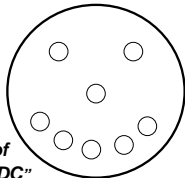


What's New in the 2006 Standards for Antimicrobial Susceptibility Testing (AST)?

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"working as a consultant with the Association of Public Health Laboratories with support from CDC"



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At the conclusion of this talk, you will be able to.....

- ◆ Outline the major changes found in the new CLSI tables (M100-S16) and standards for disk diffusion (M2-A9) and MIC testing (M7-A7).
- ◆ Discuss how to optimally use the new Disk Diffusion and MIC QC Troubleshooting Guides.
- ◆ Describe a strategy for implementing the new practice guidelines in your laboratory, as appropriate.

2



CLSI Standards - 2006

- ◆ M100-S16 Tables (2006)* **NEW!**
.....to be used with text documents explaining how to perform the tests....
- M2-A9 Disk Diffusion (2006)**
- M7-A7 MIC (2006)**

* M100 updated yearly
**M2, M7 updated every 3 years



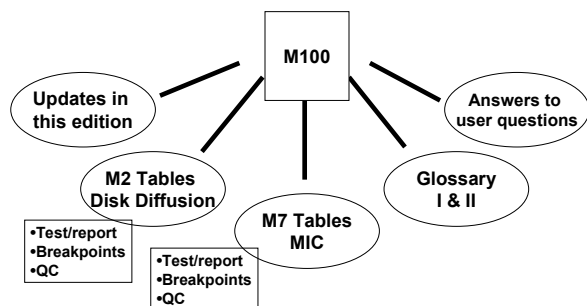
Reference Terminology

....when I refer to....

- ◆ M100 -- this means the new tables M100-S16
- ◆ M2 -- this means the new disk diffusion document M2-A9
- ◆ M7 -- this means the new MIC document M7-A7
- ◆ M45 -- this means the new fastidious organism testing document M45-P
- ◆ CLSI = formerly NCCLS

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CLSI M100 contains.....



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Updated information in M100-S16

Vol. 26 No. 3

M100-S16

Updated Information in This Edition

This document includes all of the tables from the Clinical and Laboratory Standards Institute Disk Diffusion (M2) susceptibility testing and Aerobic Dilution (M7) susceptibility testing documents. There are several important changes to the tables that have resulted from meetings of the Subcommittee on Antimicrobial Susceptibility Testing during 2005. Included below is a summary of the changes in this document, which supersedes the tables published in 2005 and in earlier years.

Summary of Major Changes in This Document

The list includes the "major" changes in this document. Other minor or editorial changes have been made to the general formatting and to some of the table footnotes. Boldface type is used to highlight the changes in each table.

Additions/Changes/Deletions

The following are additions or changes unless otherwise noted as a "deletion"

All Tables Throughout (M2, Tables 2A-3J; M7, Tables 2A-2L)

Clarification of incubation temperature (M2 and M7)

Introduction to Tables:

Speed of growth for long-term incubation (M2, Tables 1-14; M7, Tables 1-14)

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Changes 2006 CLSI M100-S16



CLSI Standard Reference Method and Breakpoints (M100-S16, page 14)

January 2006

M100-S16

It is important for users of M2-A9, M7-A7, and the M100 Informational Supplement to recognize that the standard methods described in CLSI documents are reference methods. These methods may be used for routine antimicrobial susceptibility testing of clinical isolates, for evaluation of commercial devices that will be used in clinical laboratories, or by drug or device manufacturers for testing of new agents or systems. Results generated by reference methods, such as those contained in CLSI documents, may be used by regulatory authorities to evaluate the performance of commercial susceptibility testing devices as part of the approval process. Clearance by a regulatory authority indicates that the commercial susceptibility testing device provides susceptibility results that are substantially equivalent to results generated using reference methods for the organisms and antimicrobial agents described in the device manufacturer's approved package insert.

CLSI breakpoints may differ from those approved by various regulatory authorities for many reasons, including the following: different data bases, differences in interpretation of data, differences in doses utilized in different parts of the world and public health policies. Differences also exist because the CLSI proactively evaluates the need for changing breakpoints. The reasons why breakpoints may change and the manner in which the CLSI evaluates data and determines breakpoints are outlined in CLSI document M25—Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters.

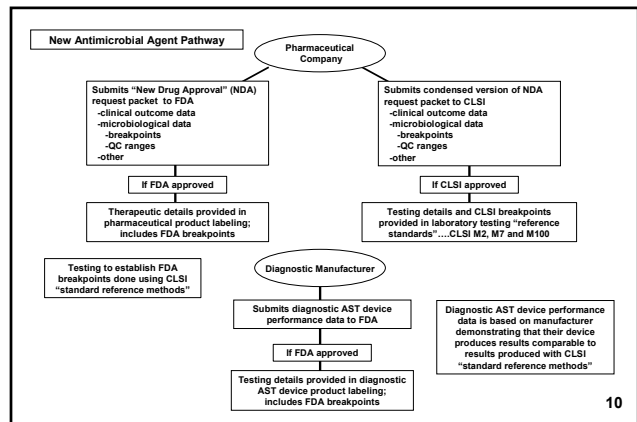
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CLSI M2, M7, M100

- ◆ Describe standard consensus “reference methods”
- ◆ U.S. clinical labs can use:
 - CLSI test method as written
 - Method that performs comparably to CLSI “reference method” (e.g. FDA-cleared diagnostic AST device)

Diagnostic AST device = commercial instrument or test used to determine antimicrobial susceptibility in vitro

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FDA vs. CLSI Breakpoints

- ◆ Nearly always agree!
- ◆ Sometimes disagree
- ◆ Sometimes only FDA breakpoints (e.g. tigecycline)
- ◆ Sometimes only CLSI breakpoints (before drug is FDA cleared or if drug used in other countries)
- ◆ Sometimes modified by CLSI (e.g., 2006, vancomycin – *S. aureus*)

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2006 Vancomycin MIC (µg/ml) Breakpoints – *S. aureus*

CLSI
≤2 4-8 ≥16

FDA
≤4 8-16 ≥32

Presently, diagnostic manufacturers must use FDA breakpoints

Clinical laboratories can use CLSI or FDA breakpoints
Caveat: if using commercial AST instrument, system uses FDA breakpoints

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Significantly with the same dose range of daptomycin as single-agent or daptomycin or daptomycin plus probenecid is co-administered with probenecid.

MICROBIOLOGY

Daptomycin is an antibacterial agent of a new class of antibiotics, the cyclic lipopeptides. Daptomycin is a natural product which has clinical utility in the treatment of infections caused by aerobic Gram-positive bacteria. The *in vitro* spectrum of activity of daptomycin encompasses most clinically relevant Gram-positive organisms. *Pseudomonas* spp. are not included.

Daptomycin has been shown to be active against most isolates of the following microorganisms both *in vitro* and in clinical infections, as described in the INDICATIONS AND USAGE section.

Aerobic and facultative Gram-positive microorganisms:

- Enterococcus faecalis* (vancomycin-susceptible strains only)
- Staphylococcus aureus* (including methicillin-resistant)
- Staphylococcus epidermidis* (including methicillin-resistant strains)
- Staphylococcus saprophyticus*
- Streptococcus agalactiae*
- Streptococcus dysgalactiae* subsp. *equisimilis*
- Streptococcus pyogenes*

The following *in vitro* data are available, but their clinical significance is unknown. Greater than 90% of the following microorganisms demonstrate an *in vitro* MIC less than or equal to the susceptible breakpoint for daptomycin versus the bacterial genus. The efficacy of daptomycin in treating clinical infections due to these microorganisms has not been established in adequate and well-controlled clinical trials.

Aerobic and facultative Gram-positive microorganisms:

- Corynebacterium jeikeium*
- Enterococcus faecalis* (vancomycin-resistant strains)
- Enterococcus faecium* (including vancomycin-resistant strains)
- Staphylococcus epidermidis* (including methicillin-resistant strains)
- Staphylococcus haemolyticus*

Daptomycin product labeling...
http://www.cubicin.com/PDF/1004-2_MARKETING_gt.pdf

Daptomycin FDA MIC Breakpoints (same as CLSI) 2006 - CLSI eliminated disk diffusion breakpoints

Table 3. Susceptibility Interpretive Criteria for Daptomycin

Pathogen	Minimal inhibitory concentration (µg/mL) ^a			Disk diffusion zone Diameter (mm) ^b		
	S	I	R	S	I	R
<i>Staphylococcus aureus</i> (methicillin-susceptible and methicillin-resistant)	≤1	(c)	(c)	≥16	(c)	(c)
<i>Streptococcus pyogenes</i> , <i>Streptococcus agalactiae</i> , and <i>Streptococcus dysgalactiae</i> subsp. <i>equisimilis</i>	≤1	(c)	(c)	≥16	(c)	(c)
<i>Enterococcus faecalis</i> (vancomycin-susceptible only)	≤4	(c)	(c)	≥11	(c)	(c)

a. The MIC interpretive criteria for *S. aureus* and *E. faecalis* are applicable only to tests performed by broth microdilution using Mueller-Hinton broth adjusted to a calcium content of 50 mg/L; the MIC interpretive criteria for *Streptococcus* spp. other than *S. pneumoniae* are applicable only to tests performed by broth microdilution using Mueller-Hinton broth adjusted to a calcium content of 50 mg/L, supplemented with 2 to 5% lysed horse blood, inoculated with a direct colony suspension and incubated in ambient air at 35°C for 20 to 24 hours.

b. The zone diameter interpretive criteria for *Streptococcus* spp. other than *S. pneumoniae* are available only to tests performed by disk diffusion.

Daptomycin product labeling...
http://www.cubicin.com/PDF/1004-2_MARKETING_gt.pdf

January 2006 Vol. 26 No. 3

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 U.S.

GROUP A PRIMARY TEST AND REPORT	Enterobacteriaceae ^a	<i>Pseudomonas aeruginosa</i> and Other Non-Enterobacteriaceae ^b	<i>Staphylococcus</i> spp.	<i>Enterococcus</i> spp. ^c
	Ampicillin ^d	Ceftazidime	Oxacillin ^e	Penicillin ^f or ampicillin
	Cefazolin ^g	Gentamicin	Penicillin ^f	
	Cephalexin ^h	Mezlocillin or ticarcillin		
	Gentamicin	Piperacillin		
GROUP B ROUTINE TEST SELECTIVELY	Amikacin	Amikacin	Azithromycin ⁱ or clarithromycin ^j or erythromycin ^k	Daptomycin Linezolid Gantrisin ^l , dalacin ^m , or vancocin ⁿ
	Amoxicillin-clavulanic acid or ampicillin-sulbactam	Cefepime		
	Piperacillin-tazobactam			
	Ticarcillin-clavulanic acid			
	Cefamandole or cefonicid or cefuroxime	Aztreonam	Clindamycin ^o	
		Cefoperazone	Daptomycin	
		Ciprofloxacin	Linezolid	
		Levofloxacin	Teicoplanin ^p	
	Cefepime	Impenem		
	Cefmetazole	Meropenem		
Cefoperazone ^q	Ticarcillin-clavulanic acid ^r			

CLSI Table 1 (M7) Drugs to Test/Report

Why are some drugs listed in Table 2 but not in Table 1?

CLSI Table 1 (M7) Drugs to Test/Report

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	Cefazolin ^g	Gentamicin	Penicillin ^f	
	Cephalexin ^h	Mezlocillin or ticarcillin		
	Gentamicin	Piperacillin		

Test Report Group	Antimicrobial Agent	MIC (µg/mL) Interpretive Standard ^a	Comments
ENTEROBACTERIACEAE	Clindamycin	<48	10 µg susceptibility only
	Clotrimazole	<16	>16
	Colistin	≤2	>2
	Colistin	≤2	>2
	Colistin	≤2	>2
	Colistin	≤2	>2
	Colistin	≤2	>2
	Colistin	≤2	>2
	Colistin	≤2	>2
	Colistin	≤2	>2
GRAM POSITIVE	Vancomycin	≤16	16-32 µg (U.S. only)
	Vancomycin	≤16	16-32 µg (U.S. only)
	Vancomycin	≤16	16-32 µg (U.S. only)

Possible Reasons why drug may be in Table 2 but not Table 1...

- ◆ Drug does not have an FDA clinical indication for organism
- ◆ Drug may not be used in the USA
- ◆ Drug is not a first-choice or alternative drug suggested for routine testing for organism

Example:

- piperacillin-tazobactam and *P. aeruginosa*

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Susceptible (“S”) Reworded

- ◆ New ...implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection.
- ◆ Old ...implies that an infection due to the strain may be appropriately treated with the dosage of antimicrobial agent recommended for that type of infection and infecting species, unless otherwise contraindicated.

CLSI M100-S16; Introduction (M2, M7) 24

Intermediate (“I”)

- ◆ The “intermediate” category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g., quinolones and β-lactams in urine) or when a higher than normal* dosage of a drug can be used (e.g., β-lactams). This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.

*previously stated “high dosage”

CLSI M100-S16; Introduction_(M2, M7) 25

Resistant (“R”) Reworded

- ◆ New –...implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate MICs that fall in the range where specific microbial resistance mechanisms are likely (e.g., β-lactamases), and clinical efficacy of that agent against the isolate has not been reliably shown in treatment studies.
- ◆ Old - ...strains are ~~not inhibited~~ by the usually achievable systemic concentrations of the agent with normal dosage schedules and/or fall in the range where specific microbial resistance mechanisms (e.g., β-lactamases) and clinical efficacy has not been reliable in treatment studies.

CLSI M100-S16; Introduction_(M2, M7) 26

“S” only Definition

rare occurrence

If only “S” criteria are specified:
For some organism/antimicrobial combinations, the absence or rare occurrence of resistant strains precludes defining any results categories other than “susceptible.” For strains yielding results suggestive of a “nonsusceptible” category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory that will confirm results using a CLSI reference dilution method.

CLSI M100-S16; Introduction_(M2, M7) 27

“Absence” vs. “rare occurrence”.... How do we know if there has EVER been a “NS” isolate?

- ◆ Check CLSI M100-S16 “Suggestions for Verification of AST Results and Confirmation of Organism Identification” [Table 4 (M2) or Table 8 (M7)]

NS, not susceptible

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Excerpt from: “Suggestions for Verification of AST Results and Confirmation of Organism Identification”

Organism or Group	Category I Verify at all labs	Category II Verify – institution specific
<i>Staphylococcus aureus</i>	daptomycin - NS linezolid – NS quin-dalfo – I or R vancomycin – I or R	oxacillin – R
<i>Streptococcus pneumoniae</i>	fluoroquinolone - NS linezolid ^c – NS vancomycin ^c – NS	penicillin - R 3 rd -gen cephalosporin - R

NS, not susceptible
Note: superscript “c” means “never reported”

CLSI M100-S16; Tables 4 (M2) and 8 (M7) 29

^cNS Example
NS, not susceptible

S. pneumoniae - Vancomycin^c

MIC (µg/ml)

Susc Int Res

vancomycin ≤ 1.0 - -

*investigate any NS isolate

..Repeat ID and AST

..Save isolate

..Send to reference lab (test by CLSI MIC method)

Note: vancomycin-NS *S. pneumoniae* have NEVER been reported

CLSI M100-S16; Table 2G (M7) 30

CLSI M45-P Guideline

Abiotrophia / Granulicatella	Lactobacillus
*Aeromonas / Plesiomonas	Leuconostoc
Bacillus spp. (not anthrax)	Listeria monocytogenes
Campylobacter jejuni / coli	Moraxella catarrhalis
Corynebacterium	*Pasteurella
Erysipelothrix	Pediococcus
HACEK Group	*Vibrio spp. (not cholera)

*disk diffusion method described in addition to MIC method

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CLSI M45-P Guideline

- ◆ “Testing should only be undertaken in consultation with infectious diseases or other expert clinicians that can assist in determining if susceptibility testing is needed in the management of a specific patient.”

CLSI M45-P; Indications

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Table 5. *Corynebacterium* spp. (including *C. diphtheriae*)—Information and Interpretive Criteria for Broth Microdilution Susceptibility Testing

Testing Conditions	Minimal QC Recommendations	Agents to Consider for Primary Testing
Medium: Cation-adjusted Mueller-Hinton broth with lysed horse blood (2.5-5%)	<i>Streptococcus pneumoniae</i> ATCC® 49619	Penicillin Vancomycin Fluoroquinolone Gentamicin
Inoculum: Direct colony suspension, equivalent to a 0.5 McFarland standard		
Incubation: 35 °C; ambient air for 24-48 hours		

General Comments

- Growth characteristics on routine media:
Nonfastidious; grows well in 3A²; ambient air; 20-24 hours
- For some organisms, antimicrobial combinations, the absence or rare occurrence of resistant strains, includes defining any results categories other than “susceptible.” For strains yielding results suggestive of a non-susceptible category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory for confirmation.

NOTE: Information in boldface type is considered tentative for one year.

Antimicrobial Class	Antimicrobial Agent	MIC (µg/mL) Interpretive Criteria				Comments
		S	I	R		
PENICILLINS	penicillin	32	4	1	2c	1) Interpretive criteria may not apply to meningitis
CEPHEMIS	ceftriaxone	32	4	1	2c	1) Interpretive criteria may not apply to meningitis
	cefepime	32	4	1	2c	
	cefepime	32	4	1	2c	
GLYCOPEPTIDES	vancomycin	32	4	1	2c	1) Interpretive criteria may not apply to meningitis
	teicoplanin	32	4	1	2c	

**CLSI M45-P Table 5
Corynebacterium spp. 39**

CLSI M45-P Table 5 (con't) *Corynebacterium* spp.

Supplemental information

Resistance:
Some species of *Corynebacterium* may exhibit resistance to multiple drug classes.

Reasons for Testing/MI Testing:
Testing of isolates from normally sterile sources (blood cultures, deep tissue, implanted prosthetic devices) may be warranted, especially in immunodeficient patients.

Derivation of Interpretive Criteria:
Interpretive criteria for penicillin and erythromycin are based primarily on MIC distributions following testing of a large number of isolates. Cephalosporin interpretive criteria are adapted from those for *Streptococcus* spp.; in solid interpretive criteria are adapted from those for *Enterococcus* spp.; remaining interpretive criteria are adapted from those for *Staphylococcus* spp. as published in the current edition of CLSI document M100.

Testing Notes:
Resistant results can be reported at 24 hours. Isolates demonstrating susceptible results for beta-lactams should be re-inoculated and results reported at 48 hours.

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Changes 2006 CLSI M100-S16 Gram Negatives

GNR

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Enterobacteriaceae

CLARIFICATIONS...

- ◆ ESBL screening breakpoints for *Proteus mirabilis*
- ◆ Warning comment for *Salmonella/Shigella*
- ◆ AST of *Salmonella* spp. from feces

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Colistin / Polymyxin B

- ◆ Currently, NO disk diffusion recommendations
- ◆ *Acinetobacter* spp.

Antimicrobial Agent	MIC (µg/ml)		
	S	I	R
Polymyxin B or colistin	≤2	-	≥4

- ◆ Breakpoints for other bugs forthcoming
- ◆ No FDA-cleared test

CLSI M100-S16; Table 2B (M7) 55

Haemophilus spp. *Neisseria meningitidis*

- ◆ Procedures in M2, M7 and M100 are for *H. influenzae* and *H. parainfluenzae*
 - CLSI M45-P for other *Haemophilus* species
- ◆ *Neisseria meningitidis*
 - Added disk diffusion procedure

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Standard Disk Diffusion Method for *Neisseria meningitidis*

NEW!

Table 2J. Zone Diameter Interpretive Standards and Equivalent Minimal Inhibitory Concentration (MIC) Breakpoints for *Neisseria meningitidis*

Testing Conditions	Minimal QC Recommendations (See Tables 3 and 3A for acceptable QC ranges.)
Medium: Mueller Hinton agar with 5% sheep blood Inoculum: Direct colony suspension, equivalent to a 0.5 McFarland standard Incubation: 35 ± 2 °C; 5% CO ₂ ; 20 to 24 hours	<i>Streptococcus pneumoniae</i> ATCC® 49619 in 5% CO ₂ ; <i>E. coli</i> ATCC® 25922 (incubated either in ambient air or 5% CO ₂) should be used for ciprofloxacin, nalidixic acid, and trimethoprim.

General Comments

(1) Recommended precautions: Biosafety Level 2 (BSL2) practices are recommended for this organism. Whenever possible, procedures likely to generate aerosols should be performed within a biological safety cabinet.

(2) For some organism/antimicrobial agent combinations, the absence or rare occurrence of resistant strains precludes defining any results categories other than "susceptible." For all cases yielding results suggestive of a "non-susceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory that will confirm results using a CLSI reference dilution method.

NOTE: Information in boldface type is considered tentative for one year.

Test Report Group	Antimicrobial Agent	Disk Content	Zone Diameter, Inherent Width (mm)			Equivalent MIC Breakpoints (µg/mL)			Comments
			R	I	S	R	I	S	

CLSI M100-S16; Table 2J (M2) 57

Neisseria meningitidis Disk Diffusion Testing

- Medium: MHA with 5% sheep blood
 Inoculum: direct colony suspension equivalent to 0.5 McFarland standard
 Incubation: 35°C +/- 2°C; 5% CO₂; 20-24h
 QC: *S. pneumoniae* ATCC 49619
E. coli ATCC 25922 (select drugs)

Biosafety Level 2 (BSL 2) safety practices; use biosafety cabinet

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Neisseria meningitidis Disk Diffusion Testing

- ◆ Therapeutic agents*
 - *Penicillin
 - *Ampicillin
 - Cefotaxime
 - Ceftriaxone
 - Meropenem
 - Chloramphenicol

All drugs listed are in Test/Report Group C "Supplemental, Report Selectively"

*No disk diffusion breakpoints; must do MIC test

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Neisseria meningitidis Disk Diffusion Testing

- ◆ "Breakpoints may be appropriate only for prophylaxis of meningococcal case contacts"
 - Azithromycin
 - Ciprofloxacin
 - Minocycline
 - Nalidixic acid (for surveillance only; may detect diminished fluoroquinolone susceptibility)
 - Rifampin
 - Trimethoprim-sulfamethoxazole (predicts susceptibility to sulfonamides also)

All drugs listed are in Test/Report Group C "Supplemental, Report Selectively"

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Changes 2006 CLSI M100-S16 Gram Positives

GPC⁶¹

Daptomycin

For.....

- Staphylococcus
- Enterococcus
- Streptococcus
- ◆ Deleted daptomycin disk diffusion breakpoints
 - Rationale for deletion – inability of disk diffusion method to consistently detect those few isolates that are non-susceptible to daptomycin
- ◆ Specify agar dilution testing has not been validated for daptomycin
- ◆ FDA-cleared for several commercial systems

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Staphylococcus spp.

Clarifications...

- ◆ For disk diffusion testing, cefoxitin disk is..
 - Preferred over oxacillin disk for detection of *mecA*-mediated resistance
 - A “surrogate” for oxacillin (report oxacillin NOT cefoxitin)
 - Should always be used for *S. lugdunensis* (do not use oxacillin disk)
- ◆ Fluoroquinolones – breakpoints for gatifloxacin, levofloxacin, moxifloxacin, and ofloxacin still tentative (for another year)

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Cefoxitin Disk for *mecA*-mediated Resistance in Staphylococci

	Cefoxitin zone (mm)	
	<u>Res</u>	<u>Susc</u>
<i>S. aureus</i>	≤19*	≥20**
<i>S. lugdunensis</i>	≤19*	≥20**
CoNS	≤24*	≥25**

- * Report as oxacillin resistant
 - ** Report as oxacillin susceptible
- CoNS, coagulase-negative staphylococci

CLSI M100-S16; Table 2C (M2) 64

MIC Breakpoints (µg/ml) Staphylococcus and Fluoroquinolones

	Old (M100-S14)*			M100-S15, M100-S16 **		
	S	I	R	S	I	R
Gatifloxacin	≤2	4	≥8	≤0.5	1	≥2
Levofloxacin	≤2	4	≥8	≤1	2	≥4
Moxifloxacin	none			≤0.5	1	≥2

- *Same as current FDA breakpoints
 - **Tentative for another year
- Check M100-S16 for corresponding disk diffusion breakpoints

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Staphylococcus spp.

Additions/changes...

- ◆ Modified vancomycin MIC breakpoints for *S. aureus*
- ◆ Table highlighting use of BHI-vancomycin agar for *S. aureus*

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CLSI Vancomycin MIC ($\mu\text{g/ml}$) Breakpoints – *S. aureus*

2006	2005
≤ 2 4-8 ≥ 16	≤ 4 8-16 ≥ 32

Notes:
 2005 same as current FDA breakpoints
 No change in disk diffusion breakpoints
 No change for coagulase-negative staphylococci

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Why did CLSI modify vancomycin breakpoints for *S. aureus*?

- ◆ To detect emerging vancomycin resistance
 - Data shows patients fail vancomycin therapy when infected with *S. aureus* with vancomycin MICs of $\geq 4 \mu\text{g/ml}$
 - Clinical labs have been advised by CDC to investigate *S. aureus* with vancomycin MICs of $\geq 4 \mu\text{g/ml}$ as potential VISA or VRSA
 - Some VISA test $4 \mu\text{g/ml}$ by some methods

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Modifying CLSI Breakpoints

CLSI has mechanism for reevaluation of breakpoints when needed as defined in CLSI M23-A2 “Development of In Vitro Susceptibility Testing Criteria and QC Parameters”

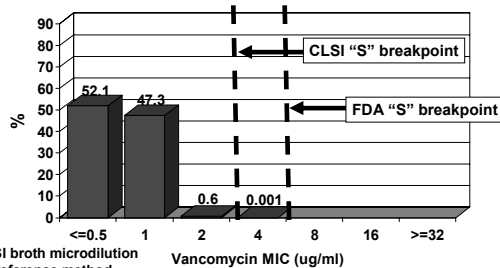
69

Will it matter what vancomycin breakpoints we use for *S. aureus*?

- ◆ “Either FDA or CLSI susceptibility interpretive breakpoints are acceptable to clinical laboratory accrediting bodies” (CLSI M100-S16, page 14)
- ◆ ...and we should all pursue *S. aureus* with vancomycin MICs $\geq 4 \mu\text{g/ml}$ since these could be VISA (or VRSA if $\geq 16 \mu\text{g/ml}$)
http://www.cdc.gov/ncidod/dhqp/ar_visavrsa_algo.html
- ◆ So, if we ALL diligently pursue vancomycin MICs of $\geq 4 \mu\text{g/ml}$ in *S. aureus*, it really won't matter

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S. aureus - Vancomycin MICs* UCLA 1/00-10/05 (n=13981)



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Table 2C. (Continued)

Screening Tests for Oxacillin Resistance and Reduced Susceptibility to Vancomycin in *Staphylococcus aureus*

Screen Test	Oxacillin Resistance	Reduced Susceptibility to Vancomycin
Medium	MURK with NaCl (4% w/v; 0.85 M)†	BHI agar
Minimal inhibitory concentration	4 $\mu\text{g/ml}$ oxacillin	4 $\mu\text{g/ml}$ vancomycin
Inoculum	Turbid colony suspension to obtain 0.5 McFarland turbidity	Turbid colony suspension to obtain 0.5 McFarland turbidity
Incubation	35°C	35°C ambient air
Readout	Zone diameter ≥ 19 mm	Protein A test: using a moist swab, spot a 10 μl drop onto agar surface. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10 to 15 mm in diameter or streak a portion of the plate. 24 hours >1 colony = presumptive reduced susceptibility Examine carefully with transmitted light for >1 colony or light film of growth. Perform vancomycin MIC using a validated MIC method to confirm reduced susceptibility.
QC recommendations	<i>Staphylococcus aureus</i> ATCC® 29213 - Susceptible <i>Staphylococcus aureus</i> ATCC® 43300 - Resistant	<i>Enterococcus faecalis</i> ATCC® 29212 - Susceptible <i>Enterococcus faecalis</i> ATCC® 81299 - Resistant

BHI-Vancomycin (6 $\mu\text{g/ml}$) Screen

- Add BHI-V screen if using automated system (unless fixed, check with manufacturer) or use CLSI MIC reference method or Etest to detect reduced susceptibility to vancomycin in *S. aureus*
- For workup of VISA and VRSA, see http://www.cdc.gov/ncidod/dhqp/ar_visavrsa_algo.html

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VRSA (as of 12/05)

	MIC (ug/ml) ¹	Source	Date	Location
1	1024	foot ulcer	4/02	Michigan
2	32	foot ulcer	9/02	Pennsylvania
3	64	nephrostomy tube	3/04	New York
4	256	foot ulcer	2/05	Michigan
5	512	wound	10/05	Michigan

¹ Reference broth microdilution MIC

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Enterococcus spp.

Additions/changes...

- ◆ Deleted vancomycin-synergy Rx comment
- ◆ Added definitions for high-level aminoglycoside resistance (HLAR) testing for disk diffusion (similar to those for MIC testing)

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Enterococcus Rx Comment Deleted

- ◆ Rx: If vancomycin is used for serious enterococcal infections, such as endocarditis, combined therapy with an aminoglycoside is usually indicated.
- ◆ Rationale for deletion – use of vancomycin should not be encouraged; ampicillin or penicillin are the preferred agents to use in combination therapy for enterococci

CLSI M100-S16; Table 2D (M2, M7)

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Enterococcus spp. Disk diffusion screen for HLAR

For Use With M100-A9—Disk Diffusion

M100-S16

Table 2D (Continued)

Test/Report Group	Antimicrobial Agent (HLAR)	Disk Content	Zone Diameter (mm)		Equivalent MIC (μg/ml)		Comments
			R ^a	S ^b	R	S	
U	Streptogramin (HLAR)	300 μg	8	12-10	1024	16	(U) If the organism is within the test is inconclusive, and an agar dilution or broth microdilution screen test should be performed to confirm resistance. See comments (2) and (3).
U	Streptogramin (HLAR)	300 μg	8	12-10	1024	16	(U) MIC—Streptogramin broth microdilution are resistant (1024 μg/ml) and for agar dilution (2000 μg/ml). See comments (2), (3) and (5).

Footnotes

- For QC of HLAR screen tests, use Enterococcus faecalis ATCC® 29212 (see Table 3, Footnote 1 [Disk Testing] for acceptable QC ranges).
- Resistant, will not be synergistic with cell-wall active agent (e.g., ampicillin, penicillin, vancomycin).
- Inconclusive, perform an agar dilution or broth microdilution test to confirm.
- Susceptible, will be synergistic with cell-wall active agent (e.g., ampicillin, penicillin, vancomycin) that is also susceptible.

See M7, Table 2D (MIC Testing) which summarizes additional screening tests for vancomycin, high-level aminoglycoside resistance, and supplemental tests for identification that may be helpful to vancomycin-resistant enterococci.

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Enterococcus spp. Disk diffusion Screen for HLAR

- ◆ Resistant -- will not be synergistic with cell-wall-active agent (e.g., ampicillin, penicillin, vancomycin)
- ◆ Inconclusive -- perform an agar dilution or broth microdilution test to confirm
- ◆ Susceptible -- will be synergistic with cell-wall-active agent (e.g., ampicillin, penicillin, vancomycin) that is also susceptible

CLSI M100-S16; Table 2D (M2)

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Streptococcus pneumoniae Modified reporting recommendations for meropenem...

- ◆ “Penicillin and cefotaxime or ceftriaxone or meropenem should be tested by a reliable MIC method and reported routinely with CSF isolates of *S. pneumoniae*.”

CLSI M100-S16; Table 2G (M2, M7)

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New Antimicrobial Agents in M100-S16*

Agent	Drug class	Route of administration	FDA approved
ceftobiprole	cephem	IV	No
faropenem	penem	PO	No

*In Glossary and QC tables only

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Ceftobiprole

- ◆ **Manufacturer:**
 - Johnson & Johnson
- ◆ **Possible clinical use:**
 - Nosocomial pneumonia
 - Complicated skin and skin structure infections

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Ceftobiprole (con't)

- ◆ **Microbiological activity:**
 - *Staphylococci* (including MRSA)
 - *Streptococci* (including penicillin-R *S. pneumoniae*)
 - *Enterococcus faecalis*
 - Most *Enterobacteriaceae*
 - *Haemophilus influenzae* (including BLNAR)
 - Many *Pseudomonas aeruginosa* and *Acinetobacter baumannii*
- ◆ **Limited activity:**
 - Many non-*Enterobacteriaceae*
 - *Enterococcus faecium*
 - ESBL-producers
 - Metallo-beta-lactamase producers
 - Beta-lactamase-producing anaerobes

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Faropenem

- ◆ **Manufacturer:**
 - Replidyne
- ◆ **Possible clinical use:**
 - Bacterial sinusitis
 - Acute exacerbations of chronic bronchitis
 - Community-acquired pneumonia
 - Uncomplicated skin and skin structure infections

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Faropenem (con't)

- ◆ **Microbiological activity:**
 - Respiratory pathogens (*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*)
 - *S. pyogenes*
 - MSSA
 - Some *Enterobacteriaceae*
- ◆ **Limited activity:**
 - Non-*Enterobacteriaceae*
 - *Enterobacter* spp.
 - *Enterococcus faecium*
 - MRSA

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Changes 2006 CLSI M100-S16 Quality Assessment / Quality Control

QA/QC

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Quality Control

ADDITIONS / Changes...

◆ Added QC ranges:

- Ceftobiprole
- Faropenem
- Drugs for testing *Campylobacter jejuni* ATCC 33560 using broth dilution method

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QC Tables

Table	M2 (disk diffusion)	M7 (MIC testing)
3	Nonfastidious	Nonfastidious
3A	Fastidious	Fastidious (broth dilution)
3B	DD QC Testing Frequency	Fastidious (agar dilution) <i>New!</i>
3C	DD Troubleshooting Guide <i>New!</i>	Fastidious (broth dilution + supplement)*
3D	NA	Fastidious (<i>Brucella</i> broth dilution)* <i>New!</i>

DD, disk diffusion; NA, not applicable;
*when testing potential agents of bioterrorism

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QC Tables (con't)

Table	M2	M7
3E	NA	MIC QC Testing Frequency
3F	NA	MIC Troubleshooting Guide <i>New!</i>

*NA, not applicable

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Table 3C. Disk Diffusion QC Troubleshooting Guide

Table 3C. Disk Diffusion QC Troubleshooting Guide

This table provides guidance for troubleshooting and corrective action for out-of-range quality control, primarily using antimicrobial susceptibility tests with Mueller Hinton Agar. Refer to M2-A9, Disk Diffusion, Section 15, Quality Control Procedures and Appendix A, Quality Control Protocol Flow Charts for additional information. Out of range quality control tests should first be repeated. If the issue is unresolved, this troubleshooting guide provides additional suggestions for troubleshooting out-of-range quality control results. In addition, if unresolved, manufacturers should be notified of potential product problems.

General Comments

(1) QC organisms manufacturers avoid repeated subcultures. Retrieve new QC strain from stock if using lyophilized strains. Follow the maintenance recommendations of the manufacturer. Some *E. coli* ATCC® 35218 and *K. pneumoniae* ATCC® 70619 each culture at 40 °C in broth and prepare working stocks in culture weekly.

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Corrective Action
Aminoglycosides	Any	Zone too small	pH of media too low	Acceptable pH range = 7.2-7.4 Avoid CO ₂ incubation which lowers pH
Aminoglycosides	Any	Zone too large	pH of media too high	Acceptable pH range = 7.2-7.4 Ca ²⁺ and/or Mg ²⁺ content too high
Aminoglycosides	<i>P. aeruginosa</i> ATCC® 27862 <i>P. aeruginosa</i> ATCC® 27633	Zone too small	Ca ²⁺ and/or Mg ²⁺ content too high	Use alternative lot of media
Aminoglycosides	<i>P. aeruginosa</i> ATCC® 27862 <i>P. aeruginosa</i> ATCC® 27633	Zone too large	Ca ²⁺ and/or Mg ²⁺ content too low	Use alternative lot of media
Amoxicillin clavulanate	<i>E. coli</i> ATCC® 35218	Zone too small	Clavulanate acid is labile	Use alternative lot of disks Check storage conditions and

New!

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Disk Diffusion QC Troubleshooting Guide Table 3C (M2) Examples:

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments / Action
Beta-lactam group	Any	Zone initially acceptable but decreases and possibly out-of-range over time	Disk has lost potency	Use alternative lot of disks. Check storage conditions and package integrity. Imipenem, cefaclor, and clavulanic acid are especially labile.
Quinolones	Any	Zone too large	pH of media too high	Acceptable pH range = 7.2-7.4

CLSI M100-S16; Table 3C (M2) 89

CLSI Standards - 2006

◆ M100-S16 Tables (2006)*

.....to be used with text documents explaining how to perform the tests....

M2-A9 Disk Diffusion (2006)**

M7-A7 MIC (2006)**

New!

* M100 updated yearly
**M2, M7 updated every 3 years



M2-A9, M7-A7 Primary Changes

- ◆ Primarily expanded discussions and detailed recommendations for test procedures in M100-S16 including those for:
 - Oxacillin-resistant staphylococci
 - *Streptococcus pneumoniae*
 - *Streptococcus* spp.
 - *Neisseria meningitidis*

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M2-A9, M7-A7 Primary Changes (con't)

- ◆ Additions to antimicrobial agent descriptions
- ◆ Additional tips for media/reagent preparation
- ◆ Supplemental QC suggestions

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Last pages of M2 and M7

Summary of Comments and Subcommittee Responses

Clinical and Laboratory Standards Institute consensus procedures include an appeals process that is described in detail in Section 5 of the Administrative Procedures. For further information, contact CLSI or visit our website at www.clsi.org.

Summary of Comments and Subcommittee Responses

M2-A9: Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard— Eighth Edition

General

1. Could you please clarify a point for me about cefuroxime testing? Your standard gives different zone size criteria for the parenteral and oral forms of the drug and specifies that different disks should be used. Is the implication that you could get an isolate testing sensitive with the one disk and resistant with the other? If this is true, could you use the parenteral (cefuroxime sodium) disk to predict sensitivity for both forms, accepting that you may miss all zones strains resistant that would respond to oral, or is it the other way around? The standard gives no details about these particular recommendations.

- Although there are two formulations for cefuroxime, one for parenteral and one for oral administration, there is only one disk for laboratory testing. Different interpretive criteria were developed based on the different pharmacodynamic/pharmacokinetic data and clinical indications for the two formulations. Tables 2A through 2J should be used to guide interpretation for individual organisms.

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Q&A Example (paraphrased)

- ◆ Q – Why is D zone test not recommended for *Streptococcus pneumoniae*?
- ◆ A – Isolates of *Streptococcus pneumoniae* can have *erm*-mediated resistance to erythromycin. However, the vast majority of these are also resistant to clindamycin (constitutive phenotype). Rare isolates of pneumococci may have inducible resistance; however the clinical significance of this has not been established. Therefore, routine testing for inducible clindamycin resistance is not recommended for this species.

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Issues Under Discussion by CLSI

- ◆ Additional recommendations for testing colistin / polymyxin B
- ◆ Disk diffusion test for *Campylobacter* spp.
- ◆ Detection of ESBLs
- ◆ Review recommendations for drugs to Test / Report (Table 1)
- ◆ Improved communication of CLSI AST Subcommittee decisions

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Material on your CD-ROM...

1. PowerPoint presentation
2. PDF of “New Antimicrobial Agent Pathway” slide (slide #10)
3. M100-S16 checklist
4. References
5. CLSI information flier
6. CLSI catalogue

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To Ask a Question....

- ◆ Please send to neoffice@nltn.org
- ◆ Questions will be compiled and answers will be published on <http://www.phppo.cdc.gov/nltn/nphct/s/ast012506.aspx>

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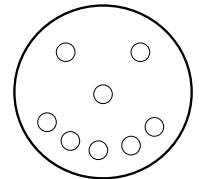
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<http://www.phppo.cdc.gov/nltn/default.aspx>

The screenshot shows the website for the National Laboratory Training Network (NLTN). At the top, it features the CDC logo and the text "Department of Health and Human Services, Centers for Disease Control and Prevention". Below this is a search bar and navigation tabs for "Health & Safety Topics", "Publications & Products", "Data & Statistics", and "Conferences & Events". The main content area is titled "NATIONAL LABORATORY TRAINING NETWORK" and includes the NLTN logo and the tagline "Quality Laboratory Practice through Continuing Education". A paragraph describes the network's mission: "The National Laboratory Training Network (NLTN), dedicated to improving laboratory practice of public health significance through quality continuing education, is a training system sponsored by the Association of Public Health Laboratories (APHL) and Centers for Disease Control and Prevention (CDC)". Below this, there are sections for "Study Modules" and "Professional Staff" with brief descriptions of the services offered. A footer note says "Search our listing of scheduled training events which includes self-study modules. Upcoming training events will be".

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Thank you!



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