Summary Minutes
Subcommittee on Antimicrobial Susceptibility Testing
Hyatt Harborside Hotel
Boston, Massachusetts
12-14 June 2011

A meeting of the CLSI Subcommittee on Antimicrobial Susceptibility Testing was held on 12-14 June 2011, at the Hyatt Harborside Hotel, Boston, Massachusetts. The following were in attendance:

Franklin R. Cockerill, III, MD **Chairholder**	**Mayo Clinic**
Matthew A. Wikler, MD, MBA, FIDSA **Vice-Chairholder**	**IASO Pharma, Inc.**
John H. Rex **Area Committee on Microbiology** **Chairholder**	**AstraZeneca**
Mary Jane Ferraro, PhD, MPH **Area Committee on Microbiology** **Vice-Chairholder**	**Massachusetts General Hospital**

Members Present

Jeff Alder, PhD	Bayer Healthcare
Michael N. Dudley, PharmD, FIDSA	Rempex Pharmaceuticals
George M. Eliopoulos, MD	Beth Israel Deaconess Medical Center
Dwight J. Hardy, PhD	University of Rochester Medical Center
David W. Hecht, MD	Loyola University Medical Center
Janet F. Hindler, MCLS, MT(ASCP)	UCLA Medical Center
Jean B. Patel, PhD, D(ABMM)	Centers for Disease Control and Prevention
Mair Powell, MD, FRCP, FRCPath	MHRA
Richard B. Thomson, Jr., PhD	Evanston Hospital, NorthShore University HealthSystem
John D. Turnidge, II, MD	SA Pathology at Women's and Children's Hospital
Melvin P. Weinstein, MD	Robert Wood Johnson University Hospital
Barbara L. Zimmer, PhD	Siemens Healthcare Diagnostics, Inc.

Advisors Present

Paul G. Ambrose, PharmD, FIDSA	ICPD/Ordway Research
Patricia A. Bradford, PhD	AstraZeneca
Steven D. Brown, PhD	The Clinical Microbiology Institute
Edward Cox	FDA Center for Drug Evaluation and Research
William A. Craig, MD	University of Wisconsin School of Medicine

Cynthia L. Fowler, MD	bioMérieux, Inc.
Ronald N. Jones, MD	JMI Laboratories
Gunnar Kahlmeter, MD, PhD	ESCMID
James S. Lewis, II, PharmD	University of Texas Health Science Center
Frederic J. Marsik, PhD, ABMM	FDA Center for Drug Evaluation and Research
Harriette L. Nadler, PhD	EUSA USA Pharmaceuticals, Inc.
Freddie Mae Poole, BS, MT(ASCP, ISCLT)	FDA Center for Devices and Radiological Health
Sandra S. Richter, MD, D(ABMM)	Cleveland Clinic
Paul A. Schwab, PhD, D(ABMM)	Quest Disagnostics, Nichols Institute
Jana M. Swenson, MMSc	Consultant
Joseph G. Toerner, MD, MPH	FDA Center for Drug Evaluation and Research

Reviewers Present

Francis Arhin	The Medicines Company
Farah Babakhani, PhD	Optimer Pharmaceuticals, Inc.
Robert E. Badal	International Health Management Associates
Caroline Baez-Giangreco	Tufts Medical Center
Harjot Bains	Siemens Healthcare Diagnostics MicroScan
Jennifer Dien Bard, PhD(ABMM), FCCM	Queens's University
Cara Bastulli	Trek Diagnostic Systems
Bret Benton, PhD	Theravance Inc.
Dr. Susanne Berglund	Siemens Healthcare Diagnostics
Sujata Bhavnani, PharmD	Ordway Research Institute
Donald Biek, PhD	Cerexa, Inc.
Johanne Blais	Theravance
Susan E. Boruchoff, MD	UMDNJ-Robert Wood Johnson Medical School
Lyn Boyer	Siemens Healthcare Diagnostics
William B. Brasso	BD Diagnostic Systems
Joyce R. Bray	Siemens Healthcare Diagnostics
Stephen M. Brecher, PhD	VA (West Roxbury) Boston Healthcare System
Dr. Derek Brown	PhD, D(ABMM), FCCM
Linda C. Bruno, MA, MT(ASCP)	University of Illinois Medical Center
Laurent Chesnel	Cubist Pharmaceuticals, Inc.
Diane M. Citron, M(ASCP)	R.M. Alden Research Laboratory
Jennifer Copeland	Theravance Inc.
Ian A. Critchley, PhD	Cerexa, Inc.
John A.Crump	Centers for Disease Control and Prevention
Sharon K. Cullen, BS, RAC	Siemens Healthcare Disagnostics
Todd Davies, PhD	Johnson & Johnson Pharm Research & Develop.
Jennifer Dawson Driscoll	Siemens Healthcare Diagnostics
Phyllis Della-Latta, PhD, MSC	Columbia University Medical Center
Michael J. Dowzicky	Wyeth Pharmaceuticals
Joanne Dzink-Fox	Novartis Institutes for Biomedical Research
Evelyn Ellis-Grosse, PhD	E2g Consulting
Anette Engelhardt	AB Biodisk
Rob Eusebio, MSHA, MT(ASCP)	Siemens Healthcare Diagnostics
Gina Ewald, CLS(CA), MT(ASCP)	Siemens Healthcare Diagnostics
John Farley	FDA Center for Drug Evaluation and Research
Sheila Farnham, MT(ASCP)	bioMerieux, Inc.

David Farrell, PhD, D(ABMM), FCCM	JMI Laboratories
Lee Ann Feeney	Achaogen
Dr. Prabhavathi Fernandes	Cempra Pharmaceuticals, Inc.
Robert K. Flamm, PhD.	Johnson & Johnson Pharm Research & Develop.
Lawrence V. Friedrich, PharmD	Cubist Pharmaceuticals, Inc.
Thomas R. Fritsche, PhD, MD	Marshfield Clinic
Marcelo Galas	Reference Centres of Latinoamerican Countries
Rboert Giacobbe	AstraZeneca
Monica Giguere	BD Diagnostic Systems
Beth P. Goldstein, PhD	Beth Goldstein Consultant
Trudy Grossman	Tetraphase Pharmaceuticals
Stephen Hawser, PhD	IHMA Europe Sàrl
Denise Holliday	BD Diagnostics
Michael D. Huband	AtraZeneca Pharmaceuticals
Dr. Romney Humphries	UCLA
Jospeh P. Iaconis	AstraZeneca Pharmaceuticals
Nilda Jacobus	Tufts Medical Center
Jack L. Johnson	International Health Management Associates
Judith Johnston, MS	Siemens Healthcare Diagnostics Inc.
James H. Jorgensen, PhD	University of Texas Health Science Center
Dr. Maria Karlsson	Centers for Disease Control and Prevention
Bradley Katz	Cubist Pharmaceuticals, Inc.
Scott B. Killian	Trek Diangostic Systems
JiYeon Kim	Massachusetts General Hospital
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Laura M. Koeth, MT(ASCP)	Laboratory Specialists, Inc.
Kevin Krause	Cerexa, Inc.
Joseph Kuti	Center for Anti-Infective Research and Development
Melinda Lacy, PharmD	Ortho-McNeil
Heidi Leister-Tebbe	Pfizer, Inc.
S. Blaine Leppanen	Blaine Healthcare Associates, Inc.
Jim Lindsey	Mast International
Dyan Luper, BS, MT(ASCP)SM	BD Diagnostic Systems
Anthony Simon Lynch	J&J Pharmaceuticals R&D
Ann Macone	Paratek Pharmaceuticals, Inc.
Hideki Maki	Shiongogi & Co. Ltd.
Linda M. Mann, PhD, D(ABMM)	Siemens Medical Solutions Diagnostics
Maureen Mansfield	Trek Diagnostic Systems
Erika Matuschek, PhD	EUCAST
Laura McDermott	T-NEMC
Brenda MCurdy, PhD	John D. Dingell VA Medical Center
Rodrigo Mendes	JMI Laboratories
Hiroshige Mikamo, MD, PhD	Aichi Medical University Graduate School of Medicine
Dr. Greg Moeck	The Medicines Company
Timothy Morris	Bausch & Lomb
Dr. Ian Morrissey	Quotient Bioresearch Ltd.
Mary R.Motyl, PhD, D(ABMM)	Merck Sharp & Dohme Corp
Ross Mulder, MT(ASCP)	bioMérieux, Inc.

Susan D. Munro, MT(ASCP)	Stanford Hospital and Clinics
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Sumathi Nambiar	FDA
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Luann Ochs, MS

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I. MEETING/OPENING REMARKS

Dr. Cockerill called the meeting to order at 8:00 a.m. on Monday, 13 June 2011. He thanked everyone for their participation in the Working Groups sessions held on Sunday, especially the Working Group chairholders, recording secretaries, and Working Group members for the significant work they do. He noted the importance of the work done by the subcommittee as it pertains to patients and patient care, as the subcommittee tries to provide best clinical practice recommendations for antimicrobial use in treating patients. Keeping this in mind, he asked the committee for efficiency and respect for the presenters and the material being discussed, since tremendous work has gone into these presentations.

Formal introductions of new reviewers/guests in the audience were provided. Ms. Janet Hindler introduced two former UCLA postdoctoral fellows, Dr. Jennifer Dien Bard from Queens University in Ontario and Dr. Romney Humphries, Assistant Director at UCLA in Los Angeles. Dr. Barb Zimmer introduced two guests from Japan, Dr. Tateda from Toho University and Dr. Mikamo from Aichi University. Dr. Mel Weinstein introduced two colleagues from Robert Wood Johnson Medical School, Dr. Tom Kirn, Associate Director of the Microbiology Laboratory and Dr. Susan Boruchoff, an Infectious Disease physician and faculty member. Another guest at the meeting was Dr. Galas from Argentina.

Dr. Cockerill discussed the recent and upcoming changes to the subcommittee and working groups including Dr. Cynthia Fowler accepting the role of Chairholder of the Fluoroquinolone Working Group; Dr. Sandy Richter assuming the role as Recording Secretary for the Staphylococcal/Streptococcal Working Group; Dr. Patricia Bradford assuming the role as Recording Secretary for the *Enterobacteriaceae* Working Group; and in discussing succession planning with Ms. Jana Swenson, Ms. Maria Traczewski accepting the role of Recording Secretary for the Text and Tables Working Group and assumption of Chairholder when Ms. Swenson retires. Dr. Cockerill thanked each of these volunteers for their willingness to serve in these new roles. He then discussed as part of the CLSI new process changes including the change of Chairholder term limits from 6 years to 4 years, his term limit will end 12/2012. In searching for an incoming Vice Chairholder, Dr. Jean Patel has graciously accepted to serve in this role for next year and will rotate to Chairholder in 2013. Dr. Matt Wikler will be stepping down as Vice Chairholder and will be assuming a new role as he was recently elected to the CLSI Board of Directors.

In an attempt to improve the processes of the AST subcommittee and based on input from the survey conducted, a small task force has been put together to evaluate the current process used by the subcommittee and discuss ways to improve efficiencies. The task force members include Dr. Jeff Alder, Dr. George Eliopoulos, Dr. Ron Jones, Dr. Susan Sharp, Dr. John Turnidge, and Dr. Barb Zimmer, along with the management teams from the AST Subcommittee, Microbiology Consensus Committee and CLSI. This group held an inaugural meeting on Sunday to brainstorm ways to improve process including possible ways to have real-time dynamic processes where work will be on-going. This task force will continue discussions and brainstorming ideas and we will update the committee on their progress.

Dr. Cockerill then thanked Dr. Barb Zimmer, Mr. Bill Brasso and all those from STMA involved in putting together and presenting the Workshop on Antimicrobial Susceptibility Testing Devices that was held on Saturday. It was very informative and provided a better understanding of device manufacturers and the process for susceptibility test instrument and antibiotic approvals.

Dr. Wikler reminded everyone the purpose of these meeting as stated in the subcommittee's mission statement that is provided in electronic tab B of the meeting CD. He emphasized the mission statement which is to "to provide useful information to enable laboratories to assist the clinician in the selection of appropriate antimicrobial therapy for patient care". He also emphasized that the values that guide this subcommittee are quality, accuracy, fairness, timeliness, teamwork, consensus, and trust. He asked that everyone keep these principles in mind during the course of these meetings. He then reminded meeting participants that the proceedings were being audiotaped per standard procedure for meetings of this subcommittee; therefore, should there be any questions on topics discussed the tapes could be reviewed.

Mr. Glen Fine, Executive Vice President of CLSI highlighted recent changes to the CLSI Board of Directors including the election of Dr. Matt Wikler as mentioned earlier. Also new to the board is Dr. Uwe Scherf from the FDA Center for Devices (microbiology devices). He then introduced new CLSI staff present at the meeting, Ms. Luann Ochs, VP of Standards Development and Ms. Marcy Anderson, Director for Education.

Mr. Fine then acknowledged Ms. Janet Hindler who recently was awarded the CLSI John V Bergen award. This award is given annually to an outstanding volunteer in recognition of advances in CLSI organizational directives and objectives, through unique and significant contributions. He also thanked those volunteers who have given talks on behalf of CLSI including Dr. Mike Dudley who spoke in Brazil, Ms. Janet Hindler who spoke in China and Hong Kong, and Ms. Susan Munro who also spoke in Hong Kong. He also thanked Dr. Jean Patel for the recent teleconference she provided on Verification of AST Methods for Implementation of the Carbapenem and Cephalosporin Breakpoints. This was a jointly sponsored teleconference with CLSI and The Joint Commission. The teleconference will be offered again on 14 September.

II. APPROVAL OF THE 9-11 JANUARY 2011 MINUTES

The Subcommittee approved the minutes with the following addition:

Page 28 under the *Enterobacteriaceae* Working Group section – add dosing comment agreed upon for the new breakpoints for piperacillin (alone and with tazobactam) and ticarcillin (alone and with clavulanic acid). **Approved 12-0**.

III. UPDATES TO THE CURRENT AST DISCLOSURE SUMMARY

Dr. Cockerill asked the members and advisors for any updates to the current disclosure summary provided on the CD of meeting materials. Below are the updates provided:

Dr. Ambrose: Consulting agreement with AstraZeneca Pharmaceuticals and GlaxoSmithKline

Dr. Bradford: Now an employee of AstraZeneca

Dr. Ferraro: Replace Targanta with The Medicines Company

IV. DORIPENEM MIC AND DISK DIFFUSION BREAKPOINT PRESENTATION

Dr. Brown presented data in support of Doripenem MIC and disk diffusion interpretive criteria. The actions were approved as follows:

***Acinetobacter* spp.:**

Antimicrobial Agent	**10 μg Disk Zone Diameter (mm)**			**MIC Range (μg/mL)**			**Vote**
	S	**I**	**R**	**S**	**I**	**R**	**MIC – Approved 11 -0; 1 abstain. Disk - Approved 12-0** **Dosing comment: Interpretive criteria are based on a dosage regimen of 500 mg every 8 h. Approved 9-3** The sponsor requested that the new interpretive criteria for Doripenem/*Acinetobacter* spp. not be published in M100 until the other carbapenems breakpoints are reassessed.
Doripenem	≥18	15-17	≤14	≤1	2	≥4	

The *Enterobacteriaceae* Working Group is charged with reviewing and reassessing the other carbapenem breakpoints for *Acinetobacter*.

In determining "I" for the MIC interpretive criteria for *Acinetobacter* spp. the following rationale points were made:

- Historically, *Acinetobacter* MIC breakpoints have generally been the same as *Enterobacteriaceae*
- It is logical to build in a buffer zone to account for testing variation that occurs but generally this is only one MIC dilution
- The exceptions for a wider "I" range eg, have to be well rationalized (it was acknowledged that EUCAST has an intermediate range of 2-4 and this would be different now in the CLSI tables)
- The CLSI decision was based on the 1 hour infusion and not the 4 hour infusion as the 4 hour infusion is not in the US FDA label

- There were no clinical data at MIC = 2 presented for review (except for one complicated UTI) that would fit the subcommittee's definition of "I" where a higher than normal dosage of drug can be used.
- The subcommittee did not see any data on the MICs that would result with carbapenemases in *Acinetobacter*. It is suspected that they could be as low as MIC = 4 with certain carbapenemases.
- The "S" breakpoint selected (MIC = ≤1) covers all doses and mode of administration.
- The subcommittee did not review any data that would allow to conclude that the "I" range should include the different dosage regiments.
- The target attainment rates for *Acinetobacter* are more like *Enterobacteriaceae* but this specific data was not presented.
- No animal model data was presented.

Staphylococcus spp.:

Antimicrobial Agent	**10 μg Disk Zone Diameter (mm)**			**MIC Range (μg/mL)**			**Vote**
	S	**I**	**R**	**S**	**I**	**R**	**Disk and MIC Approved 8-3; 1 abstain**
Doripenem	≥30	–	–	≤0.5	–	–	Add in Table 2C with other carbapenems as Test/report group O. Add the following footnote and also refer user to the "S" only comment (7): (X) Interpretive criteria for methicillin-susceptible staphylococci only. No dosing comment is to be added.

The Staphylococcal Working Group was charged with reviewing breakpoints for other carbapenems and cephalosporins against staphylococci.

***Streptococcus pneumoniae*:**

Antimicrobial Agent	MIC Range (μg/mL)			Vote
	S	**I**	**R**	**MIC – Approved 11 -0; 1 abstain.**
Doripenem	≤1	–	–	Add in Table 2G as Test/report group O. Add the following footnote and also refer user to the "S" only comment (4):

***Streptococcus* spp. Viridans Group:**

Antimicrobial Agent	MIC Range (μg/mL)			Vote
	S	**I**	**R**	**MIC only – Approved 7 -4; 1 abstain.**
Doripenem	≤1	–	–	Add in Table 2H-2 as Test/report group O. Add the following footnote and also refer user to the "S" only comment (4):

***Streptococcus* spp. β-hemolytic Group:**

Antimicrobial Agent	MIC Range (μg/mL)			Vote
	S	**I**	**R**	**MIC only – Approved 10 -0; 2 absent.**
Doripenem	≤0.12	–	–	Add in Table 2H-1 as Test/report group O. Add the following footnote and also refer user to the "S" only comment (5):

Anaerobes:

Antimicrobial Agent	MIC Range (μg/mL)			Vote
	S	**I**	**R**	**Approved 9-2; 1 abstain**
Doripenem	≤2	4	≥8	List in Test/report Group A with other carbapenems

***Haemophilus* spp.**

Antimicrobial Agent	10 μg Disk Zone Diameter (mm)			MIC Range (μg/mL)			Vote
	S	**I**	**R**	**S**	**I**	**R**	**Approved 9-2; 1 abstain**
Doripenem	≥16	–	–	≤1	–	–	List in Test/report Group O Refer user to "S" only comment (7)

Refer to page 34 of the minutes for the approved MIC and Disk Diffusion interpretive criteria for Doripenem as well as the other carbapenems for *Pseudomonas aeruginosa*.

V. REPORT OF THE TEXT AND TABLES WORKING GROUP

***Minutes Submitted by Jana Swenson and Maria Traczewski* (Electronic Tab D in the Meeting Agenda)**

Chairholder – Jana Swenson

Recording Secretary – Maria Traczewski

Working Group Members present – David Farrell, Janet Hindler, Judy Johnston, Dyan Luper, Linda Mann, Susan Munro, Jeffrey Schapiro, Dale Schwab, Tom Thomson, and Mary York

Working Group Members absent – Fred Marsik, Flavia Rossi, Al Sheldon, Mel Weinstein

Major Changes of M02/M07:

1. Modification of β-lactamase section for staphylococci to include use of zone edge or cloverleaf.

2. Major revision of Gram-negative Bacilli section for discussion of ESBLs, AmpCs, and carbapenemases.

Following revision of the M02/M07, the documents were circulated to the subcommittee for review and comment prior to the June meeting.

Subcommittee input from review of M02/M07:

1. Include Nitroimidazole class as separate section in AA section (M02 and M07 sections 6) because no longer single agent.

   "6.2.2.9 Nitroimidazoles

Nitroimidazoles, including metronidazole and tinidazole, are bactericidal agents that are converted intracellularly in susceptible organisms to metabolites that disrupt the host DNA; they are only active against strictly anaerobic bacteria."

Working Group: Agreed to suggestion above. **Subcommittee agreed for change to be made in the appropriate sections of M02 and M07.**

In addition, it was discovered that new classes now appear in the glossary (eg, thiazolide [2 agents] and glycolipodepsipeptide [1 agent]) that are not included in this section. Should we add these to section 6.2.2?

Subcommittee suggested waiting to add classes for new anaerobes.

2. In M02 Section 11.1.1 and M07 Section 12.1.1, insert sentence (2nd sentence in new paragraph):
"Some β-lactamase producing staphylococcal isolates test susceptible to penicillin. Because staphylococcal β-lactamase is readily inducible, there is a risk of this occurring if penicillin were used to treat such strains. For this reason, it is recommended…"

The subcommittee agreed to the suggested edits.

3. Omit KPC and NDM from title for section 11.3.4 in M02 and 12.3.4 in M07 as shown below:

~~KPC- and NDM-type~~ Carbapenemases (Carbapenem-resistant Enterobacteriaceae)

The subcommittee agreed to the suggested edits.

4. Revise footnote to table in section 11.3.4 in M02 and section 12.3.4 in M07 as follows:

"Carbapenemases ~~are~~ have not yet been found in Class C"

The subcommittee agreed to the suggested edits.

5. Revise section 6.3 in both M02 and M07, paragraph 2, sentence 3 as follows:

"This means combined major and very major errors are fewer than 3% and minor errors are fewer than 10%, based on a large ~~population of bacteria~~ collection of random clinical isolates tested."

The subcommittee agreed to the suggested edits.

M100:

1. Suggested to eliminate repeated information in M100 in the following tables:

   • Introduction I A = Table 1A NOTE 1 = Table 1B NOTE 1
   "Selection of the most appropriate antimicrobial agents to test and to report is a decision . . ."

   • Introduction I B = Table 1A NOTE 2 = Table 1B NOTE 2
   "The listing of drugs together in a single box designates clusters of agents . . . ."

   • Neither appear in Table 1C

   • Introduction C 2 = Table 1A fn d = Table 1B fn b
   "Group B comprises agents that may warrant primary testing. However, they . . . ."

   • Introduction C 3 = Table 1A fn e = Table 1B fn c
   "Group C comprises alternative or supplemental antimicrobial agents that may require …"

   • Neither appear in Table 1C

   Actions to suggestions above:

   • Delete duplicate information in Tables 1 and 2
   • Create new NOTE 1 in all Table 1s that refers to the information in the Introduction as follows:
   "NOTE 1: For information about the selection of appropriate antimicrobial agents; explanation of Test and Report Groups A, B, C, and U; and explanation of the listing of agents within boxes, including the meaning of "or" between agents, refer to the Introduction to Tables 1 and 2 that precede Table 1A."
   • Revise title of Introduction to: "Instructions for Use of Tables 1 and 2"

   The subcommittee agreed with the all the suggested edits.

2. Why is term "Interpretive Standard" used for Table 2 MIC column headings and "Breakpoint" used for Disk diffusion column headings?

   Working Group edits: all headings changed in M100 to say Interpretive Criteria
   The subcommittee agreed with the suggested edits.
3. Breakpoints = Interpretive Criteria? Should we define or state that they are equivalent?
   Working Group proposal: add the definition of breakpoint/interpretive criteria used in CLSI document M39 to M02/M07 Definitions section and to the M100 Introduction II as follows:

   breakpoint criteria/interpretive criteria – minimal inhibitory concentration (MIC) or zone diameter value used to indicate susceptible, intermediate, and resistant as defined above.

   For example, for antimicrobial X with interpretive criteria of:

	MIC (μg/mL)	Zone Diameter (mm)
Susceptible	≤4	≥20
Intermediate	8-16	15-19
Resistant	≥32	≤14

"Susceptible breakpoint" is 4 μg/mL or 20 mm.
"Resistant breakpoint" is 32 μg/mL or 14 mm.

The subcommittee agreed with this addition.

4. Delete footnote a in Table 2A-S1 (ESBL screening and confirmatory tests): a. Screening of *Proteus mirabilis* for ESBL production is recommended only when it is deemed clinically relevant (eg, a bacteremic isolate).

Justification - ESBL test now mainly used for epidemiological purposes

The subcommittee agreed with this change.

5. Table 2E suggestions:

- Consider the deletion of *H. parainfluenzae* from Table 2E because it does not require HTM for growth
- Include *H. parainfluenzae*, and *H. parahaemolyticus* with *A. aphrophilus* in M45.
- Consider adding *H. haemolyticus* to Table 2E because it requires X factor

The working group and subcommittee agreed to refer these suggestions to the M45 working group, but for now change column heading in Table 1B to "*H. influenzae* and *H. parainfluenzae*"

6. Change wording in Table 2E comment (3) as follows:

(3) For isolates of *H. influenzae* from CSF, only results of testing with ampicillin, one of the third-generation cephalosporins, chloramphenicol, and meropenem ~~should be reported~~ are appropriate to report routinely.

Justification: no reported resistance to third-generation cephalosporins or meropenem and chloramphenicol is rarely used.

The subcommittee agreed with this change.

7. Suggest revising Table 2E comment (11):

(11) ***Rx*:** Rifampin should not be used alone for antimicrobial therapy.

Two possible suggestions:
1. (11) Used for prophylaxis in post-meningitis exposure, not treatment.

2. (11) May be appropriate only for prophylaxis of meningitis case contacts. These interpretive criteria do not apply to therapy of patients with invasive disease. (from meningitis table)

Working Group Suggestion: Revise comment to read: "May be appropriate only for prophylaxis of case contacts. These interpretive criteria do not apply to therapy of patients with invasive *H. influenzae* disease" **The subcommittee agreed with this suggested change.**

8. In Table 2G, comment (5) add "penicillin (oral or parenteral)" to list of agents for which penicillin can predict susceptibility as follows:

(5) <u>For nonmeningitis isolates,</u> the penicillin MIC can predict susceptibility to other β-lactams as follows:
Penicillin MICs ≤ 0.06 μg/mL (or oxacillin zones ≥ 20 mm) indicate susceptibility to ampicillin (oral or parenteral), ampicillin-sulbactam, cefaclor, cefdinir, cefditoren, cefpodoxime, cefprozil, ceftizoxime, cefuroxime, imipenem, loracarbef, ~~and~~ meropenem, <u>and penicillin (oral or parenteral.</u>

The subcommittee agreed with this suggestion.

9. Tables 2H-1 and 2H-2:

- Suggest adding new general comment to Table 2H-1 and 2H-2 with reference included for each agent in body of Table:

"( ) Reliable disk diffusion susceptibility tests do not yet exist for penicillin, ampicillin, cefepime, cefotaxime, ceftriaxone, ertapenem, meropenem, and daptomycin. Their in vitro activity is best determined using an MIC method."

To address suggestion possibly:

- Create a generic explanation to explain lack of disk diffusion breakpoints in Introduction and remove existing comments from all Tables 2 where no disk breakpoints exist or are not reliable.
- In the same vein, remove the comment explaining the Susceptible Only designation and all references to it from all Tables 2 and move to the Introduction

Working Group Proposal: Wait to make these changes until they can be really reviewed and then suggestions for changes proposed. Possibly create a new working group to review this.

No change at this time.

10. In Appendix A, problem exists for information given for *Salmonella* spp. in Table versus what is given in footnote d:

		Category I	Category II	Category III
Salmonella spp.[d]	**Cephalosporin III and/or fluoroquinolone – R**		**x**	

[d] When submitting the report to a public health department, include antimicrobial susceptibility results for *Salmonella* spp. that are intermediate or resistant to 3rd-generation cephalosporins (cephalosporin III) and/or intermediate or resistant to fluoroquinolone or resistant to nalidixic acid.

Proposed Change:

- Appendix A: make *Salmonella* row apply to both *Salmonella/Shigella* with separate rows for Ceph III and FQ:
- Retain footnote d

		Category I	Category II	Category III
***Salmonella* and *Shigella* spp.[d]**	Cephalosporin III – I or R		x	
	Fluoroquinolone – I or R		**x**	

The subcommittee agreed with this suggestion.

11. Information in M02 11.1.2.4 bullet 4 is not included in M100—suggest adding this information as comment in Table 2C.

11.1.2.4 Oxacillin-Based Methods

If oxacillin-intermediate results (disk diffusion testing) are obtained for *S. aureus*, perform testing for *mec*A or PBP 2a, the cefoxitin MIC or cefoxitin disk test, an oxacillin MIC test, or the oxacillin-salt agar screening test. Report the result of the alternative test rather than the oxacillin intermediate result (see below for reporting oxacillin when using cefoxitin as a surrogate test).

Working Group proposal: insert in Table 2C as new comment opposite oxacillin disk breakpoints.

The subcommittee agreed with this suggestion.

12. Suggestion that we really think about explaining old (will be 2 years old in 2012) vs. revised breakpoints in the beginning of each document…maybe in a "black box". When teaching M2, M7, M100 it is tough when this is not clearly defined.

- New wording proposed in Commercial vs Reference method box in M02, M07 and M100 and new table with dates to be added in M100 as follows (new text underlined):

CLSI Reference Methods vs Commercial Methods and CLSI vs FDA Interpretive Criteria (Breakpoints)

It is important for users of M02-A10, M07-A8, 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 AST 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 databases, differences in interpretation of data, differences in doses used in different parts of the world, and public health policies. Differences also exist because CLSI proactively evaluates the need for changing breakpoints. The reasons why breakpoints may change and the manner in which CLSI evaluates data and determines breakpoints are outlined in CLSI document M23—*Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*.

Following a decision by CLSI to change an existing breakpoint, regulatory authorities may also review data in order to determine how changing breakpoints may affect the safety and effectiveness of the antimicrobial agent for the approved indications. If the regulatory authority changes breakpoints, commercial device manufacturers may have to conduct a clinical laboratory trial, submit the data to the regulatory authority, and await review and approval. For these reasons, a delay of one or more years may be required if an interpretive breakpoint change is to be implemented by a device manufacturer. In the United States, laboratories that use Food and Drug Administration (FDA)–approved susceptibility testing devices are allowed to use existing FDA interpretive breakpoints. Either FDA or CLSI susceptibility interpretive breakpoints are acceptable to clinical laboratory accrediting bodies. Policies in other countries may vary. **Laboratories should check with the manufacturers of their antimicrobial susceptibility test system for additional information on the breakpoints used in their system's software.**

Following discussions with appropriate stakeholders, such as infectious disease practitioners and the pharmacy department, as well as the Pharmacy and Therapeutics and Infection Control committees of the medical staff, newly approved or revised breakpoints may be implemented by clinical laboratories. CLSI disk diffusion test breakpoints may be implemented as soon as they are published in M100. If a device includes antimicrobial test concentrations sufficient to allow interpretation of susceptibility and resistance to an agent using the CLSI breakpoints, a laboratory could, after appropriate validation, choose to interpret and report results using CLSI breakpoints.

CLSI Breakpoint Additions / Revisions Since 2010

Antimicrobial Agent	Date of Revision*	Comments

	(M100 version)	
Enterobacteriaceae		
Aztreonam	January 2010 (M100-S20)	
Cefazolin	January 2010 (M100-S20) January 2011 (M100-S21)	Breakpoints were revised twice since 2010
Cefotaxime	January 2010 (M100-S20)	
Ceftazidime	January 2010 (M100-S20)	
Ceftizoxime	January 2010 (M100-S20)	
Ceftriaxone	January 2010 (M100-S20)	
Doripenem	June 2010 (M100-S20U)	No previous CLSI breakpoints for doripenem
Ertapenem	June 2010 (M100-S20U) **January 2012 (M100-S22)**	**Breakpoints were revised twice since 2010.**
Imipenem	June 2010 (M100-S20U)	
Meropenem	June 2010 (M100-S20U)	
Ciprofloxacin – Salmonella only	January 2012 (M100-S22)	
Pseudomonas aeruginosa		
Piperacillin-tazobactam	January 2012 (M100-S22)	
Ticarcillin-clavulanate	January 2012 (M100-S22)	
Ticarcillin	January 2012 (M100-S22)	
Piperacillin	January 2012 (M100-S22)	

*previous breakpoints can be found in M100 version that precedes document listed here; example old breakpoints for aztreonam are listed in M100-S19 (January 2009)

The subcommittee agreed with this suggestion.

13. Q&A for inclusion in M02 and M100

1. Many years ago, weren't there guidelines for determining whether or not a Kirby Bauer plate had enough inoculum before measuring the zone sizes? I seem to remember using a plastic template for determining the zone sizes on a 12-disk plate, after determining that the density of growth on the plate was adequate, eg, barely able to read the print on the template through the plate. This has become a competency issue and I am looking for a reference. It is no longer adequate to just ensure the density of the inoculum suspension and length of incubation. Swabs are now a problem. Some are not very absorbent and seem to vary from brand to brand. When one presses the swab against the side of the tube to remove excess, based on the appearance of the plate after incubation, insufficient inoculum was delivered.

- Cotton swabs are specified for use in M02; polyester-tipped swabs are not recommended. No guidelines for adequacy of inoculum have ever been published except to state that the lawn should be confluent. If individual colonies are apparent, the inoculum was too light.

The subcommittee agreed with the suggested response.

14. Revise Table 2F comment (8) for penicillin to read:

(8) Gonococci with 10-unit penicillin disk zone diameters of ≤ 19 mm are likely to be β-lactamase–producing strains. However, the β-lactamase test remains preferable to other susceptibility methods for rapid, accurate recognition of this plasmid-mediated form of penicillin resistance.

Working Group proposal: review all Table 2F comments for accuracy and relevance in 2012.

Also to be addressed in 2012:

- Fix the inconsistencies for use of oxacillin disk diffusion for *S. pneumoniae* in Table 1B footnote k, Table 2G comment (2), and Table 2G comments (5) and (6).

  Create a temporary sub-Working Group of T&T group to clean up Table 2G and 1B oxacillin disk screen comments.

15. Suggestion to review selective reporting for certain species within an organism group.

    At January 2011 meeting, the subcommittee recommended that rules be established to guide when selective reporting should be used so that they can be applied consistently. It was suggested that a working group should be formed to consider this.

    Working Group proposal: create new Working Group (Qualified Reporting Working Group) to develop a strategy for when this is done in the future and use it clean up M100.

    The subcommittee approved all changes from the Text and Tables Working Group as noted (**Approved 11-0; 1 absent**)

VI. REPORT OF THE FLUOROQUINOLONE BREAKPOINT WORKING GROUP

Minutes Submitted by Cynthia Fowler **(Electronic Tab E in the Meeting Agenda)**

Chairholder – Cynthia Fowler
Recording Secretary (ad hoc) Barbara Zimmer

Working Group Members present - Jeff Alder (new), Sujata Bhavnani, George Eliopoulos, Robert Flamm, Mair Powell, Barth Reller, Helio Sader, Mel Weinstein (new)

Working Group Members absent – Karen Bush

Note: Karen Carroll has resigned. Two new members have been recruited (Jeff Alder and Mel Weinstein). The working group needs a recording secretary. Barb Zimmer volunteered for this meeting only.

Items Proposed for Vote

1. **Breakpoints for levofloxacin against extraintestinal *Salmonella* sp.**

   Rationale:

   Background: At the January 2011 meeting the AST Subcommittee approved changes to the ciprofloxacin breakpoints for extraintestinal *Salmonella* sp. (≤0.06/0.12-0.5/≥1), with the intention that the MIC and disk diffusion breakpoints for other fluoroquinolones used to treat *Salmonella* infections, including levofloxacin, ofloxacin, and gatifloxacin would be assessed prior to publishing the revised ciprofloxacin breakpoints.

   The recommended changes to the levofloxacin break points are consistent with the process used to develop the ciprofloxacin break points including review of available PK/PD analyses, MIC distribution data, and clinical reports. The PK/PD analysis demonstrates that the MIC breakpoints associated with achieving ≥ 90% probability of PK-PD target attainment for a 500 mg daily dose and a 750 mg daily dose of levofloxacin are 0.25 and 0.5 µg/mL respectively (Bhavnani – Tab E Agenda Book June 2011). The data presented on MIC distributions was obtained from the SENTRY data (Sader Tab C Agenda book January 2011) and from EUCAST wild type distributions (Kahlmeter and www.EUCAST.org) The clinical data on levofloxacin is limited, but there are recent published reports using the racemic mixture (ofloxacin) with good results for strains with MICs to ofloxacin of ≤0.125 µg/mL (Parry et al in press see Agenda Book June 2011 Tab E). The intermediate breakpoint allows for the use of fluoroquinolones based on probability of target attainment and the available clinical data. This susceptibility breakpoint would be in harmony with the EUCAST breakpoints, and would separate bacterial populations into those without resistance mechanisms from those with some form of resistance.

   a. **MIC breakpoints for levofloxacin against *Salmonella* sp. were proposed as:**

   MIC: S = ≤0. 125 µg/mL
   I = 0.25 – 1 µg/mL
   R = ≥ 2 µg/mL

   These were <u>not approved</u> by the subcommittee (2-8; 1 abstain, 1 absent).

   b. **Disk breakpoints for levofloxacin against *Salmonella* sp.**

Rationale:

These disk diffusion breakpoints were recommended based on scattergrams from JMI as presented by H. Sader. No major or very major errors were observed. The minor error rate was 6.7%.

Proposed:

S = ≥ 29 mm
I = 19-28 mm
R = ≤ 18 mm

These were <u>not approved</u> by the subcommittee (2-8; 1 abstain, 1 absent).

B. Reinstate the comment in Table 2A (previous comment 31 in S21) recommending use of nalidixic acid test to predict fluoroquinolone utility in treating extraintestinal infections caused by *Salmonella* sp.

The comment will read as follows (bolded text new):

In addition to testing urine isolates, nalidixic acid may be used to test for reduced fluoroquinolone susceptibility in isolates from patients with extraintestinal *Salmonella* infections. **Strains of *Salmonella* that test resistant to nalidixic acid may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with extraintestinal salmonellosis.**

However, nalidixic acid may not detect all mechanisms of fluoroquinolone resistance. Therefore, *Salmonella* strains may also be tested with ciprofloxacin and reported using the *Salmonella* sp. interpretive criteria above. Approved (8-3; 1 absent).

Rationale:

Background: At the January 2011 meeting the AST Subcommittee approved removing these comments.

The decision to remove may have been premature as the test may well have utility in some settings in some geographic area. There were representatives from Latin America who claim it is useful to them. They also pointed out that the NA disk test is easy to read whereas the fluoroquinolone disk diffusion tests are more difficult to read. There was discussion about how the NA test should be used if it is reinstated and that perhaps the comment should be altered.

<u>Items for Discussion and Input</u>

1. Could the NA disk test be "tweaked" to provide better information?
2. Are there issues with the FQ disk tests?
3. Would some combination of NA/FQ disk testing be useful?
4. Is it necessary to establish *Salmonella* breakpoints for other FQs?

<u>Items for Information Only</u>

The following will be investigated and discussed in upcoming sessions

- FQ MIC and Disk Diffusion breakpoints for *Enterobacteriaceae*, including *Salmonella*
- Reevaluation of groupings quinolones and fluoroquinolones in Table 1
- Value/utility of NA test for *Salmonella*, if retained, most appropriate comment

It was agreed that the working group would meet several times via teleconference in order to better prepare for the January 2012 meeting.

VII. REPORT OF THE TOPICAL AGENTS WORKING GROUP

Minutes Submitted by Mair Powell **(Electronic Tab F in the Meeting Agenda)**

Chairholder – Mair Powell

Recording Secretary – Fred Marsik

Working Group Members present - Jeff Alder, Farah Babakhani, Ian Morrissey, Harriet Nadler, Jeffrey Shapiro, Lauri Thrupp

Working Group Members absent **–** Robert Rennie

This fourth meeting aimed to:

- Discuss the possible relevance of biofilm formation to various types of infections that are often or routinely treated using a topical route of administration and
- Reach a decision on the future of the Working Group.

The additional agenda item mentioned in the meeting request letter had to be cancelled due to lack of availability of the presenter.

A presentation was given by Dr. W. Costerton who is Director of Microbial Research in the Department of Orthopaedics, Allegheny General Hospital, and also Director of Biofilm Research at the Center for Genomic Sciences, Allegheny-Singer Research Institute.

The presentation considered the changes in gene expression and resulting effects on bacterial metabolic status when organisms form biofilms. Biofilms release planktonic cells at variable rates. When released in sufficient numbers, planktonic cells can usually be cultured from suitable specimens without difficulty using routine laboratory methods. Their detection may be enhanced by using certain culture methods. Susceptibility testing can assist in predicting the effect of systemic antibacterial therapy on planktonic cells but does not predict the effect on the biofilms from whence they came. Hence in due course, and with variable time intervals, planktonic cells are released again in sufficient numbers that they can be picked up on culture of suitable specimens. Depending on the site and content of the biofilm, intermittent releases of considerable numbers of planktonic cells may be associated with re-appearance of clinical signs and symptoms and cumulative local tissue damage.

Biofilms rarely disperse spontaneously. It is sometimes possible to disrupt and clear biofilms by achieving very high local concentrations of an antibacterial agent.

There are novel methods available for detecting the presence of biofilms even when no planktonic cells are cultured. A method was described that can detect bacterial nucleic acid and genes encoding specific resistance mechanisms. However, it is not possible to ascribe the resistance gene to a particular species if there is more than one present and detecting a gene encoding a resistance mechanism does not necessarily mean that there is ongoing expression of that gene.

The working group discussed that the presence of biofilms is very pertinent but is not confined to the treatment of bacterial infections by a topical route of administration. The fact that susceptibility testing does not predict effects of antibacterial agents on underlying biofilms adds to the complexities of attempting to determine whether or not treatment by a topical route would be successful. At present the field is also hampered by the lack of standardized methodologies for assessing drug penetration into biofilms and antibacterial activity within biofilms.

The working group then revisited the proposals made in January 2011 for proceeding with the assessment of the potential for setting breakpoints for any mode of topical application of antibacterial agents. It was reiterated that the investigations and experience of WG members thus far has not identified any type of topical application of antibacterial agents for which there are sufficient and reliable data available to set either PK/PD or clinical breakpoints. The MIC distributions can be documented but with no evidence of a strong relationship between in-vitro susceptibility testing and clinical outcome it is only possible to identify epidemiological cut-off values and, in some cases, the limited solubility of test agents limits even this approach.

The working group voted unanimously (8 present; one absent) to cease regular meetings. However, it was agreed that the working group should become a "virtual" group charged with monitoring any important scientific developments that might trigger further meetings on an *ad hoc* basis.

The working group discussed that the Working Group Chair's report to the AST subcommittee should summarize the deliberations of the four meetings and propose a vote to endorse the discontinuation of regular meetings without disbanding the group.

The subcommittee agreed to not disband this working group but agreed that they would only convene as necessary. Also it was agreed that the working group would prepare a summary of the findings and challenges of the working group that could potentially be published for the medical community. **Approved 10-0; 2 absent.**

VIII. REPORT OF THE STAPHYLOCOCCAL AND STREPTOCOCCAL WORKING GROUP - *Minutes Submitted by Sandy Richter* (Electronic Tab G in the Meeting Agenda)

Chairholder - Jean Patel

Recording Secretary – Sandy Richter

Working Group Members present - Bill Craig, Mel Weinstein, Jana Swenson, Patricia Bradford, Maria Traczewski, George Eliopoulos, Susan Sharp

Working Group Members absent - Mike Dudley, Dan Sahm

Presenters: Robert Skov, Jim Jorgensen

Items Proposed for Vote

A. β-Lactamase Detection in Staphylococci: Replace the recommendation to use a nitrocefin-based test for ß-lactamase detection with a recommendation to evaluate the zone edge of a penicillin disk diffusion test.

Background: MSSA isolates that test susceptible to penicillin may still possess a β-lactamase that could lead to therapeutic failure if penicillin is used (Clin Microbiol Infect 2008; 14:614-616). Current CLSI recommendations are to perform an induced nitrocefin-based test to detect β-lactamase production in isolates with a "susceptible" penicillin MIC (≤0.12 µg/mL).

Presentation: Robert Skov presented data to the working group that was also presented at the January meeting showing two alternative tests for ß-lactamase detection (cloverleaf test and zone edge penicillin disk test) are more sensitive than the nitrocefin-based test currently recommended in M100. Four ß-lactamase test methods (cefinase, Dryslide, cloverleaf, zone edge of penicillin disk diffusion) were compared to PCR for *bla*Z. A total of 348 isolates were tested and of these 303 isolates were negative for a functional *bla*Z (i.e., 300 isolates were *bla*Z negative by PCR and 3 isolates were *bla*Z PCR positive but significant mutations were identified in the sequence); 45 isolates were PCR positive for *bla*Z and expression of *bla*Z could be detected by at least one phenotypic test. The performance characteristics of the ß-lactamase methods in comparison to *blaZ* PCR were:

Test	Sensitivity	Specificity
Cefinase	77%	100%
Dryslide	88%	100%
Cloverleaf test	100%	100%
Zone edge of penicillin disk (sharp = positive; fuzzy = neg)*	96%	100%

*A fuzzy zone edge ("beach", shown below) indicates no β-lactamase production; a sharp zone edge ("cliff") indicates β-lactamase production (Gill, J Clin Microbiol 1981; 14:437-440).

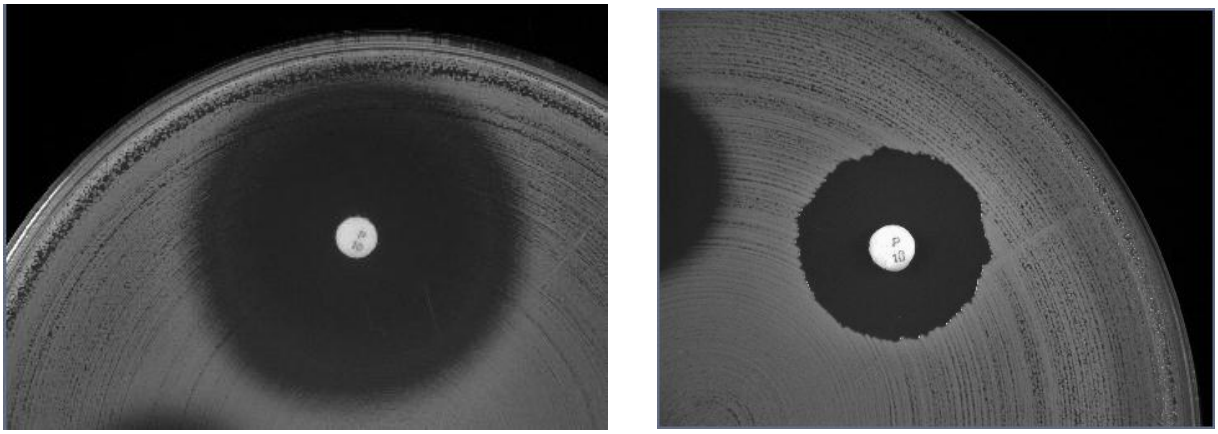


Fig 1. Negative (fuzzy) zone edge ß-lactamase test on the left and a positive (sharp) zone edge β-lactamase test on the right.

Although the cloverleaf test demonstrated the best performance, the test was hard to read and did not have reproducible results between three labs. The zone edge test had good reproducibility between the three labs using a 10U penicillin disk. A comparison of MICs with *blaZ* PCR results is below:

MIC	blaZ functional	
(mg/L)	Negative	Positive
0.008	2	
0.016	15	
0.032	180	1
0.064	90	5
0.125	15	17
0.25	1	14
0.5		4
2		2
4		1
Total	303	45

Working group discussion: Because most of the isolates (53%) with penicillin MICs of 0.125 µg/ml were *blaZ* positive, many attending the working group suggested lowering the penicillin breakpoint may be the optimal solution. However, it was clear that the steps required to change the breakpoint change would not allow this to be done at the current meeting. As an alternative, the working group passed a motion to replace the nitrocefin-based test in table 2C-S4 and 2C-S5 with the penicillin zone edge test.

Subcommittee discussion and vote: There was support for possibly lowering the penicillin breakpoints in the future to address the problem of false negative ß-lactamase test results. There was concern expressed regarding the lack of data for the performance of the zone edge test with *S. lugdunensis* and other coagulase negative staphylococci. Because labs were already using the

nitrocefin-based test, the committee voted to add the penicillin zone edge test to Table 2C-S4 for *S. aureus* only (**Approved 10-0: 2 absent**). Data regarding the performance of the penicillin zone edge test for ß-lactamase detection in *S. lugdunensis* and other coagulase negative staphylococci will be generated by the CDC. Table 2C edits were circulated to the working group and then the subcommittee for approval after the June meeting (see appendix A – to be finalized after circulation and review of SC).

B. Inducible Clindamycin Resistance in Streptococci: Modifications to the supplemental table describing when to test and how to report inducible clindamycin-resistance in beta-hemolytic streptococci.

Background: At the January 2011 meeting Jim Jorgensen brought data demonstrating the accuracy of a D-zone and broth-based test to detect inducible clindamycin resistance in *S. pneumoniae*. The subcommittee voted to include the inducible clindamycin tests in M100 but deferred a decision on how results should be reported until the June 2011 meeting. It was also decided that the reporting recommendations for beta-hemolytic streptococci be revisited in June as well (Approved 8-0; 1 oppose [J. Turnidge]; 3 absent).

Presentation: Jim Jorgensen provided wording for reporting a positive inducible clindamycin test (beta-hemolytic streptococci and pneumococci) for the working group to consider that was included in the agenda book (Attachment 1).

Working group discussion: There was extensive discussion regarding the clinical significance of inducible clindamycin resistance in streptococci. Although many expressed doubts of significance for colonizing isolates of group B streptococci (GBS) because of the short term therapy, CDC guidelines instruct laboratories to test isolates from β-lactam allergic patients for inducible clindamycin resistance. Situations where knowing whether an isolate was inducible clindamycin resistant could be important include β-lactam allergic patients with necrotizing fasciitis who might be treated with clindamycin alone or in patients where clindamycin is being considered for follow up oral therapy of an invasive infection. Bill Craig has beta-hemolytic streptococci and pneumococcal strains that he is planning to test in animal models to generate data for presentation at the next meeting. The working group decided to delay adding the inducible clindamycin resistance test for pneumococci to M100 until animal or other data supporting clinical significance becomes available. There was concern that the reporting of beta-hemolytic streptococci as "resistant" despite limited clinical evidence that inducible clindamycin resistance is clinically important could take away a potentially effective agent. To more clearly communicate this uncertain significance to clinicians the working group passed a motion to change reporting guidelines for beta-hemolytic streptococci in Table 2H (other than colonizing strains of GBS) from "report as resistant" to "report as inducible clindamycin resistant" and to let individual laboratories determine how to enter this nonstandard interpretive result in the electronic patient record. Colonizing strains of GBS would continue to be reported as "resistant" in order to avoid a conflict with CDC guidelines. A footnote was also moved to the top of Table 2H and instructions to "test all invasive isolates" was softened to say laboratories "may choose to test invasive isolates" to allow decisions for testing to be made at the institution level.

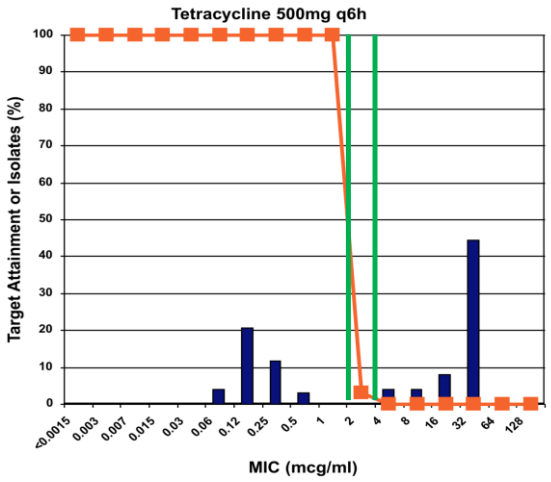
After presentation of the proposal to the Subcommittee, a motion by George Eliopoulos to accept the working group recommendation was voted on, but not passed. Concerns expressed included difficulty of LIS reporting for "inducible clindamycin resistant" and not wanting to make any change in reporting until animal data was available. A motion by Barb Zimmer to accept the working group recommendation regarding moving and editing the footnote with addition of the MMWR citation passed (**Approved 8-0; 4 absent**). Table 2H edits were circulated to the working group and then the subcommittee for approval after the June meeting (see appendix B). Bill Craig will present animal data when available.

Items for Discussion and Input

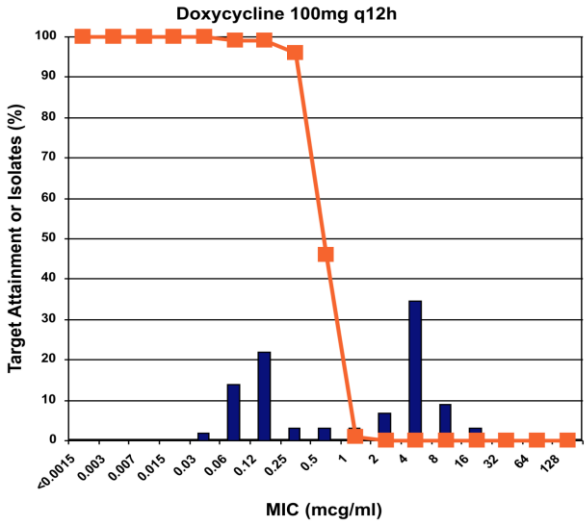
A. Doxycycline and Tetracycline Breakpoints for *Streptococcus pneumoniae:*

Background: Currently there are no doxycycline breakpoints for *S. pneumoniae*, yet this drug is recommended for treatment of community acquired pneumonia caused by *S. pneumoniae*. Instead tetracycline susceptibility results are used to predict doxycycline susceptibility. Using tetracycline susceptibility to predict doxycycline susceptibility could result in an inaccurate estimation of resistance, especially if mechanisms of resistance other than *tetM* were to emerge in *S. pneumoniae*.

At the January 2011 meeting Jim Jorgensen presented reference broth microdilution (BMD) and disk diffusion (DD) data generated at UTHSC for 101 *S. pneumoniae* isolates selected from 2009-10 CDC ABC surveillance. Dr. Jorgensen also presented limited PK/PD data available from the literature (Burgess, et al. CMI, 2006). A Monte Carlo simulation using drug levels collected from 6 male subjects were used with a PK/PD target of AUC/MIC ≥ 25.



Burgess, et al, 2006



Burgess, et al, 2006

At the January meeting the working group and subcommittee were generally in favor of setting doxycycline breakpoints and re-evaluating tetracycline interpretive criteria. Additional antimicrobial susceptibility data was requested from another laboratory using a different lot of media. There was also a request for more PK/PD data.

Presentation: Dr. Jorgensen presented data that included 78 additional isolates tested at CDC using Hardy media. The following new breakpoints were proposed:

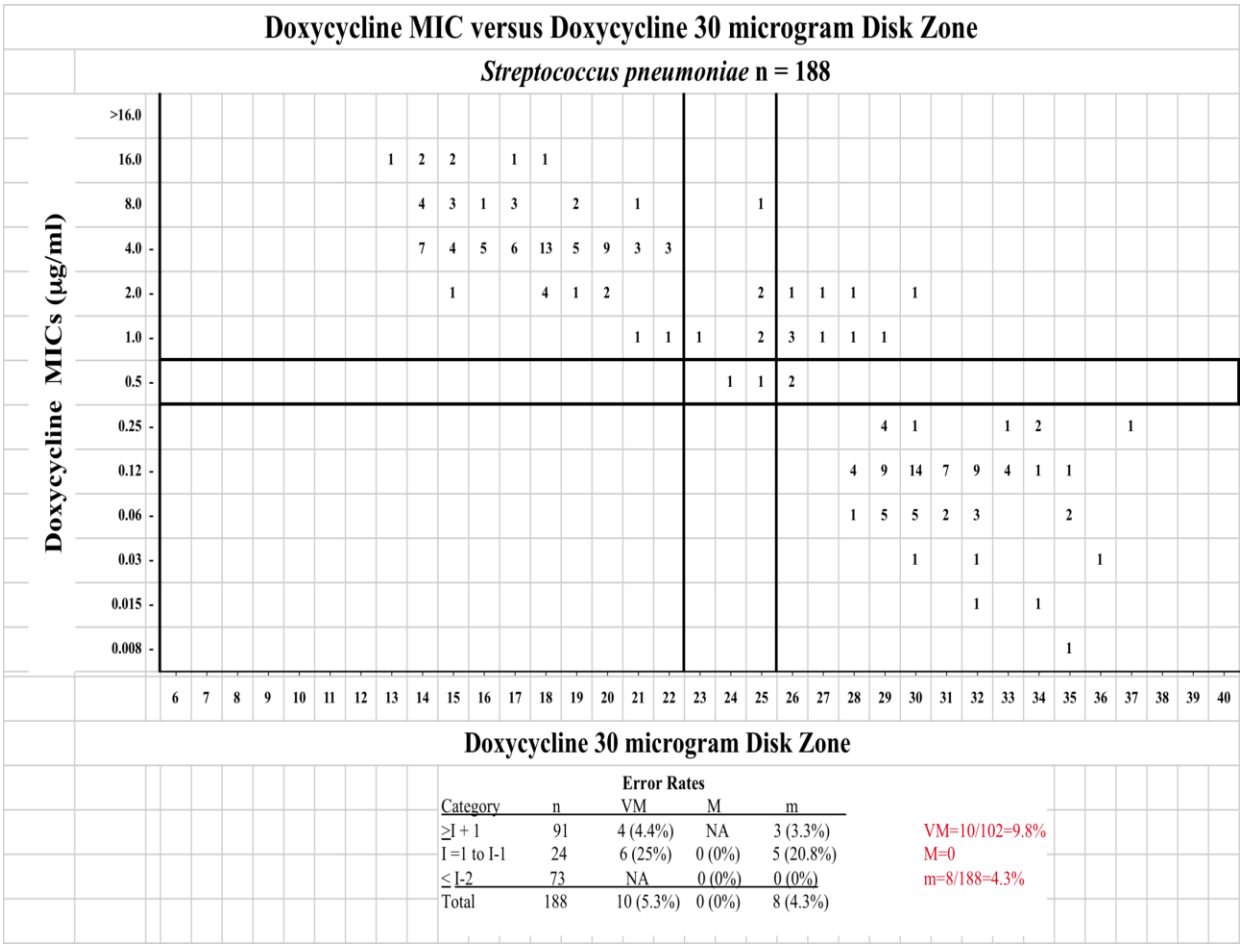
Tetracycline MIC S ≤1, I = 2, R ≥ 4 µg/ml
DD S ≥26, I = 23-25, R ≤ 22 mm

Doxycycline MIC S ≤ 0.25, I = 0.5, R ≥ 1 µg/ml
DD S ≥ 26, I = 23-25, R ≤ 22 mm

Correlation of *tetM* with MIC data was presented to support the proposed breakpoints:

No. isolates	Tetracycline MIC	Doxycycline MIC	Presence of tetM
3	>16	16	2 of 3
2	>16	8	2 of 2
6	>16	4	5 of 6
6	>16 or 16	2	5 of 6
1	8	2	1 of 1
5	8	1	5 of 5
1	4	2	1 of 1
4	4	0.5	4 of 4
1	0.5	0.25	0 of 1
1	0.5	0.12	0 of 1
8	0.25	0.12	1 of 8
Total of 38 isols			
24	≥ 4		22 of 25 or 88%
6	≤ 0.5		1 of 10
		≥ 0.5	22 of 25 or 88%
		≤ 0.25	1 of 10

Scattergrams were shown comparing BMD results to DD results for each drug for the initial lab (UTHSC) and then combined with the new CDC data for possible breakpoints. Test results from CDC on Hardy media had unacceptably high very major error (VME) rates for doxycycline (23% VME for breakpoint of ≤0.25 µg/mL for CDC data alone; 9.8% when combined with UTHSC data).



A comparison of disk zones from the two labs revealed larger doxycyline zone sizes for Hardy media. A comparison of MH agars performed at UTHSC (shown in table below) confirmed larger zones with Hardy media and QC was also out of range.

STATE #	Tet M	Tet-BBL	Tet - Remel	Tet - Hardy	Doxy - BBL	Doxy - Remel	Doxy - Hardy
TNF6324	+	15	12	15	16	16	18
TNF6357	+	15	16	19	20	20	23
TNF6332	+	13	13	16	17	18	20
CTK0414	+	13	13	16	15	15	17
CTK0350	+	10	9	12	14	14	17
OR10022	+	15	15	18	20	20	23
OR09419	+	14	12	16	16	15	19
CTK0255	+	23	22	25	27	25	28
CTK0081	-	33	32	35	33	30	35
CTK0192	+	25	25	27	25	25	28
49619	-	32	31	32	30	30	30

Working group discussion: The working group consensus was that if the media was noncompliant with M6 standards then the testing performed on the media would need to be repeated. An alternative view was that the results may represent real world variability and that breakpoint adjustment could be attempted to minimize errors.

Subcommittee discussion: Dr. Jorgensen reported that he had contacted the Hardy technical director who seemed unfamiliar with M6 standards for MH agar. The package insert did not include any statement of M6 compliance. The subcommittee consensus was that if further investigation confirmed the media was M6 noncompliant then the testing performed on the media would need to be repeated. After the meeting, further information from Hardy indicated they did not have the MHA physical standard to compare to their own MHA base powder. Dr. Jorgensen is arranging for the evaluation using the same 87 strains to be repeated at CDC using Remel agar. CDC will also test a subset of nine strains using all three brands of MHA to determine if media effect led to the poor correlations of disk data.

B. A Screen Agar for the Detection of Vancomycin-Intermediate *S. aureus*

A protocol for a multicenter study to identify a new screen agar for VISA detection was presented to the subcommittee. The study will be lead by Robert Skov with seven participating laboratories and financial support from multiple companies. The subcommittee was supportive of the study as outlined below:

A challenge set of 60 isolates (to be sent blinded to each site) will be chosen from the strain collection at CDC based on the following criteria:

10 isolates with BMD MIC of 2 µg/L - not hVISA by PAP/AUC
10 isolates with BMD MIC of 2 µg/L - hVISA by PAP/AUC
30 isolates with BMD MIC of 4 µg/L
10 isolates with BMD MIC > 4 µg/L

In addition each laboratory will test 10 consecutive blood isolates from their own routine collection.

QC strains**:** *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, MU50 (GISA), MU3 (hGISA)
Composition of VISA screen agar: BHI with 16g/L casein and 4 µg/L vancomycin from different manufacturers. A 10 µL inoculum (0.5 McFarland) will be spotted on each agar plate. Screening plates will be incubated and read at 24 and 48 hours. A positive will be defined as greater than one colony of growth.

The reference method will be frozen broth microdilution panels containing MH broth with vancomycin at 0, 1, 1.5, 2, 3, 4, 6, and 8 µg/L. Oxacillin will also be included on the trays.
Screening agar results that are discordant with BMD will be repeated at the participating lab and also sent to a central laboratory for testing.

C. Report of MRSA with Novel *mecA* Element

Robert Skov presented a brief summary of recent publications (Lancet Infect Dis, June 3, 2011) and an ECCMID poster to the subcommittee describing a new MRSA strain with a novel *mecA* homologue. The strain is not detected by primers in current commercial molecular tests for MRSA. Current PBP2a latex agglutination tests are also negative when testing this MRSA strain. The initial strain was recovered from a dairy cow in England with mastitis. Human isolates of this novel strain have been identified in patients from Scotland, England, Denmark, Ireland, Germany, Sweden and France. Most human isolates occurred after 2004, but an isolate obtained as early as 1975 has been found.

IX. REPORT OF *ENTEROBACTERICEAE/Pseudomonas aeruginosa* WORKING GROUP

Minutes Submitted by Patricia Bradford **(Electronic Tab H in the Meeting Agenda)**

Chairholder – Mike Dudley

Recording Secretary – *Enterobacteriaceae* – Patricia Bradford

Working Group Members present - Paul Ambrose, Bill Craig, Dwight Hardy, Ron Jones, Jim Lewis, Paul Schreckenberger, Lauri Thrupp, Mel Weinstein, Barb Zimmer

Working Group Members absent - Steve Jenkins

Items for vote

A. Zone diameter correlated for new breakpoints for ticarcillin/clavulanate and piperacillin/tazobactam against *Pseudomonas aeruginosa*

At the previous meeting, new breakpoints for these extended spectrum penicillin/β-lactamase inhibitor combination products were approved. The outstanding business was to develop zone diameter correlates (cutoffs) for these agents.

A presentation was made by Dr. Ron Jones presenting disk and MIC correlates on over 400 isolates of *Pseudomonas aeruginosa* from a wide variety geographical locations. Many different resistance genotypes and phenotypes were included.

1. Zone diameter interpretive criteria for both piperacillin-tazobactam and ticarcillin-clavulanate were selected that minimized the error rates when compared to MICs with the new breakpoints.

2. It was noted that there were no differences in the data obtained from different regions of the world.

3. Two data points were included for each organism to correct for reader error.

A motion was made and approved by the Working Group (10/0/0) to accept disk breakpoints for Pseudomonas as follows:

piperacillin-tazobactam: susceptible ≥21 mm, intermediate 15-20 mm, resistant ≤14 mm.
ticarcillin-clavulanate: susceptible ≥25 mm, intermediate 17-24 mm, resistant ≤16 mm.

A question was raised regarding a disk correlate for piperacillin and ticarcillin without the inhibitor. The current breakpoints in the book might make an isolate appear to be piperacillin susceptible but resistant to piperacillin-tazobactam. A suggestion was made to have the zone diameters for piperacillin alone published as the same as the breakpoints for piperacillin-tazobactam for one year. A discussion point was made that there were no data presented to support these breakpoints.

The subcommittee voted to accept the disk diffusion interpretive criteria for piperacillin-tazobactam and ticarcillin-clavulanate (shown above) and remove the disk diffusion disk breakpoints for piperacillin and ticarcillin in Table 2B-1 until suitable experiments can be conducted (Approved 9-1; 2 absent). These experiments may be conducted prior to the publication deadline and thus could be reviewed and approved via email in 2011 for inclusion in the M100 2012 edition.

B. Carbapenem breakpoints

1. Carbapenem breakpoints for *Pseudomonas aeruginosa* with doripenem

Dr Ron Jones and colleagues have conducted a study of MICs and zone diameters for carbapenems vs. *Pseudomonas aeruginosa* to support potential MIC breakpoint decisions.

a) A presentation was made by Dr. Steve Brown on behalf of Johnson & Johnson regarding doripenem breakpoints for *P. aeruginosa* and *Acinetobacter* spp. J&J requests that the breakpoints for *P. aeruginosa* are not published until the Working Group has finished reviewing all of the carbapenems and publishes all together.

The proposed MIC and disk diffusion breakpoints are as follows:

Organism	MIC (μg/mL)			Zone diameter (mm)		
	S	I	R	S	I	R
P. aeruginosa	≤2	4	≥8	≥19*	17-18	≤16

* FDA susceptible disk susceptible breakpoint for *P. aeruginosa* is ≥24 mm .

b) With the sponsor presentation, Joseph Kuti from Hartford hospital presented preliminary data on a population PK study for doripenem dosed with 1 or 4 hr infusion. This data was not provided in the agenda book and the Working Group and subcommittee was advised to consider this in the discussion.

c) A discussion was held on which dosage regimen should be used to set the breakpoints. The 4 hr infusion was used to support the PK/PD, however this infusion time is not approved in the USA. There was also a discussion regarding the % Target Attainment (PTA) and target exposures. Data for a single strain for doripenem in the mouse thigh model in the agenda package suggest exposures are less for *Pseudomonas aeruginosa* than that for *Enterobacteriacea*e. Data for imipenem were also included in the agenda materials. It was pointed out that the design of the animal model did not consider selection of resistance during treatment as an endpoint, which is an important component that has led to clinical failure in the treatment of human infections due to *Pseudomonas aeruginosa*.

A motion in the Working Group was made to accept the recommended MIC breakpoints of ≤2, 4 and 8 µg/mL for S, I and R with *P. aeruginosa*. **Motion did not carry 2/7/1**
A motion was made for MIC breakpoints* of ≤1, 2 and 4 µg/mL for S, I and R with *P. aeruginosa*, with a note for the dosage of 500 mg, q8hr with a 1 hr infusion. **Approved by the Working Group 6/3/1**

Meropenem, Imipenem breakpoints for *P. aeruginosa*

No sponsor data received for either of these two drugs.

M. Dudley reviewed PK/PD data and Monte Carlo simulations performed by ICPD for imipenem and meropenem that was presented previously. Most were looking to a T>MIC of 35% and an acceptable PTA of > 85% as being desirable for P. aeruginosa.

A motion in the Working Group was made to recommend MIC breakpoints for imipenem, meropenem (1gm, q8rh), **AND** doripenem (500 mg, q8hr) of ≤2, 4 and 8 µg/mL for S, I and R with *P. aeruginosa* . **Working Group Vote: 6/2/3 NOTE: This negates the previous working group vote shown above**

- **The subcommittee agreed and approved the above MIC breakpoints (2, 4 and 8 µg/mL) with dosing comments for all three carbapenems for *P. aeruginosa* (Doripenem, Imipenem, and Meropenem) – Approved 6-3; 1 abstain, 2 absent. Disk diffusion breakpoints of ≤15mm, 16-18 and ≥19 were also approved for R, I and S, respectively – Approved 9-1; 2 absent.**

Disk breakpoint correlates

Materials are in the agenda book show an excellent correlation between zone size and MIC. Statistics for breakpoints for susceptible for all drugs as ≤2 mg/L are summarized in the agenda book and error rates were small and within the M23 guidance.

Ertapenem breakpoints for *Enterobacteriaceae*

A follow up discussion of ertapenem breakpoints as part of the 1 year provisional status of new breakpoints was held.

Dr. Jim Lewis gave an update with regards to the creatinine levels and a measure of renal function for the patients that were included in his previous study. There was no indication of renal failure (creatinine clearances expec ted to be ~ 60 mL/min) , and therefore would not have had markedly higher levels of drug than expected. Their hospital continues to see ESBL-producing *Enterobacteriaceae* that result in an ertapenem MIC of 0.5 µg/mL that are called resistant, but have only ESBLs.

Mr. Bob Badal presented SMART data from Merck's surveillance and molecular characterization of β-lactamases. No carbapenemases were detected in organisms with MICs less than 2 µg/mL.

A motion was made to recommend MIC breakpoints for ertapenem of ≤0.5, 1 and 2 µg/mL for S, I and R with *Enterobacteriaceae*. **Approved by the working group 7/1/2**

- **The subcommittee agreed and approved the above revised MIC breakpoints for ertapenem with *Enterobacteriaceae* (Approved 8-0; 1 abstain, 3 absent). An action item is to review previously reviewed zone diameter vs. MIC correlations to identify corresponding disk breakpoints.**

  Follow up note 6/20/11: analysis shows the corresponding disk breakpoints are S: ≥ 22mm; I: 19-21 mm; R: ≤ 18 mm. These values provide 0% very major or major errors, and a 6.1% minor error. This proposal has been approved by the WG. The proposed disk breakpoints were then circulated on 21 June – 27 June for subcommittee review and comment then voted on by the members 28 June – 5 July and approved (12-0).

Items for discussion

Azithromycin breakpoints for enteric pathogens

Dr. John. Crump and Dr. Maria Karlsson from CDC presented some data with a request of the WG to examine testing of azithromycin and breakpoints invasive *Salmonella* and *Shigella* infections. Several publications were provided that showed azithromycin can be used to treat these infections. MIC and zone diameter distributions were presented as well as MIC vs. disk scattergrams. It was noted that disk diffusion tests are sometimes difficult to read because of a double zone phenomenon with some strains. The Working Group was asked to determine if M23 criteria have been met for examination of these breakpoints. It was noted that there is currently no QC strain approved for use with azithromycin. It was suggested that the group should work with the QC working group to determine the best testing methods for this drug/bug combination.

Text and Tables

This Working Group has requested that dosages for cefoxitin and cefmetazole be included in Table 2A as with other cephalosporins. This will be recommended to the full committee.

- **The subcommittee voted to add a comment for cefoxitin "the breakpoint for cefoxitin is based on a dose of at least 8 gm per day (e.g., 2 g every 6 hr). For cefmetazole, a comment will be added that states there was insufficient data to review interpretive criteria (Approved 10-0; 2 absent).**

Rationale documents for the changes made to cephalosporin and carbapenem breakpoints:

This will be completed by J. Lewis. Comments are due to Jim Lewis by 3pm on June 14 james.lewis@uhs-sa.com

X. REPORT OF THE QUALITY CONTROL WORKING GROUP

Minutes Submitted by Sharon Cullen **(Electronic Tab I in the Meeting Agenda)**

Co-Chairholder - Steven Brown

Co-chairholder - Sharon Cullen

Working Group Members present- Bill Brasso, Stephen Hawser, Janet Hindler, Michael Huband, Ron Jones, Ann Macone, Ross Mulder, Susan Munro, Frank Wegerhoff (replaced Paul Oefinger who is working on assignment)

Working Group Members absent – Jean Patel, Bob Rennie

M23 Tier 2 Studies

The Quality Control Working Group reviewed 74 organism/QC combinations!

Finafloxacin								
Previous ID				Abbrev	FIN		WG Vote	
Solvent	Water	Diluent	Water	Rev History	Tier 2			
Route of Admin	IV PO Topical (eardrops)	Class	fluoro-quinolone	Sub-class	8-cyano-fluoro-quinolone	Mfg: OmniChem (Wetteren, Belgium). MerLion Pharmaceuticals. Presented by IHMA		
QC Strain (ATCC)	Range	# mm or dil	% In range	Mode/ Median	Shoulder %	Variability/Comments	10-0-2	
Staphylococcus aureus 29213	0.03-0.25	4	98.3	0.06	78%	Lot C 1 dil lower mode, very few results at 0.25, pg 29	10-0-2	
Enterococcus faecalis 29212	0.25-1	3	100	0.5		Lot C 1 dil lower mode	10-0-2	
Escherichia coli 25922	NR	NA	NA	0.03		No range, 28.5% of values were at lower limit of testing ≤0.015µg/ml, pg 17 Will do new study with lower dilutions	10-0-2	
Pseudomonas aeruginosa 27853	1-8	4	99.2	4	94%	Lot C 1 dil lower mode, Labs with 1 dil lower mode, pg 25	10-0-2	
Streptococcus pneumoniae 49619	0.25-1	3	99.2	0.5			10-0-2	
Haemophilus influenzae 49247	NR	NA	NA	0.004		No range, 36.2% of values were at lower limit of testing ≤0.002µg/ml. Excluded Lab G as outlier Lot B 1 dil lower, pg 37	10-0-2	
B. fragilis 25285 (Agar)	0.12-0.5	3	100	0.25	24%	96% 0.12-0.5, Lab H 22@0.25, 8@1 Lab H mode @ 0.5, pg 45 Range finder 0.012-0.5.	9-2-1	
B. thetaiotaomicron 29741 (Agar)	1-4	3	100	2	27%		10-0-2	
E.lentum 43055 (Agar)	0.12-0.5	3	100	0.25			10-0-2	
C. difficile 700057 (Agar)	1-4	3	97.6	2	21%	Lab H had 2 dil lower outliers at 0.12 with one lot media pg 53	10-0-1	

The subcommittee approved the proposed QC as listed above for Finafloxacin **(Approved 7-0; 5 absent)**

Beta lactam/Beta lactamase inhibitors								
QC Strain (ATCC): *K. pneumoniae* 700603	Range	# mm or dil	% In range	Mode/ Median	Shoulder %	Variability/Comments:	WG Vote	
Amoxacillin/ clavulanic acid	4-16		99.1	8		Lab 5 mode @ 16	9-0-2	
Ampicillin/ sulbactam	8-32		100	16				
Piperacillin/ tazobactam	8-32		100	16				
Ticarcillin/ clavunanic acid	32-128		100	64	50	Excluded Lab 5 (out high) due to outlier mean, mode and median by outlier, pg 9		
Amoxacillin	>128		100	>128		Off scale high (all results >128), pg 13		
Ampicillin	>128		100	>128		Off scale high (all results >128), pg 12		
Piperacillin	NR		NA			No range, results off scale (only tested 128). Data not presented		
Ticarcillin	>256		100	>256		Off scale high (all results >256), pg 14		

The subcommittee approved the above QC ranges (**Approved 8-0; 4 absent**). The approved ranges will be published in the minutes only (not yet in M100). Need to look at current and newer β-lactam/β-lactamase combinations to make recommendations for routine and/or supplemental testing of *K. pneumoniae* 700603 and *E. coli* 35218. Presented by CMI.

Telavancin							
Previous ID				Abbrev	TLV	Mfg: Theravance. Presented by JMI.	WG Vote
Solvent	DMSO	Diluent	DMSO	Rev History			
Route of Admin	IV	Class	glycopeptides	Subclass	lipoglycopeptide		
Similar to dalbavancin, the sponsor recommends preparing intermediate dilutions in DMSO and including polysorbate-80 with final concentration 0.002% in the wells. Without surfactants, telavancin may not be completely solubilized when serial dilutions are performed and it sticks to plastic thereby the MIC underestimates the availability of the drug. **QC ranges and associated footnotes describing the method were approved for inclusion of the minutes only at this time. However, inclusion of the revised method and QC ranges will be coordinated in the future with proposal of updated breakpoints.** Table 4A: Add Footnote "g" to telavancin "Quality control ranges reflect MICs obtained when CAMHB is supplemented with 0.002% P-80." Table 5A: DMSO should replace Water as diluent. Add footnote g to telavancin in diluent column: "Starting stock solutions should be prepared at concentrations no higher than 1600 µg/ml. Intermediate 100x concentrations should be diluted in DMSO. Final 1:100 dilutions should then be made directly into CAMHB supplemented with 0.002% (v/v) polysorbate-80,							10-0-0
QC Strain (ATCC)	Range	# mm or dil	% In range	Mode	Shoulder %		
Staphylococcus aureus 29213	0.03-0.12	3	100	0.06		Current CLSI range (w/o surfactant) is 0.12-1	10-0-0
Enterococcus faecalis 29212	0.03-0.12	3	100	0.06		Current CLSI range (w/o surfactant) is 0.12-0.5	
Streptococcus pneumoniae 49619	0.004-0.015	3	100	0.008		Current CLSI range (w/o surfactant) is 0.004-0.03	

The subcommittee approved the above QC ranges for telavancin (**Approved 7-0; 2 abstain, 3 absent**). These will only be published in the minutes at this time as noted above in the table.

Solithromycin								
Previous ID			CEM-101	Abbrev	SOL, SOLI	Mfg: Cempra	WG Vote	
Solvent	Water	Diluent	Water	Rev History				
Route of Admin	IV PO Ophthalmic drops (topical)	Class	macrolide	Subclass	fluoro-ketolide			
QC Strain (ATCC)	Range	# mm or dil	% In range	Mode/ Median	Shoulder %	Variability/Comments	10-0-0-	
Staphylococcus aureus 29213	0.03-0.12	3	96.6	0.06	51	Note: no results at 0.03, pg 6		
Enterococcus faecalis 29212	0.015-0.06	3	95.6	0.03		3 labs with mode of 0.06, pg 7		
Streptococcus pneumoniae 49619	0.004-0.015	3	99.3	0.008				
Haemophilus influenzae 49247	1-4	3	99.7	2				
Staphylococcus aureus 29523	22-30	9	97	25		Labs median 25-28, Gavin statistic range 23-29 with 94.9% in range, Lot median 26-27, pg 7. Double Zone of inhibitiion in most labs. Data was analyzed both ways, but recommended as complete inhbition (i.e.inner zone) per routine reading recommendations.	10-0-0	
Streptococcus pneumoniae 49619	25-33	9	97.7	28		Lab A excluded (if included 97.7% in range), Lab medians range from 27-31, pg 11 Gavin statistic range 25-31 with 95% in range. Lot median 27-28	10-0-0	
Haemophilus influenzae 49247	16-23	8	97.6	19		Lab median ranged from 18-20. Gavin statistic range 16-22 with 96.3% in range. Lot median 19-20, Pg 15	10-0-0	

The subcommittee approved the above QC ranges for solithromycin (**Approved 9-0; 3 absent**).

JNJ463								
Previous ID			JNJ-32729463, JNJ463, JNJ-Q2	Abbrev		Mfg: Furiex	WG Vote	SC Vote
Solvent	Water	Diluent	Water	Rev History				
Route of Admin		Class	fluoro-quinolone	Subclass				
QC Strain (ATCC)	Range	# mm or dil	% In range	Mode/ Median	Shoulder %	Variability/Comments		
Staphylococcus aureus 29213	0.004-0.015	3	100	0.008	46	2 lots mode @0.04, 2 lots mode @ 0.08, pg 7	10-0-0	
Enterococcus faecalis 29212	0.015-0.06	3	100	0.03				
Escherichia coli 25922	0.008-0.03	3	100	0.015				
Pseudomonas aeruginosa 27853	0.5-2	3	98.8	1				
Streptococcus pneumoniae 49619	0.004-0.015	3	100	0.008		Some lab variability (3 with higher MICs), pg 12		
Haemophilus influenzae 49247	0.002-0.015	4	100	0.008	82			
Staphylococcus aureus 25923	32-38	7	99.6	35		Lab median ranges from 33-36, pg 31		
Escherichia coli 25922	30-36	7	96.5	20		Lab median ranges from 19-21, pg 33 All outliers from Lab E	8-2-0	
Pseudomonas aeruginosa 27853	17-23	7	100	33		Lab median ranges from 30-34, pg 32 Range finder 18-23 w/99.8% in range		
Streptococcus pneumoniae 49619	28-35	8	95.2	32		Lab median ranges from 29-35, lot median ranges from 31-32, pg 35 Range finder 27-36 w/100% in range		
Haemophilus influenzae 49247	31-39	9	98.3	35		Lab median ranges from 33-36, lot median ranges 34-36, pg 34 Range finder 31-38 w/98.1% in range		

The subcommittee approved the above QC ranges for JNJ463 (**Approved 9-0; 3 absent**).

Fusidic Acid								
Previous ID			CEM-102	Abbrev	FA, FC	Mfg: Cempra	WG Vote	
Solvent	Water	Diluent	Water	Rev History				
Route of Admin	PO IV Topical	Class	Steroidal	Subclass	Fusidanes			
QC Strain (ATCC)	Range	# mm or dil	% In range	Mode/ Median	Shoulder %	Variability/Comments		
Staphylococcus aureus 25923	24-32	9	99.8	28		Lab D excluded per Rangefinder, (if included would be 92.1% in range), Lab median ranges from 27-30. Lot median 28-30. pg 6	9-1-0	
Streptococcus pneumoniae 49619	9-16	9	revised	12		Lab median 10-14, Lot median 12-14, pg 10	9-1-0-	
Staphylococcus aureus 29213	0.06-0.25	3	97.8	0.12			10-0-0	
Streptococcus pneumoniae 49619	4-32	4	100	8	61	3 Labs with mode of 16. Alternative 4-16 99.7% in range, pg 33		
TP-434								
Previous ID				Abbrev		Mfg: Tetraphase	WG Vote	
Solvent	Water	Diluent	Water	Rev History				
Route of Admin	IV/Oral	Class	tetracycline	Subclass	fluoro-cycline?			
QC Strain (ATCC)	Range	# mm or dil	% In range	Mode/ Median	Shoulder %	Variability/Comments		
Staphylococcus aureus 29213	0.015-0.12	4	99.2	0.06	61	Excluded Lab E (mode 0.25), 88% in range including Lab E Lab modes ranged from 0.03-0.12, shoulder @ 0.03, Broth mode 0.03-0.06, pg 9 Rangefinfer 0.015-0.12	10-0-0	
Enterococcus faecalis 29212	0.015-0.06	3	100	0.03		Labs read two ways, with haze (0.004-0.015) and complete inhibition of growth (0.015-0.06). Recommend complete inhibition per routine reading instructions. pg 12-13	10-0-0	
Escherichia coli 25922	0.03-0.12	3	100	0.06			10-0-0	
Pseudomonas aeruginosa 27853	2-16	4	100	8	72	Lot and lab modes range from 4-8, Shoulder @ 4, pg 22	10-0-0	
Streptococcus pneumoniae 49619	0.004-0.03	4	100	0.08	87	Lab mode ranges from 0.04-0.015, Lot mode range from 0.08-0.015, pg 17	10-0-0	
Haemophilus influenzae 49247	0.06-0.5	4	100	0.25	56	3 Labs with mode of 0.12, Lab mode ranges from 0.12-0.25, Shoulder @ 0.12, 2 Lots also had significant size of shoulder Rangefinder 0.12-0.5, pg 24	10-0-0	

The subcommittee approved the above QC ranges for fusidic acid and TP-434 (**Approved 9-0; 3 absent**).

Torezolid								
Previous ID			TR-700	Abbrev	TED	Mfg: Trius Therapeutics	WG Vote	
Solvent	DSMO	Diluent	Water	Rev History				
Route of Admin		Class	oxa-zolinidone	Subclass				
QC Strain (ATCC)	Range	# mm or dil	% In range	Mode/ Median	Shoulder %	Variability/Comments		
Staphylococcus aureus 25923	22-29	8	99.6	25		Range from Gavin statistic 22-28 with 97.7% in range. Lab median 24-27, Lot median 25-26, Read with transmitted light. pg 6 Read with reflected light, one lab and one lot gave higher zone sizes with only 90.9% in range, pg 18-19	10-0-0	
Streptococcus pneumoniae 49619	24-30	8	98.7	27		Lab median 25-28, Lot median 27-28, Gavin statistic range 24-30 with 98.7% in range. Range finder 24-31 but no results at 31		
PMX-300603								
Previous ID				Abbrev		Mfg: Polymedix	WG Vote	
Solvent	DMSO	Diluent	DMSO			Note: Need to determine which footnotes apply to diluent.		
Route of Admin	IV/topical	Class	BAAC	Subclass		BAAC: biomimetic of amphiphilic antimicrobial peptide.		
QC Strain (ATCC)	Range	# mm or dil	% In range	Mode/ Median	Shoulder %	Variability/Comments		
Staphylococcus aureus 29213	0.5-2	3	99.5	1	49	3 Labs with mode 0.5. Shoulder 0.5, Lot modes 0.5-1, pg 7	10-0-0	
Enterococcus faecalis 29212	1-4	3	95.9	2	44	Lot modes 1-2, All out of range from Lab F, pg 9		
Streptococcus pneumoniae 49619	4-16	3	99.6	8		Lab E mode 32 (if included would be 89.6% in range). pg 11. Lab E was also out of control with control drug pg 20		

The subcommittee approved the above QC ranges for torezolid and PMX-300603 (**Approved 9-0; 3 absent**).

Ceftaroline								
Previous ID				Abbrev	CPT	Mfg: Cerexa	WG Vote	SC Vote
Solvent	DMSO to 30% of vol	Diluent	Saline	Rev History			8-1-1	
Route of Admin		Class		Subclass	Cephalosporin with anti-MRSA activity			
QC Strain (ATCC)	Range	# mm or dil	% In range	Mode/ Median	Shoulder %	Variability/Comments		
B. fragilis 25285 (Broth)	2-16	4	96.9	4	89	Lab mode 4-16, Lot mode 4-8, Shoulder at 8, pg 9		
B. fragilis 25285 (Agar)	4-32	4	96	8	80	Lot and Lab modes 8-16, Shoulder at 16, pg 10		
B. thetaiotaomicron 29741 (Broth)	8-64	4	97.6	32	71	Shoulder at 16, Lab and Lot modes 16-32, pg 11		
B. thetaiotaomicron 29741 (Agar)	16-128	4	100	32	16	Lab mode 16-128, Only 91.1% in range with 16-64. Range finder 16-128, pg 12		
E.lentum 43055 (Broth)	NR					0.5-2 only 72% in range. Lab mode 1-16, Lot mode 1, pg 13		
E.lentum 43055 (Agar)	8-32	3	100	16	46	Lab mode 8-16, Shoulder at 8, pg 14		
C. difficile 700057 (Broth)	0.5-4	4	99.1	1	73.6	Lot and lab modes 1-2, Shoulder at 2, pg 15		
C. difficile 700057 (Agar)	2-16	4	99.8	4	55.3	Alternative range 2-8 with 93.8% in range, Lab modes 2-8, Shoulder at 2, pg 16		

The subcommittee approved the above QC ranges for ceftaroline (**Approved 9-0; 3 absent**).

Ceftaroline-NXLavibactam								
Previous ID				Abbrev	CPA	Mfg: Cerexa	WG Vote	SC Vote
Solvent	Water	Diluent	Water	Rev History			8-0-2	
Route of Admin	IV	Class		Subclass				
QC Strain (ATCC)	Range	# mm or dil	% In range	Mode/ Median	Shoulder %	Variability/Comments		
B. fragilis 25285 (Broth)	0.06/4-0.5/4	4	93.1	0.12/4		Lab mode 0.12/4-0.5/4, Shoulders at 0.25/4 and 0.5/4, Lot modes 0.12/4-0.5/4, No statistical outliers. Rangefinder 0.06/4-1/4 (5 dilutions), pg 9		
B. fragilis 25285 (Agar)	0.12/4-0.5/4	3	100	0.25/4		Excluded Lab 3 with mode of 2 (87.2 if included), Lab modes 0.25/4-0.5/4, Rangefinder 0.06/4-2/4 (6 diltutions), pg 11		
B. thetaiotaomicron 29741 (Broth)	2/4-8/4	3	100	4/4				
B. thetaiotaomicron 29741 (Agar)	4/4-16/4	3	100	8/4	47.6	Shoulder 4/4, pg 17		
E.lentum 43055 (Broth)	4/4-16/4	3	100	8/4				
E.lentum 43055 (Agar)	4/4-16/4	3	100	8/4	33	Lab modes 4-16, pg 21		
C. difficile 700057 (Broth)	0.25/4-1/4	3	95	0.5/4		Lab modes 0.5/4-1/4, Lab 6 represented 11 of 12 out of range results, Lab modes 0.5/4-1/4, Rangefinder 0.12/4-2/4 (5 dilution range) pg 23		
C. difficile 700057 (Agar)	0.5/4-4/4	4	99.1	1/4	71	Shoulder at 2/4, Lab modes 1/4-2/4, pg 25		

The subcommittee approved the above QC ranges for ceftaroline/NXL104 (**Approved 9-0; 3 absent**).

M23 Tier 3 Recommendations						
Tier 3 QC Recommendations	Current range		Proposed range	Comments	WG Vote	SC Vote
Colistin and E. coli 25922 and MIC	0.5-2		0.25-2	Original range 0.25-1. Revised to 0.5-2 in 2010 Considered expansion to include 0.25 which was previously included in range as temporary improvement which would address some (but not all) out of range low results (but not approved). Data shown that depending upon the electrical charge on the trays, the MICs can vary considerably. Untreated plastic had lowest MICs. The drug will also stick to glass in preparation of stock solutions and filling processes. MICs wtih surfactants generally are 1 dilution lower (but less significant factor than plastic/glass). It is not certain if differences in preparation of panels have impact (e.g. autoclaved cations added to warm media vs add cations to cold media then autoclave). Initial study proposed to share reference panels made by various sources, with and without pluronic in inoculum tested with 2 QC and 8 clinical strains to demonstrate the variablility between labs. Further studies will be defined based on outcome.	10-1	Did not pass
Tobramycin and P. aeruginosa 27853 and Disk	19-25		19-26 or 27	Increase by 1-2 mm or request addn media lot/mfg	No vote	No vote
Ampicillin and E. coli 25922 with Disk	16-22		Same	Double zones seen at or just under 18 hrs. reading or add note to troubleshooting to read ≥18 hours incubation	No vote	No vote
Gentamicn and P. aeruginosa with Disk	16-21		17-23	Increase in range from 87% to 100%	No vote	No vote
Teicoplanin and E. faecalis 29212 and MIC	0.06-0.25		0.25-2 or 0.12-1	Mode at 1 with 65% should at 0.5. 27% out low with current range.	No vote	No vote

User QC questions

A preliminary discussion occurred regarding User QC questions and potential responses. There was insufficient time to obtain consensus on specific recommendations. An offline meeting will be arranged to continue discussion on this topic will be deferred to the January meeting.

Frequency: Table 3C and 4F

- When doing 5 day "verification", what is needed if out of range result obtained (Table 3A and 4F)?
  - Proposal: Single out of range: repeat 1X or 2X
  - Proposal: Multiple out of range, investigate and take corrective action

- After modification/corrective action for AST, what QC testing is needed?
  - Proposal: 5 day "verification"
- What QC frequency is needed when adding new antimicrobial agent?
  - Current: 20-30 day testing
  - Proposal: 5 day "verification" if antimicrobial agent doesn't raise new questions regarding user QC responsibilities (see M07-A8, 16.7.2 or M02-A10, 15.7).
    - Not less labile than other antimicrobial agents currently tested with the same system
    - No new/special instructions for reading, inoculum preparation or other test conditions.
    - Document justification or test 20-30 days

What Strains to Test and with What Frequency?

- Some routine QC strains are not likely to detect problems and adds unnecessary costs (e.g., positive QC strains for screening tests, strains with very high/off scale MICs).
- Manufacturers test an extensive battery of QC organisms with each lot to ensure the test is prepared properly.
- QC testing by labs can/should then be focused on the areas of risk and procedural/technique variables (see M7 Section 16.2)
- QC strains identified as "routine" and "supplemental" in CLSI documents

Proposed Strain Selection and Frequency

- Manufacturer tests each lot with routine QC (and generally with additional QC strains)
- User tests each lot with routine QC
- User selects useful strains for routine (e.g., daily) testing.
  - The strain that most closely resembles the genus and growth requirements of the isolate being tested (e.g., S. pneumonia ATCC 49619 for Streptococcus sp.)
  - Indicators of deterioration (e.g., negative QC for screens, QC strains whose acceptable limits are on scale, E. coli ATCC 35218 with β-lactam/β-lactamase inhibitors)
  - Indicators to confirm proper technique or testing conditions

Tier 3 QC Monitoring

Colistin

- Original range 0.25-1.
- Revised to 0.5-2 in 2010
- QCWG considered inclusion of 0.25 (previously included in range), would address some (but not all) out of range low results.
- Depending upon the electrical charge on the trays, the MICs can vary considerably. Untreated plastic had lowest MICs. Also sticks to glass in preparation of stock solutions and filling processes.
- MICs with surfactants generally are 1 dilution lower (but less significant factor than plastic/glass).
- Initial study proposed to share reference panels made by various sources, test with 2 QC and 8 clinical strains with and without pluronic in inoculum to demonstrate the variability between labs.

- Compile differences in preparation to try to assess impact (e.g. autoclaved cations added to warm media vs add cations to cold media then autoclave).
- Further studies will be defined based on outcome.

Other Antimicrobial Agents: Please submit additional data to Sharon Cullen to compile for January 2012 meeting.

Antimicrobial Agent	QC Strain	Method	Current Range	Proposed Range	Comment
Tobramycin	P. aeruginosa 27853	Disk	19-25	19-26 or 27	Increase by 1-2 mm or collect additional lot/lab
Ampicillin	E. coli 25922	Disk	16-22	Same	Double zones seen if read just under 18 hrs (troubleshooting)
Gentamicn	P. aeruginosa 27853	MIC	16-21	7-23	Increases in range from 87% to 100%
Teicoplanin	E. faecalis 29212	MIC	0.06-0.25	0.25-2 or 0.12-1	Mode at 1 with 65% shoulder at 0.5. 27% out low with current range

XI. REPORT OF THE M39 WORKING GROUP

Minutes Submitted by Janet Hindler **(Electronic Tab J in the Meeting Agenda)**

Chairholder – Janet Hindler

Working Group Members present – Michael Barton, Dyan Luper, Judy Johnston, Jim Lewis

Working Group Members absent – Sharon Erdman, Alan Evangelista, Steve Jenkins, Ron Master, Graeme Nimmo, John Stelling

The working group reviewed the current (near final) draft document of M39-A4. M39 has undergone significant expansion since M39-P was published in 2000. Now, because of concerns with redundancy and potential difficulties for users of M39 to discern elements of the "basic antibiogram" compared to "enhanced" antibiograms, it was decided to separate the guideline into two sections: Section 1) will include all elements necessary to generate a basic antibiogram; and Section 2) will include suggestions for enhanced antibiograms that may be appropriate in certain settings. The section on handling cumulative antibiograms when a laboratory practices selective reporting has also been enhanced significantly.

Members of the working group will finalize the reformatted version of M39-A4 by early Fall at which time it will be circulated to the full AST Subcommittee and subsequently to the Microbiology Consensus Committee. The intent is to have voting complete such that publication of M39-A4 will occur in early 2012.

XII. GOALS FOR THE INTRINSIC RESISTANCE WORKING GROUP

Minutes Submitted by Barb Zimmer **(Electronic Tab K in the Meeting Agenda)**

Chairholder – Barb Zimmer

Recording Secretary – Dyan Luper

Working Group Members present – Jeff Alder, Eliana Armstrong, Sandy Richter, Susan Sharp, Carole Shubert, Paul Schreckenberger, Tom Thomson, Kate Murfitt

The Working Group did not meet at the June SC meeting. Per an off-line discussion, the Working Group requested time during the plenary session of the full Subcommittee to ask for feedback in these areas:

1. There has been discussion over the inclusion of this table in a standards document over a guideline.

   There was no further discussion about this at the Subcommittee.

2. There was discussion over the meaning of "Intrinsic Resistance" and inclusion (or not) of some drug classes and bug/drug combinations. Our definition is "that laboratories should definitely report as "R". Working Group would like direct feedback if there were inappropriate combinations.

   The full Subcommittee affirmed that decision.

3. At the full Subcommittee plenary session, we discussed the use of references such as the Manual of Clinical Microbiology, particularly as it utilizes the genus "*Citrobacter*" instead of species, and agreed that individual species would be more appropriate.

4. At the full Subcommittee plenary session, we agreed that:

   - Tables for other organisms will be drafted for the next document
   - Will include references as provided to CLSI

XIII. VOTE ON DOCUMENTS M2-A11, M7-A9, and SUPPLEMENTAL TABLES M100-S22

Dr. Cockerill requested comments from meeting participants regarding the voting drafts: M2-A11, *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eleventh Edition,* M7-A9, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition,* and corresponding M100-S22 *Supplemental Tables.* The subcommittee members voted to accept the documents with the changes approved at the January and June meetings and recommend M2-A11, M7-A9, and corresponding M100-S22 Supplemental Tables to the Consensus Committee on Microbiology for approval to be published.

A tally of the votes follows:

Total Subcommittee Members	= 12
Votes to Accept	= 12 (J. Alder, M. Dudley, G. Eliopoulos, D. Hardy , D. Hecht, J. Hindler, J. Patel, M. Powell, R. Thomson, J. Turnidge, M. Weinstein, B. Zimmer)
Votes to Accept with Comment	= 0
Votes to Reject	= 0
Votes not Received	= 0

XIV. AGENDA BOOK SUBMISSIONS FOR 22-24 JANUARY 2012 MEETING

Materials for the January meeting will be distributed to the subcommittee on a CD prior to the meeting. The meeting rooms will be equipped with power strips for those who prefer to view the material on their computer instead of printing the material.

To meet the schedule for completing and shipping the CDs, submission due dates and requirements must be met. In order to present at the 22-24 January 2012 meeting please:

1) Submit agenda materials electronically as a PDF file **on or before Thursday, 1 December 2011.**

   Please Note: For QC submissions based on M23 Tier 2 Studies please make sure to include information for the solvent and diluent to include in Table 5, antimicrobial class and subclass, antimicrobial agent abbreviation, and route of administration for inclusion in Glossary I and II.

2) E-mail proposed agenda topics to Franklin R. Cockerill, III, MD (cockerill.franklin@mayo.edu) please copy his Administrative Assistant JoAnn Brunette (Brunette.Joann@mayo.edu) and also to Tracy Dooley (tdooley@clsi.org) for review.

XV. ADJOURNMENT – The meeting adjourned at 2:05 p.m. on Tuesday, 14 June 2011.

Respectfully submitted,

Tracy A. Dooley, BS, MLT (ASCP), Senior Standards Administrator

Appendix A:

Table 2C comment (11) edits:

11) Penicillin-resistant strains of staphylococci produce β-lactamase, and the testing of penicillin instead of ampicillin is preferred. Penicillin should be used to test the susceptibility of all staphylococci to all penicillinase-labile penicillins, such as ampicillin, amoxicillin, azlocillin, carbenicillin, mezlocillin, piperacillin, and ticarcillin. Perform test(s) to detect ~~an induced~~ β-lactamase production on staphylococci ~~all *S. aureus* isolates~~ for which the penicillin MICs are ≤ 0.12 μg/mL or zone diameters ≥ 29 mm before reporting the isolate as penicillin susceptible. Rare isolates of staphylococci that contain genes for β-lactamase production may~~not produce a~~ appear negative ~~positive induced~~ by β-lactamase tests. Consequently, for serious infections requiring penicillin therapy, laboratories should perform MIC tests and ~~induced~~ β-lactamase testing on all subsequent isolates from the same patient. PCR testing of the isolate for the *bla*Z β-lactamase gene may be considered. See Supplemental Table 2C-S4 and Table 2C-S5 at the end of Table 2C.

Appendix A continued:

Table 2C Supplemental Table 1. Screening Tests for β-Lactamase Production, Oxacillin Resistance, and *mecA*-Mediated Oxacillin Resistance Using Cefoxitin in the *Staphylococcus aureus* Group for Use with Table 2C

Screen Test	β-Lactamase[a, b]		Oxacillin Resistance	*mecA*-Mediated Oxacillin Resistance Using Cefoxitin	
Organism group	*S. aureus* with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm[a,c]	*S. aureus*[a,c] and *S. lugdunensis*[b] with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm	*S. aureus*	*S. aureus* and *S. lugdunensis*	
Test method	Disk diffusion (Penicillin zone-edge test)	Nitrocefin-based test	Agar dilution	Disk diffusion	Broth microdilution
Medium	MHA	NA	MHA with 4% NaCl	MHA	CAMHB
Antimicrobial concentration	10 U penicillin disk	NA	6 µg/mL oxacillin	30 µg cefoxitin disk	4 µg/mL cefoxitin
Inoculum	Standard disk diffusion recommendations	Induced growth (ie, growth taken from the zone margin surrounding an oxacillin or cefoxitin disk test on either MHA or a blood agar plate after 16–18 hours of incubation)	Direct colony suspension to obtain 0.5 McFarland turbidity. Using a 1-µL loop that was dipped in the suspension, spot an area 10 to 15 mm in diameter. Alternatively, using a swab dipped in the suspension and expressed, spot a similar area or streak an entire quadrant.	Standard disk diffusion recommendations	Standard broth microdilution recommendations
Incubation conditions	35 ± 2 ºC; ambient air	Room temperature	33–35 °C; ambient air. (Testing at temperatures above 35 °C may not detect MRSA.)	33–35 °C; ambient air. (Testing at temperatures above 35 °C may not detect MRSA.)	33–35 °C; ambient air. (Testing at temperatures above 35 °C may not detect MRSA.)
Incubation length	16-18 hours	Up to 1 hour for nitrocefin-based test or follow manufacturer's directions	24 hours; read with transmitted light	16–18 hours	16–20 hours

Screen Test	β-Lactamase[a, b]		Oxacillin Resistance	*mecA*-Mediated Oxacillin Resistance Using Cefoxitin	
Results	Sharp zone edge ("cliff") = β-lactamase positive. Fuzzy zone edge ("beach") = β-lactamase negative.	Nitrocefin-based test: conversion from yellow to red/pink = β-lactamase positive.	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = oxacillin resistant.	≤ 21 mm = *mecA* positive ≥ 22 mm = *mecA* negative	>4 µg/mL = *mecA* positive ≤ 4 µg/mL = *mecA* negative
Further testing and reporting	β-Lactamase-positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins. .		Oxacillin-resistant staphylococci are resistant to all β-lactam agents; other β-lactam agents should be reported as resistant or should not be reported	Cefoxitin is used as a surrogate for *mecA*-mediated oxacillin resistance. Isolates that test as *mecA* positive should be reported as oxacillin (not cefoxitin) resistant; other β-lactam agents should be reported as resistant or should not be reported. Because of the rare occurrence of oxacillin resistance mechanisms other than *mecA,* isolates that test as *mecA* negative, but for which the oxacillin MICs are resistant (MIC ≥ 4 µg/mL), should be reported as oxacillin resistant.	
QC recommendations	*S. aureus* ATCC® 25923 for routine QC of disks *S. aureus* ATCC® 25923 negative penicillin zone-edge test (fuzzy edge = "beach") Use the following for supplemental QC (see table 3A) *S. aureus* ATCC® 29213 – positive pencillin zone edge test (sharp edge = "cliff")	*S. aureus* ATCC® 29213 – positive *S. aureus* ATCC® 25923 – negative (or see manufacturer's recommendations)	*S. aureus* ATCC® 29213 – Susceptible *S. aureus* ATCC® 43300 – Resistant	*S. aureus* ATCC® 25923 – *mecA* negative (zone 23–29 mm) *S. aureus* ATCC® 43300 – *mecA* positive (zone ≤ 21 mm)	*S. aureus* ATCC® 29213 – *mecA* negative (MIC 1–4 µg/mL) *S. aureus* ATCC® 43300 – *mecA* positive (MIC >4 µg/mL)

Appendix A continued:

Footnotes

a. The penicillin disk diffusion zone edge test was shown to be more sensitive than nitrocefin-based tests for detection of β-lactamase production in *S. aureus*. The penicillin zone-edge is recommended if only one test is used for β-lactamase detection. However, some labs may choose to perform a nitrocefin-based test first and if this test is positive report the results as positive for β-lactamase (or penicillin resistant). If the nitrocefin test is negative, the penicillin zone edge test should be performed before reporting the isolate as penicillin susceptible in cases where penicillin may be used for therapy (e.g., endocarditis).

b. In a three lab study that tested 168 clinical isolates of *S. lugdunensis* showed that all β-lactamase producing isolates tested resistant using CLSI reference broth microdilution MIC and disk diffusion methods and all were β-lactamase positive with the induced nitrocefin assay. The penicillin disk zone edge test was inferior to the induced nitrocefin assay and should not be used for *S. lugdunensis*.

   If a laboratory is using a method other than one of the CLSI reference methods and are unsure if this method can reliably detect penicillin resistance with contemporary isolates of *S. lugdunensis,* the laboratory should perform an induced nitrocefin assay or other CLSI reference method on isolates that test penicillin susceptible before reporting the isolate as penicillin susceptible.

c. References:

Kaase M, Lenga S, Friedrich S, Szabados F, Sakinc T, Kleine B, Gatermann SG. Comparison of phenotypic methods for penicillinase detection in *Staphylococcus aureus*. *Clin Microbiol Infect.* 2008;14:614-616.

Gill VJ, Manning CB, and Ingalls CM**.** Correlation of penicillin minimum inhibitory concentrations and penicillin zone edge appearance with staphylococcal beta-lactamase production. *J. Clin. Microbiol.* 1981;14:437-440.

Figure 1. A positive penicillin disk zone edge test for β-lactamase detection. The zone edge is sharp or like a “cliff” indicating β-lactamase production.

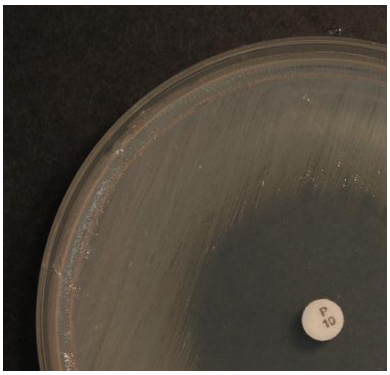


Figure 2. A negative penicillin disk zone edge test for β-lactamase detection. The zone edge is fuzzy or like a “beach” indicating no β-lactamase production.

Appendix B: ~~Supplemental~~ Table 2H-1-Supplemental Table 1. Screening Test for Inducible Clindamycin Resistance in *Streptococcus* spp., β-Hemolytic Group for Use with Table 2H-1

NOTE: Since the clinical significance of inducible clindamycin resistance among β-hemolytic streptococci is unclear, it may not be necessary to perform **tests for inducible Clindamycin resistance** on all isolates that are erythromycin resistant and clindamycin susceptible. Isolates from invasive infections **may be considered for testing**. **The 2010 CDC guidelines on prevention of group B streptococcal disease in neonates recommends that colonization isolates from pregnant women with severe penicillin allergy (high risk for anaphylaxis) should be tested for inducible clindamycin resistance.**[a] (See comment [10] in Table 2H-1.)

Screen Test	Inducible Clindamycin Resistance	
Organism group	**β-hemolytic *Streptococcus* spp. resistant to erythromycin and susceptible or intermediate to clindamycin**	
Test method	**Disk diffusion**	**Broth microdilution**
Medium	**MHA supplemented with sheep blood (5% v/v) or TSA supplemented with sheep blood (5% v/v)**	**CAMHB with LHB (2.5%–5% v/v)**
Antimicrobial concentration	**15-µg erythromycin disk and 2-µg clindamycin disk spaced 12 mm apart**	**1 µg/mL erythromycin and 0.5 µg/mL clindamycin in same well**
Inoculum	**Standard disk diffusion recommendations**	**Standard broth microdilution recommendations**
Incubation conditions	**35 ± 2 °C; 5% CO_2**	**35 ± 2 °C; ambient air**
Incubation length	**20–24 hours**	**20–24 hours**
Results	**Flattening of the zone of inhibition adjacent to the erythromycin disk (referred to as a D-zone) = inducible clindamycin resistance.** **Hazy growth within the zone of inhibition around clindamycin = clindamycin resistance, even if no D-zone apparent.**	**Any growth = inducible clindamycin resistance;** **No growth = no inducible clindamycin resistance**
Further testing and reporting	**Report isolates with inducible clindamycin resistance as "clindamycin resistant"** **An optional comment that may be included "This isolate is presumed to be clindamycin resistant based on detection of inducible clindamycin resistance. Clindamycin may still be effective in some patients".**	
QC recommendations	***S. pneumoniae* ATCC® 49619 for routine QC of disks;** **See Appendix C for use of supplemental QC strains.**	***S. pneumoniae* ATCC® 49619** ***S. aureus* ATCC® BAA-976 or *S. aureus* ATCC® 29213 – no growth** ***S. aureus* ATCC® BAA-977 – growth**

Abbreviations: ATCC, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; LHB, lysed horse blood; MHA, Mueller-Hinton agar; QC, quality control; TSA, tryptic soy agar.

[a] ~~**Since the clinical significance of inducible clindamycin resistance among all β-hemolytic streptococci is unclear, it may not be necessary to perform this induction test on all isolates that are erythromycin resistant and clindamycin susceptible. however, all isolates from invasive infections should be tested. When a Group B streptococcus is isolated from a pregnant woman with severe penicillin allergy (high risk for anaphylaxis), clindamycin and erythromycin should be tested and reported (see comment [10] in Table 2H-1).**~~

[a] **Reference**

Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease – revised guidelines from CDC, 2010. *MMWR Recomm Rep.* 2010;59(RR-10):1-36.