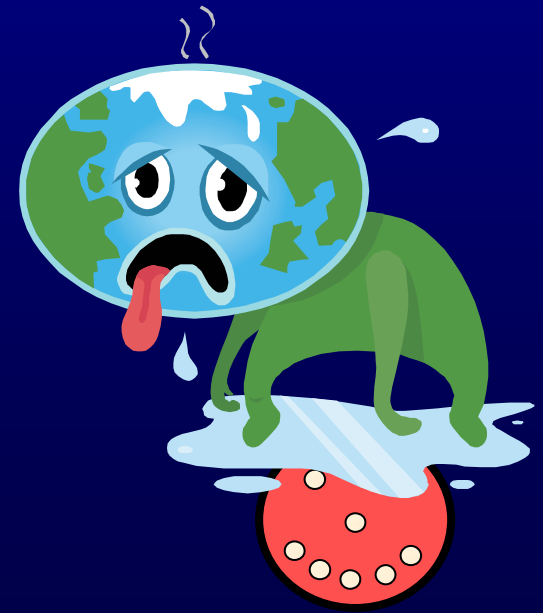
\*listed with “Warning” comment

CLSI M100-S19.

***Editing rules are based on ID and AST results being accurate!!! So, when editing “S” to “R”, we must consider....***

- ◆ **Competency** of personnel doing testing
  - Follow procedural steps precisely
- ◆ Testing of pure cultures – use **purity plates** for broth MIC tests
- ◆ Reliability of **organism ID**
- ◆ Reliability of **AST results** that invoke edit
- ◆ Impact to patient management
- ◆ ***Situations where it would be wise to confirm initial results prior to reporting edited results***

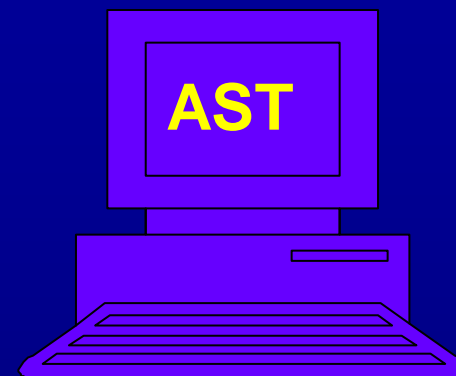
***How can we handle  
all of this????***



# Need.....

***“Artificial Intelligence” to:***

- ◆ Flag atypical / inconsistent results
- ◆ Suggest confirmatory tests
- ◆ Report appropriate drugs
- ◆ Edit “S” or “I” results to “R”
- ◆ Add comments to report

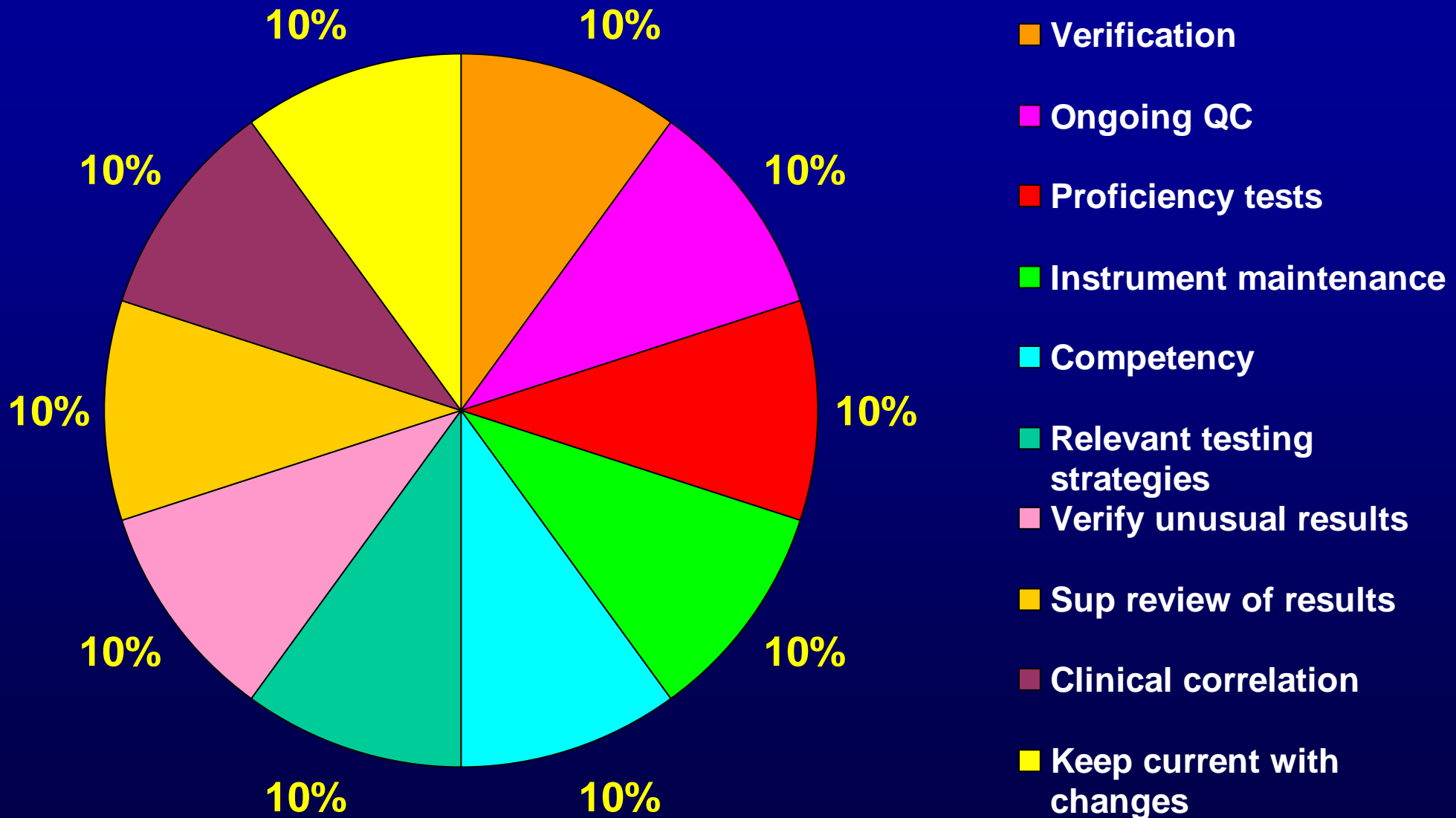


**and**

***“Informed Clinical  
Laboratory Scientists!”***



# Summary - Ensuring Quality of AST



# Summary (1)

- ◆ Both the “Manufacturer” of AST systems and the “User” play a role in obtaining “quality” AST results.
- ◆ The “User” must make sure: materials are stored properly; testing is performed according to protocol; and staff are properly trained.
- ◆ “QC strains” should be tested daily, weekly, or concurrent with testing patient’s isolates.
- ◆ “Supplemental QC strains” may be tested in select circumstances.

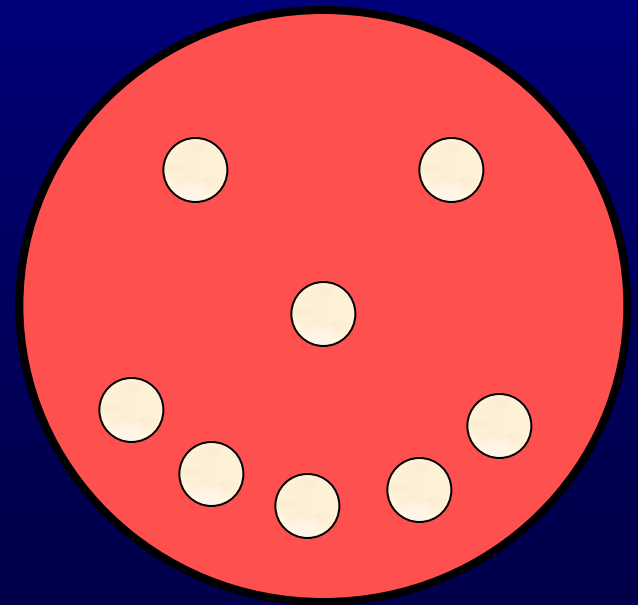
# Summary (2)

- ◆ Once a laboratory has demonstrated satisfactory performance of daily QC, **weekly QC** is acceptable.
- ◆ If QC results are **out-of-control**, patient results must not be reported until the out-of-control results are investigated.
- ◆ QC strains must be **stored properly** to ensure their antimicrobial susceptibility patterns are maintained.
- ◆ **Patient results** may be incorrect even when QC is in control.

# Summary (3)

- ◆ All patient's results must be examined before reporting. Uncommon results should be **confirmed or verified**.
- ◆ **“Artificial intelligence”** or **“Expert”** software can be extremely helpful to highlight potential problem results.

**Thank you!**



**Example 1:**  
**Ensure results are accurate:**

## ***Klebsiella pneumoniae***

<b>Drug</b>	<b>Result</b>
amikacin	R
cefepime	R
ceftriaxone	R
ciprofloxacin	R
gentamicin	R
imipenem	R
piper-tazobactam	R
tobramycin	R
trimeth-sulfa	R

**If not previously seen in your facility, verify results**

**Example 2:**  
**Ensure results are accurate:**

## *Klebsiella pneumoniae*

Drug	Isolate	
	A	B
amikacin	R	R
cefepime	R	R
ceftriaxone	R	R
ciprofloxacin	R	R
gentamicin	S	R
imipenem	R	R
piper-tazobactam	R	R
tobramycin	R	R
trimeth-sulfa	R	R

**A – KPC-producing *K. pneumoniae* endemic strain in ICU**

**B – new patient with KPC-producing *K. pneumoniae* in ICU;  
verify gentamicin (and other?) results**