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Summary Minutes
Subcommittee on Antimicrobial Susceptibility Testing
Westin San Diego Gaslamp Quarter
San Diego, California
29 June – 1 July 2014

A meeting of the CLSI Subcommittee on Antimicrobial Susceptibility Testing was held on 29 June-1 July 2014, at the Westin San Diego Gaslamp Quater, San Diego, California. The following were in attendance:

Jean B. Patel, PhD, D(ABMM) **Chairholder**	**Centers for Disease Control and Prevention**
Franklin R. Cockerill, III, MD **Vice-Chairholder**	**Mayo Clinic**
Richard B. Thomson, Jr., PhD, D(ABMM), FAAM **Consensus Committee on Microbiology** **Chairholder**	**Evanston Hospital, NorthShore University HealthSystem**

Members Present

Patricia A. Bradford, PhD	AstraZeneca Pharmaceuticals
George M. Eliopoulos, MD	Beth Israel Deaconess Medical Center
Janet A. Hindler, MCLS, MT(ASCP)	UCLA Medical Center
Stephen G. Jenkins, PhD, D(ABMM),F(AAM)	New York Presbyterian Hospital
James S. Lewis, II, PharmD	University of Texas Health Science Center
Brandi Limbago, PhD	Centers for Disease Control and Prevention
Linda A. Miller, PhD	GlaxoSmithKline
David P. Nicolau, PharmD, FCCP, FIDSA	Hartford Hospital
Mair Powell, MD, FRCP, FRCPath	MHRA
John D. Turnidge, MD	SA Pathology At Women's and Children's Hospital
Melvin P. Weinstein, MD	Robert Wood Johnson Medical School
Barbara L. Zimmer, PhD	Siemens Healthcare Diagnostics Inc.

Advisors Present

Jeff Alder, PhD	Bayer HealthCare
William B. Brasso, BS	BD Diagnostic Systems
Michael N. Dudley, PharmD,FIDSA	The Medicines Company

Marcelo F. Galas	National Institute of Infectious Diseases, Ministry of Health, Argentina
Dwight J. Hardy, PhD	University of Rochester Medical Center
Romney M. Humphries, Ph.D., D(ABMM)	UCLA David Geffen School of Medicine
Howard Gold, MD	Beth Israel Deaconess Medical Center
Tony Mazzulli, MD, FRCPC, FACP	Mt. Sinai Hospital
Sumathi Nambiar, MD, MPH	FDA/CDER
Robin Patel, MD	Mayo Clinic
Cathy A. Petti, MD	University of South Florida
Sandra S. Richter, MD, D(ABMM)	Cleveland Clinic
Helio S. Sader, MD, PhD	JMI Laboratories
Michael Satlin, MD, MS	Weill Cornell Medical College
Paul C. Schreckenberger, PhD, D(ABMM), F(AAM)	Loyola University Medical Center
Audrey N. Schuetz, MD, MPH, D(ABMM)	Weill Cornell Medical College/ NewYork-Presbyterian Hospital
Susan Sharp, PhD, D(ABMM)	Kaiser Permanente-NW/ASM Representative
Ribhi M. Shawar, PhD, D(ABMM)	FDA Ctr. for Devices/Rad. Health (CDRH)
Kerry Snow, MS, MT(ASCP)	FDA/CDER
Maria M. Traczewski, BS, MT(ASCP)	The Clinical Microbiology Institute
Hui Wang, PhD	Peking University People's Hosptial

CLSI Reviewers Present

Paul G. Ambrose, PharmD, FIDSA	Ordway Research Institute
Robert E. Badal	IHMA, Inc
Sujata M. Bhavnani, PharmD	Ordway Research Institute
Donald Biek, PhD	Cerexa, Inc.
Steven D. Brown, PhD, ABMM	Consultant
Ian A. Critchley, PhD	Cerexa, Inc.
Michael J. Dowzicky	Pfizer Inc
German Esparza, BSc	Hospital Santa Clara
Robert Eusebio, MSHA, MT(ASCP)	Siemens Healthcare Diagnostics Inc.
Gina L. Ewald-Saldana, CLS(CA), MT(ASCP)	Siemens Healthcare Diagnostics Inc.
Mary Jane Ferraro, PhD, MPH	Massachusetts General Hospital
Robert K. Flamm, PhD	JMI Laboratories
Lawrence V. Friedrich, PharmD	Cubist Pharmaceuticals, Inc.
Meredith Hackel	IHMA, Inc.
Patricia Hogan, MT(ASCP), MBA	Pfizer Inc
Denise Holliday, MT(ASCP)	BD Diagnostic Systems
Michael D. Huband	AstraZeneca Pharmaceuticals
Nilda V. Jacobus	Tufts Medical Center
James H Jorgenson, PhD	University of Texas Health Science Center
Jack L. Johnson	IHMA, Inc.
Ronald N. Jones, MD	JMI Laboratories
Scott B. Killian	Thermo Fisher Scientific

Laura M. Koeth, MT(ASCP)	Laboratory Specialists, Inc.
Brigitte Lefebvre	Laboratoire de sante, publique du Quebec
Sara Blaine Leppanen	Blaine Healthcare Associates
Dyan Luper, BS, MT(ASCP)SM	BD Diagnostic Systems
Linda M. Mann, PhD, D(ABMM)	Consultant
Maureen Mansfield	Thermo Fisher Scientific
Amy J Mathers, MD	University of Virginia Medical Center
Susan D. Munro, MT(ASCP)	Consultant
Susan O'Rourke	BD Diagnostic Systems
Elizabeth Palavecino, MD	Wake Forest University Baptist Medical Center
Samir Patel, PhD, FCCM	Public Health Ontario
L. Barth Reller, MD	Duke University Medical Center
Darcie E. Roe-Carpenter, PhD, CIC, CEM	Siemens Healthcare Diagnostics Inc.
Daniel F. Sahm, PhD	IHMA, Inc.
Dale A. Schwab, PhD, D(ABMM)	Quest Diagnostics, Nichols Institute
Katherine Sei	Siemens Healthcare Diagnostics
Sharon Shinn	Siemens Healthcare Diagnostics Inc.
Dee Shortridge, PhD	BioMerieux, Inc.
Phillip B. Sonke	Innovative Molecular Diagnostics Check-Points
Jana M. Swenson, MMSc	
Susan Thomson	MAST Group
Lauri D. Thrupp, MD	University of California Irvine Medical Center
Yun F. Wang, MD, PhD	Emory University Hospital
Paige E. Waterman	Armed Forces Health Surveillance Center
Nancy Watz	Stanford Hospital and Clinics
Matthew A. Wikler, MD, MBA, FIDSA	The Medicines Company

Observers/Guests Present

April Abbott	University of Washington
Francis Arhin	The Medicines Company
Paul Bien, MS	Cubist Pharmaceuticals
April Bobenchik	SCPMG Regional Lab (Kaiser)
Lynn Boyer	Siemens Healthcare Diagnostics
Regina Brookman	Siemens Healthcare Diagnostics
Linda C. Bruno MA, MT(ASCP)	ACL Laboratories
Sysan Butler-Wu	University of Washington
Christina Chantell	Accelerate Diagnostics, Inc
Katie Coyle	Becton Dickinson
Evelyn Ellis-Grosse, PhD	E2g Consulting
Sheila Farnham	bioMerieux
Barb Gancarz	bioMerieux
Akinobu Ito	Shionogi & Co., Ltd

Maria Karlsson	Centers for Disease Control and Prevention
Susan M. Kircher, MS, MT (ASCP)	BD Diagnostic Systems
Roberta Knefel	bioMerieux
Peggy Kohner	Mayo Clinic
Christine Lascols	Centers for Disease Control and Prevention
Erika Matuschek, PhD	ESCMID
Greg Moeck	The Medicines Company
Pamela Moise PhD	Cubist Pharmaceuticals Medical Affairs
Martie Morgan	Cedars-Sinai Medical Center
Ross Mulder	bioMerieux
Susan M. Novak-Weekley, PhD	
Jennifer O'Connor	Siemens Healthcare Diagnostics
Kiyofumi Ohkusu	Tokyo Medical University
Pritty Patel, MS, MBA	Covance Central Labs
Chris Pillar	Micromyx
Shannon Popson	Siemens Healthcare Diagnostics
Janet Raddatz	Cubist
Nilia Robles-Hernandez	bioMerieux
Carole Shubert	bioMerieux
Alisa Serio	Achaogen
David Smith	Alere Canada
Roger Stephens	Forest Laboratories
Gina Thomson	University of Louisville
Ken Thomson	Covance, Inc.
Mikala Wang, MD, PhD	Aarhus University Hospital
Collette Wehr	Siemens Healthcare Diagnostics
Natalie Williams-Bouyer, PhD	UTMB Health Dept. of Pathology
Teresa Wong	Siemens Healthcare Diagnostics
Sara Wood	Siemens Healthcare Diagnostics

CLSI Staff

Tracy A. Dooley, BS, MLT (ASCP)
Erica Berlanger
Glen Fine, MS, MBA, CAE
Marcy Hackenbrack, MCM, M(ASCP)
Luann Ochs, MS
Jack Zackowski, PhD, , FACB, CLSI President Elect

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I. MEETING/OPENING REMARKS

Dr. Jean Patel called the meeting to order at 8:00 a.m. on Monday, 30 June 2014. She stated the purpose of this subcommittee is to develop standards for antimicrobial susceptibility testing for laboratories. These standards help ensure accurate testing for improved patient care.

Dr. Patel introduced Dr. Cathy Petti, who is an independent consultant for HealthSpring Global and adjunct professor at University of South Florida. Dr. Petti is a new advisor on the subcommittee.

She then thanked all the working groups (WG) for the all the on-going work being done outside of the January and June meetings. All of the WGs have been busy over the past few months, accomplishing much of the work thru conference calls and e-mail, making this meeting more efficient.

II. CLSI UPDATE

Ms. Luann Ochs, Senior Vice President of Operations with CLSI welcomed everyone to the meeting, introduced CLSI staff, and then gave an overview of some of the updates within CLSI.

Ms. Ochs then introduced CLSI staff present at the meeting as follows:

- Mr. Glen Fine, Executive Vice President;
- Tracy Dooley – Senior Project Manager and Staff Liaison to the Consensus Committee on Microbiology and;
- Marcy Hackenbrack – Senior Project Manager and Staff Liaison to the Consensus Committee on Molecular Methods who also assists with various projects under; and
- Erica Berlanger – Meeting Manager who coordinates all the logistics for these meetings.

Ms. Ochs also introduced Dr. Jack Zakowski, CLSI President Elect and Director of Scientific Affairs and Professional Relations at Beckman Coulter in Brea CA.

What's New:

- CLSI is continuing to make progress with streamlining our consensus process. As it relates to this subcommittee, we have received positive feedback regarding the Working Group (WG) structure that Dr. Patel has put in place for this subcommittee allowing more volunteers to actively participate in various WGs and Ad Hoc WGs that have been formed. These WGs are working throughout the year, allowing the work here at these meetings to run more efficiently.

- The field of laboratory medicine continues to change and as such, CLSI is doing so as well. The CLSI Board of Directors has approved the consolidation/merge of the Hemotology Consensus Committee with the Inmmunology/Ligand Assay Consensus Committee as well as the Quality Management Systems Subcommittee moving under the Quality Systems and Laboratory Practices Consensus Committee. These consolidations eliminate several face-to-face meetings each year, resulting in substantial cost savings. CLSI is working on other initiatives for continual improvement and cost savings as well, ensuring that CLSI standards and guidelines are developed and published on schedule.

- CLSI remains committed to achieving our vision of "Quality Practices for Better Health", and our mission to "Develop clinical and laboratory best practices, and promote their use worldwide". Any ideas you have on how CLSI can better fulfill our vision and mission are always welcome.

In closing, Ms. Ochs thanked everyone again for all that this committee does for CLSI. Without the volunteers of this committee, CLSI would not be able to meet our vision and mission.

III. UPDATES TO THE CURRENT AST DISCLOSURE SUMMARY

Dr. Patel asked the members and advisors for any updates to the current disclosure summary provided on the CD of meeting materials – Dr. Cathy Petti provided updates that will be added to the summary.

IV. APPROVAL OF THE JANUARY 2014 MEETING MINUTES

Summary minutes of the 12-14 January 2014 subcommittee meeting were approved: **(12-0)**

V. REPORT OF THE BREAKPOINT WORKING GROUP (Electronic Folder 5)

Co-Chairholder – Dr. George Eliopoulos
Co-Chairholder – Dr. Jim Lewis

Members Present: Marcelo Galas, Amy Mathers, Michael Satlin, Paul Schreckenberger, Lauri Thrupp, Audrey Schuetz, Hui Wang, Mel Weinstein, Barb Zimmer (served as Recording Secretary for this meeting)
Members Absent: Karen Bush, Simone Shurland

SDD Ad Hoc WG (Co Chairholders Dr. Jim Jorgensen and Dr. Mel Weinstein)

- Presented list of antimicrobial agents for *Enterobacteriaceae*, and associated doses if SDD is to be expanded:
  a. Cefotaxime
  b. Ceftriaxone
  c. Cefoxitin
  d. Ceftazidime
  e. Ceftizoxime (drug may not be available worldwide) Note – no current active drug label at FDA
  f. Aztreonam

- Recommendation of Ad Hoc WG is to see how cefepime SDD rollout went and wait for a later date to look at others. Further data would be results of renewed survey once cefepime SDD breakpoint has been out a little longer.
- Requested motion: Would we like to look at SDD for list of drugs above for Enterobacteriaceae? No motion made

- **Action item**: There is interest in resurveying an extended audience, after there is more experience with cefepime SDD, now that there are FDA BPs for cefepime which correlate "Intermediate" with "SDD", even if "I" is used and not "SDD".
- WG Motion – Recommend SDD Ad Hoc WG examine Cefepime, Ceftazidime, and Aztreonam MICs with *P. aeruginosa* to see whether or not SDD is applicable. WG vote Passed. (9-0-0)

Fluoroquinolone Ad Hoc WG (Dr. Romney Humphries) What disk can best predict reduced S to fluoroquinolones?

1. Presented data for pefloxacin as a surrogate disk that most reliably identifies Salmonella isolates that are not susceptible to fluoroquinolones based on current ciprofloxacin susceptible or not susceptible (I+R) MIC breakpoints. Assumption is that ciprofloxacin MIC is able to accurately differentiate those isolates.
2. Recommendation of ad hoc WG is pefloxacin disk is a surrogate for ciprofloxacin resistance, with breakpoints of ≤23mm R; ≥ 24mm S. The WG discussed whether the nalidixic acid and ciprofloxacin disk tests should be removed from the document. Those in favor of removing these tests noted that the pefloxacin disk test is more accurate for detecting the most common plasmid-mediated resistance mechansims. Those against removing noted that some types of resistance (e.g., AAC(6′)-Ib-cr) will not be detected by pefloxacin disk testing but may be detected by ciprofloxacin disk testing.
3. Note M23 QC for pefloxacin disk being presented at this meeting.
4. No votes or further discussion on pefloxacin.

   Subcommittee votes:

   For Salmonella spp, add pefloxacin 5 µg disk test (zone ≤ 23 mm = non-susceptible) as a surrogate test for detecting non-susceptibility to ciprofloxacin with a comment as follows:

   Strains of *Salmonella* that test nonsusceptible to ciprofloxacin, levofloxacin, ofloxacin, pefloxacin, and/or nalidixic acid may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with salmonellosis. Multiple fluoroquinolone resistance mechanisms have been identified in *Salmonella* spp. and no single test will detect resistance resulting from all possible fluoroquinolone resistance determinants.

   If a ciprofloxacin, levofloxacin, or ofloxacin MIC test cannot be done, pefloxacin disk diffusion as a surrogate test is the preferred alternative. A ciprofloxacin disk alone or both ciprofloxacin and nalidixic acid disks could also be tested.

   Approved by Subcommittee 12-0. Also ciprofloxacin and nalidixic acid will remain in the tables.

Azithromycin – Salmonella Ad Hoc WG (Dr. John Turnidge and Mr. Marcelo Galas, Co-chaiholders)

1. Azithromycin MIC distribution data for *Salmonella* Typhi and *Salmonella* Paratyphi A was presented for >1000 strains tested in Asia. The data were mainly generated using E test. Data showed an epidemiological cutoff value (ECV) of 16µg/mL for S. Typhi and 32µg/mL for S. Paratyphi A. In addition, an evaluation of Etest vs. MICs data were presented for 98 isolates. There was a 63% correlation of MICs, Etest read 1 doubling dilution higher for 24% of the isolates and 1 doubling dilution lower for 13% of the isolates. There was a tendancy for Etest MICs to be highter by Etest. Reference broth microdilution MIC data from CDC were reported for 100 S. Typhi and 96 S. Paratyphi A isolates. For both species the ECV was 16 µg/mL.
2. A PK/PD model for azithromycin was not presented because of azithrmycin's distribution properties. This drug likely exerts most antimicrobial activity in the intracellular spaces where Salmonella reside.
3. Data from a clinical trial was presented, but there were no isolates with MICs above the ECOFF and few clinical failures. A single case report of a treatment failure as presented. The infecting isolate's azithromycin MIC was >64µg/mL. The lack of PK/PD data and informative clinical trial data meant that the breakpoints would primarily be based upon MIC distribution data.
4. The ad hoc WG proposed MIC breakpoint for *S. typhi* and S. paratyphi A of S ≤16; NS ≥32. No intermediate breakpoint was recommended because of the rarity of strains with MICs >16µg/mL, except for a small proportion of S. Paratyphi A tested by Etest. They proposed a correlative disk zone breakpoint of S ≥13 mm NS ≤12.
5. Discussion: MIC distribution data are different for *S. typhi* and S. paratyphi with a tendancy for S. Paratyphi A isolates to demonstrate slightly higher MICs. This difference was statistically significant when using Etest. Disk correlates, work for S. Typhi, but the same correlates result in an extremely high very major error rate for S. Paratyphi A.
6. The ad hoc WG voted for S. Typhi breakpoints only: S BPs of ≤16 and NS ≥32 and corresponding disk diffusion BPs zone S ≥13 mm NS ≤12 (WG vote Passed 10-1-0). There was one abstaining vote: the WG member stated that treatment is predictable and there is no practical way for labs to test susceptibility.

Subcommittee Vote – Approved for Salmonella Typhi only with no intermediate range – MIC – S, ≤ 16 R, ≥32; Disk – S , ≥13, R ≤12. A comment stating breakpoints were based on WT cutoff and limited clinical data will be added as well. Approved 9-1; 2 abstain.

VI. M45 WORKING GROUP UPDATE (Electronic Folder 6)

Co-Chairholder – Dr. Sandra Richter
Co-Chairholder – Ms. Janet Hindler

Working Group Members: Kathy Bernard, Sonia Bodeis-Jones, Mariana Castanheira, Diane Citron, Marc Couturier, Tom Fritsche, Romney Humphries, Jim Jorgensen, Scott Killian, Peggy Kohner, Erika Matuschek, Samir Patel; Advisor: Pat McDermott

Dr. Richter and Ms. Hindler gave an overview of the updates that the M45 WG are working on as they revise the third edition of the document.

For all organisms/tables the WG is going through each of the current 16 tables and 5 new tables and making updates as follows:

- Reviewing literature and updating current list of references for the Table being updated or created. Determining if any current references should be deleted .
- Contacting individuals who may have unpublished data.
- Determining if method, QC ranges, and breakpoints are appropriate.
- For each new organism, determine if existing CLSI methods can be used.
- Determining if a limited amount of supplemental testing would be useful for the proposed changes or new additions (eg, growth studies, broth microdilution, disk diffusion).
- Updating Supplemental Information section of table.
- Some new tables for the next edition include tables for *Aerococcus* spp., *Gemella* spp., *Lactococcus* spp., *Micrococcus* spp., and *Rothia mucilaginosa.*

Some specifc major changes for certain tables include:

Table 4. *Campylobacter jejuni*/*coli*

- Disk diffusion media & incubation:
  - Reassessed media using MHA, MHA-5% sheep blood and HTM
  - Confirmed MHA-5% sheep blood growth is best
  - Determined 42°C & 24 h incubation is best (delete 36-37°C for 48 h)
- Disk diffusion breakpoints
  - Adding S, I breakpoints for ciprofloxacin, erythromycin
  - Adding S, I, R breakpoints for tetracycline
  - Developed by testing 307 isolates: *C. jejuni* (N=206), *C. coli* (N=101)
  - 205 retail meat (120 *C. jejuni*, 85 *C. coli*)
  - 102 human (86 *C. jejuni*, 16 *C. coli*).
  - MIC-zone diameter regression analysis: no VM or major errors, and only 1-1.4% minor errors
- Adding statement: "Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline."

Table 8. *Helicobacter pylori*

- Peggy Kohner performed growth studies and confirmed that aged sheep blood for agar dilution results in better growth than fresh sheep blood: "Aged blood was shown to provide superior growth to blood plates prepared with fresh blood."

- Add footnote: "*In vitro* resistance to metronidazole under these test conditions does not reliably predict *in vivo* treatment failure; therefore, testing for metronidazole is not recommended."

Additional updates are summarized in the table below:

Organism Group	Addition/Modification for M45-A3
***Aeromonas* spp.**	• Move *Plesiomonas shigelloides* to M100 (*Enterobacteriaceae*) • Harmonize β-lactam breakpoints with those of *Enterobacteriaceae* in M100 (include dosage information)
***Bacillus* spp. (not *B. anthracis*)**	• Tentative - add comment that Table applies to closely related genera (now identifiable with MALDI): *Brevibacillus, Cohnella, Lysinibacillus, Paenibacillus, Solibacillus* and *Sporolactobacillus* • Add breakpoints for meropenem (≤4 S, 8 I, ≥16 R µg/mL; same as imipenem)
HACEK	• Growth studies in various broths to include CAMHB-5% LHB, HTM, BB-5% LHB + Vit K and hemin (BB), EUCAST's MH-F, suggests: – MH-F > HTM > CAMHB-5% LHB for *Aggregatibacter* – HTM > MH-F > CAMHB-5% LHB for *Cardiobacterium* – BB > CAMHB-5% LHB for *Eikenella* • Representative MIC studies in progress
***Lactobacillus* spp.**	• Tentative – add comment that meropenem MICs are 2-3 dilutions greater than imipenem MICs – Add meropenem breakpoints (≤1 S, 2 I, ≥4 R µg/mL)
Listeria monocytogenes	• Change T-S breakpoint to "S only" (≤0.5/9.5 µg/mL) • Add meropenem "S only" breakpoint (≤0.25µg/mL)
Moraxella catarrhalis	• Remove cefaclor breakpoints
***Vibrio* spp.**	• Harmonize β-lactam breakpoints with those of *Enterobacteriaceae* in M100 (include dosage information)
***Aerococcus* spp.**	• New table with breakpoints adapted from viridans streptococci • Includes *Aerococcus urinae, Aerococcus viridans* and *Aerococcus sanguinocola* • CAMHB with LHB (2.5% to 5% v/v); CO_2 • Testing isolates from normally sterile sources (blood cultures) may be warranted • "*A. urinae* are intrinsically R to TMP-SMX but may test S *in vitro*"
***Gemella* spp.**	• New table with breakpoints adapted from viridans streptococci (and *Abiotrophia/Granulicatella*) • CAMHB with LHB (2.5% to 5% v/v); ambient air • Testing isolates from normally sterile sources (blood cultures) may be warranted

The M45 WG is also working on ways to communicate these changes. Some ideas that they are working on include drafting a comprehensive rationale document, possibly a paper in CID microbiology section, and a session during ASM's 2015 annual meeting.

VII. REPORT OF THE QUALITY CONTROL WORKING GROUP (Electronic Folder 7)

Co-Chairholder – Dr. Steven Brown
Co-Chairholder – Ms. Sharon Cullen (absent)

Members Present: Bob Flamm, Janet Hindler, Denise Holliday, Michael Huband, Erika Matuschek, Ross Mulder, Susan Munro,
Members Absent: Patti Conville, Stephen Hawser, Bob Rennie, Frank Wegerhoff, Mary York

1. AZD0914 vs. *N. gonorrhoeae* ATCC® 49226 via agar dilution = 0.06-0.5 µg/mL.

Drug Information:

Name	Prev ID	Abbrev	Class	Subclass	Solvent	Diluent
AZD0914	N/A	TBD	Spiropyrim-idinetrione	TBD	DMSO	DMSO

Summary of QC Study Results:

# mm or	% In range	Mode	Shoulder	WG Vote	Variability/Comments
0.06-0.5	100.0%	0.12	57% @ 0.25	Votes(For/Opposed/Abstained/Not present) Working Group (WG) Vote – Total 6/1/1/6.	RangeFinder proposed range was 0.06-0.25. The size of the shoulder was the reason for the single no vote.

Subcommittee Vote on AZD0914 vs. *N. gonorrhoeae* ATCC® 49226 via agar dilution – Approved 9-0; 3 abstain

2. TD1607 via broth microdilution. No surfactant was used in this study.

   S. aureus ATCC® 29213 = 0.002-0.008 µg/mL

   E. faecalis ATCC® 29212 = 0.002-0.008 µg/mL

   S. pneumoniae ATCC® 49619 = 0.001-0.004 µg/mL

Drug Information:

Name	Prev ID	Abbrev	Class	Subclass	Solvent	Diluent
TD1607	N/A	TBD	**glycopeptide-cephalosporin heterodimer**	TBD	**10 mM phosphate buffer pH6.0**	**10 mM phosphate buffer pH6.0**

Summary of QC Study Results:

QC Strain (ATCC®)	Dilutions (µg/mL)	% In range	Mode	Shoulder	WG Vote (For/Opposed/Abstain/Not present)	Variability/ Comments
***S. aureus* ATCC® 29213**	**0.002-0.008**	**100.0%**	**0.004**	None	7/0/0/6	**NO SURFACTANT FOR THIS DRUG FOR ALL ORGANISMS**
***E. faecalis* ATCC® 29212**	**0.002-0.008**	**100.0%**	**0.004**	None		
***S. pneumoniae* ATCC® 49619**	**0.001-0.004**	**100.0%**	**0.002**	None		

Subcommittee Vote – Approved 11-0;1 abstain

3. Pefloxacin vs. *E. coli* ATCC® 25922 disk diffusion using 5 µg disks = 25-33 mm.

Summary of QC Study Results:

QC Strain (ATCC®)	# mm	% In range	Mode	Shoulder	WG Vote (For/Opposed/Abstain/Not present)	Variability/ Comments
***E. coli* ATCC® 25922**	**25-33 mm**	**99.2%**	**28**	None		**Gavan and RangeFinder method agree. This range was approved by a vote of 5/2/1/6. Dissenting votes were due to a desire to achieve harmony with EUCAST. MH abstained.**

Note: The EUCAST QC range is 26-32mm, but a M23 QC study was not performed. The range noted here is consistent with M23 guidelines.

Subcommittee Vote – Approved 12-0

4. Update table of *K. pneumoniae* ATCC 700603 for routine testing - Not replacing *E. coli* but just updates table. Working Group vote 7/0/0

NOTE: The AST subcommittee expressed interest in including *K. pneumoniae* ATCC® 700603 into the full Table 5A for MIC testing and Table 4A for disk diffusion testing. It is important to emphasize that that *K. pneumoniae* ATCC® 700603 is REQUIRED for testing aztreonam-avibactam, ceftaroline- avibactam, ceftazidime-avibactam and ceftolozane-tazobactam. The use of *K. pneumoniae* ATCC® 700603 is SUPPLEMENTAL for testing any of the older β-lactam/ β-lactamase inhibitor combination drugs.

Existing wording is show in BLACK. Newly proposed wording and ranges are shown in BLUE. Deleted wording is shown with a strikethrough. There are

updates to the respective tables only. There is no proposal at this time to try to replace *E. coli* ATCC® 35218 with *K. pneumoniae* ATCC®700603.

Page 156, M100-S24, supplement to Table 5A. MIC (µg/ml) Quality Control Ranges for *Klebsiella pneumoniae* ATCC® 700603* ~~as supplemental~~ QC.

Antimicrobial Agent	*Klebsiella pneumoniae* ATCC 700603 (µg/mL)
Amoxicillin	>128
Amoxicillin-clavulanic acid	4/2-16/8
Ampicillin	>128
Ampicillin-sulbactam	8/4-32/16
Aztreonam	8–64
Aztreonam-avibactam*	0.06/4–0.5/4
Biapenem	0.03–0.12
Ceftaroline	2-8
Ceftaroline-avibactam*	0.25/4–1/4
Ceftazidime	16-64
Ceftazidime-avibactam*	0.25/4–2/4
Ceftolozane-tazobactam*	0.5/4–2/4
Piperacillin	--**
Piperacillin-tazobactam	8/4-32/4
Ticarcillin	>256
Ticarcillin-clavulanic acid	32/2-128/2

**K. pneumoniae* ATCC 700603 must be used for routine QC of ceftazidime-avibactam, ceftaroline-avibactam, aztreonam-avibactam and ceftolozane-tazobactam. Either *K. pneumoniae* ATCC 700603 or *E. coli* ATCC 35218 can be used for routine QC of other β-lactam/β-lactamase inhibitor combination agents.

K. pneumoniae ATCC® 700603 should be tested against ceftazidime-avibactam and ceftazidime alone, ceftaroline-avibactam and ceftaroline alone or aztreonam-avibactam and aztreonam alone to confirm the activity of avibactam in the combination and to ensure that the plasmid encoding the ß- lactamase has not been lost in this strain. Currently, there are no MIC QC ranges for ceftolozane alone. ~~The acceptable range for ceftazidime alone is >16 µg/ml~~. Any of the above β-lactams can be used to test the strain for loss of plasmid. It is not necessary to test each one.

**No range recommended due to off-scale results on the low end.

The Subcommittee approved adding the *K. pneumoniae* ATCC® 700603 ranges in a new column with appropriate footnotes that would be added in Table 5A instead of having as a separate table as currently found in M100. **Approved 9-1; 1 abstain, 1 absent.**

Add as a a new row in Table 4A, page 143, M100-S24 (similar what was approved above for Table 5A, page 156).

Disk Diffusion Quality Control Ranges (mm) for *Klebsiella pneumoniae* ATCC® 700603

Antimicrobial Agent	*Klebsiella pneumoniae* ATCC® 700603 (mm)
Ceftaroline-avibactam*	21-27
Ceftazidime-avibactam*	21-27
Ceftolozane-tazobactam	17-25

**K. pneumoniae* ATCC 700603 must be used for routine QC of ceftaroline-avibactam and ceftazidimeavibactam. Either *K. pneumoniae* ATCC 700603 or *E. coli* ATCC 35218 can be used for routine QC of ceftolozane-tazobactam.

Eliminate footnotes (d) and (e) to Table 4A, page 143 of M100-S24.

The Subcommittee approved adding the *K. pneumoniae* ATCC® 700603 ranges in a new column with appropriate footnotes that would be added in Table 4A in M100. **Approved 9-1; 1 abstain, 1 absent.**

5. User QC questions and proposed revisions. –
None at this time.

6. Quality Control Tier 3 Monitoring:

REQUEST FOR DATA

The QC Working Group requests MIC data for meropenem and/or clarithromycin vs. *S. pneumoniae* ATCC® 49619. Please submit this data directly to Sharon Cullen (sharon.k.cullen@siemens.com).

VIII. REPORT OF THE METHODOLOGY WORKING GROUP (Electronic Folder 8)

Co-Chairholder - Brandi Limbago
Co-Chairholder - Stephen Jenkins

Members Present: Romney Humphries, Laura Koeth, Sandra Richter, Darcie Roe-Carpenter, Katherine Sei, Susan Sharp, Ribhi Shawar, John Turnidge

Members Absent: Seth Housman

1. Report from Anaerobe Ad Hoc Working Group (WG): Darcie Roe-Carpenter, Chairholder
   Ad Hoc WG members: Diane Citron, Audrey Schuetz, Karen Anderson, Cindy Knapp, Meredith Hackel, Joanne-Dzink-Fox, Nilda Jacobus, Stephen Jenkins, Hanna Wexler, Laura Koeth

   a. Establishment of Anaerobe vancomycin epidemiological cutoff value (ECV) based on published studies from multiple sources:

   - MIC distribution data from 12 studies (U.S., Europe, Kuwait) that used reference AST methods, 2004 - 2014
   - Aggregate data (generated using many methods) suggest that MIC values for most wild-type isolates are ≤ 4 µg/mL

   WG Discussion:
   Question: What is the mechanism of vancomycin resistance in anaerobes? (Mostly unknown)
   Comment: ECV of 4 µg/mL looks to be very close to the normal distribution of isolates
   Comment: (John Turnidge): Not a true ECV if this is applied to multiple species of anaerobes
   Question: What is the purpose for establishing this? Really driven by needed to identify new vancomycin resistance in *Propionibacterium acnes* and *Clostridium difficile*
   Comment: Either call this something different from ECV or establish an ECV for *C. difficile* and *P. acnes*

Motion from WG: Like to see *P. acnes* and *C. difficile* broken out and re-presented
In favor: 10; Opposed: 0

***At plenary session, the Subcommittee agreed that ECV for *P. acnes* was appropriate, but did not approve ECV for *C. difficile* (Subcommittee Approved 9-1; 2 absent). The ECV for *P. acnes* will be placed in a separate table with additional information within M100 to education labs on the use of ECVs vs. clinical breakpoints (Refer to Appendix A at the end of these minutes to see additions to M100 that will be made).**

   b. Proposed several revised comments for M100, Table 2J and Table 1C. **Referred to Text & Tables Working Group for implementation.**

      i. Table 2J, General comment 1 (re: Intermediate range) - Proposed change to comment to make it consistent with other statements about 'Intermediate range' in the document. **WG Motion to accept as proposed. In favor: 10; Opposed: 0**

      ii. Table 1C, Note 2: Recommended to reduce comment to "If testing is requested in a polymicrobial infection, the most resistant strain should be reported (e.g., *Bacteroides fragilis* group isolates)

Comment: language unclear – how would one know which was most resistant? Or does this direct the user to test all and only report the most resistant?

WG Motion to modify to "Only the organism most likely to be resistant (eg, *B. fragilis* group) should be tested and results reported." In favor: 10; Opposed: 0
Motion to further revise comment to add: "Testing may not be necessary for polymicrobial anaerobic infections." In favor: 10; Opposed: 0

iii. Table 1C, Note 3: Recommended to reduce comment to 'Specific *Clostridium* species (e.g., *Clostridium perfringens, Clostridium septicum, Clostridium sordellii*) may be the singular cause of an infection, are typically susceptible to penicillin and ampicillin, and should be tested and the results reported. **WG Motion to accept as proposed In favor: 10; Opposed: 0**

iv. It was brought to the attention of the group that there is good guidance in M11 about when to test and how to report. Perhaps we should consider inserting that language rather than trying to wordsmith these. (**Text &Tables Working Group to review this**).

c. Proposed revisions to Quality Control (QC) recommendations for Table 2J (**Refer to Text & Tables WG minutes**)

i. Proposed revision to "For commercial system, refer to the manufacturer's instructions for QC recommendations and ranges. For Reference methods: Agar dilution – Test two of the following QC stains; BMD – test one or more QC strains.

Questions: Why two for agar dilution but just one for BMD? (Historic; no one knows). Laura proposed we only test the minimum number of strains to get an on-scale result.

d. Anaerobe antibiogram in previous versions was outdated; proposed new version with updated information. **Motion to update antibiogram with more current information. In favor: 10; Opposed: 0**

e. Broth microdilution for bacteria other than *B. fragilis* – Ran out of time this year: **Tabled for future work.**

2. Report from Broth Microdilution Working Group – Bill Brasso, Chairholder (Informational Only)
Ad Hoc WG members: Susan Kircher, Cindy Knapp, Laura Koeth, Katherine Sei, Ribhi Shawar, John Turnidge, Michael Ullery, Halsey Boyd, Bob Rennie

Goals of the BMD ad hoc Working Group are to:

a. Define main sources of variability in BMD assay
b. Demonstrate how these impact reproducibility
c. Determine if acceptable level of variability should be defined for BMD
d. Publish as appropriate

Taking two tracks: Technical aspects, statistical aspects.

Technical issues: To be addressed by review of QC data and expanding with challenge set data, administer survey for laboratories that perform BMD (especially those who contribute to QC data and/ or M23 studies).

Statistical issues: We know there is variability in the performance of our QC strains, but what about clinical isolates? Single clinical isolate tested in replicate can demonstrate 4-dilution variation. Team looking into new ways to deal with variance based on statistical methods.

Issues with reading: Guidance from M07 states:

- that MIC is supposed to be read at the concentration that 'completely inhibits growth'
- 'for a test to be valid, there must be at least 2mm of growth in the positive control well'
- for SXT 'read at 80% reduction'
- 'for skipped well, read and report highest MIC after the skip'

Suggest that text to be added re: reading of trailing endpoints; read MIC at first well that shows prominent reduction in growth

- Proposal to add pictures for how to read MICs
- **Action items**: 1) prepare and administer survey; 2) clarify issues about test performance from M07; provide examples and pictures.
- Interest in re-examining skipped wells, maybe changing guidance to 'repeat' or 'don't report unless repeated'.

3. Report from Joint CLSI/EUCAST Polymyxins Working Group: John Turnidge, Chairholder (Informational only**)**

WG members: Alasdair MacGowan, Johan Mouton, Stephen Jenkins, Roger Nation, Luis Martinez-Martinez

a. Importantly, it has recently come to light that there are issues with the estimation of protein binding in mouse plasma. This obviously impacts on the estimations of $fAUC/MIC$ ratio estimates, a necessary step towards settling on PK-PD cutoff values. This has led to

Some further work, still ongoing, in the mouse thigh and lung models to ensure that the new protein binding estimates in mice lead to the correct choice of fAUC/MIC.

b. We are also have data on the direct comparability, or otherwise of colistin versus polymyxin B MICs from Helio Sader at JMI laboratories. Preliminary analysis of 15,377 strains of Gram-negative bacteria collected world-wide through the SENTRY program shows that polymyxin B MICs are generally higher that those of colistin against wild-type strains, while the reverse applies to strains with MICs above the wild type (see attached scattergram). This suggests that the two agents are not interchangeable, and that colistin is not a simple surrogate for polymyxin B. We speculate that this difference may be an artefact of the testing system, and may be driven by increased binding of polymyxin B at lower concentrations, but overall greater potency that manifests at the higher concentrations. The source(s) of the reagents used in the Trek plates is also being checked, given that both colistin and polymyxin B are mixtures with two predominant components, whose ratio varies depending on manufacturer.

4. Report from the CarbaNP working group: Robin Patel, Chairholder
WG members: Maria Traczewski (co-chairholder), Scott Cunningham, Shawn Vasoo, Romney Humphries, Janet Hindler, Dan Sahm, Meredith Hackel, Elizabeth Palavecino, Audrey Schuetz, Stephen Jenkins, Karen Anderson, Brandi Limbago

   a. The evaluation included 80 organisms (Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter* spp.) tested in 7 different sites for a total of 560 results.
   b. A summary of results:
      i. The rate of invalid results was 1.6% (1 carbapenemase positive isolate and 9 carbapenemase negative isolate)
      ii. The specificity of the test was 98%
      iii. The sensitivity of the test by carbapenemase was:
         1. NDM (N=13), 99%
         2. KPC (N=10), 93% (Note – false negatives occurred for two isolates with low cabapenem MICs)
         3. VIM+IMP (N= 12), 99%
         4. SME (N=2), 100%
         5. SPM (N=1), 86%
         6. OXA (N=6), 24%
   c. WG discussion
      i. Some concerns re: the reading of endpoints. (An invalid result was a common outcome.)

ii. Additional information re: OXA48 - seems to correlate with MIC; some have suggested this could be overcome with higher inoculum, but that would require re-validation of the entire study.
iii. Solution A labile - may not keep more than 24 hours (vs. expected 3 days)
iv. Phenol red powder crashes out of solution (could start with liquid phenol red)
v. Imipenem is expensive - went through entire vial of powder over course of study
vi. Question: Could pharmacy-grade imipenem be used? Price much lower than powder

Pros: Rapid, good performance overall (except OXA); good specificity
Cons: Cumbersome; expensive; may not be suitable for routine use

Subcommittee voted to include Carba NP procedure in M100-S25; Approved 11-1. Refer to Appendix B at the end of these minutes to see additions to M100 that will be made in incorporate this screen test.

5. Report from Ad Hoc Table 1 Clean-up Working Group: Stephen Jenkins (reporting for Mary York)
Roster: Mary York (chairholder), Barth Reller, Tom Thomson, Dwight Hardy, Tony Mazzulli, Susan Munro (recording secretary), Stephen Jenkins

The purpose of this ad hoc WG is to identify outdated text in M100.

There is a need for algorithm for when/ how to delete drugs in M100. Their proposal:

- In order **to stay in Table 1**, an antibiotic must be at least FDA-cleared and available in the U.S.
- In order for a drug **to stay in Table 2**, it must be used somewhere in the world to treat active infections.
- If in doubt, delete!

They followed this algorithm using FDA, Wikipedia, and Medical websites as the primary sources to determine availability. Some pharmaceutical and chemical web sites were also checked, as well as China and India sites

a. Proposed list for **deletion from Tables 1** (discontinued in US, but not internationally):
azlocillin, mezlocillin, <u>ticarcillin</u>, cloxacillin, mecillinam, cephradine, cefamandole, cefoperazone, enoxacin, <u>lomefloxacin</u>, and sparfloxacin

Subcommittee approved to delete those shown above (<u>underlined are the drugs actually in Table 1s that were deleted</u>) **Approved 12-0**

b. Proposed list for deletion in Tables 1 and 2 (not officially discontinued but not thought to be used clinically):

carbenicillin, methicillin, **cephalothin**, cephapirin, **cefonicid**, **ceftizoxime**, **cefmetazole**, **moxalactam**, loracarbef, spectinomycin, dirithromycin, cinoxacin, grepafloxacin, and trovafloxacin

Also:

i. telithromycin (black box warning; banned in many countries);
ii. gatifloxacin (ophthalmic only in US; available in China)
iii. ofloxacin - not used in US; available in China. Can't confirm use.

**Cefonicid, ceftizoxime, cefmetazole, and moxalactam (still used in Asia); cephalothin in Australia.

WG Discussion:
Comment: John Turnidge recommends using Martindale as a reference for drugs registered and in use.

Question from Dwight Hardy: We should investigate whether or not our approach (that is, our algorithm) is the appropriate one.

Comment from Barth Reller: This is based on the same process that removed ceftriaxone testing vs. staphylococci. Our data for breakpoints are likely unreliable and, therefore, may do more harm than good. There was agreement up front (before the data were examined) about Table 1 criteria - drugs must be AVAILABLE in the U.S. for Table 1, but leave some in for drugs that are available and used in other countries. This needs to be done on a more regular basis, and according to specified criteria. Start with more conservative approach now, but develop a more regular approach and review.

Comment from Susan Sharp: probably too early to remove cephalothin from document
Comment from Akinobu Ito: Cefonicid, ceftizoxime, cefmetazole, moxalactam are still used in Asia.

WG Motion: That the Working Group consider going back through the lists and determine more completely what is available outside US.
In favor: 9; 0pposed: 0, Absent: 1
WG Motion: Approve proposed removal from Table 1 only (Item A above)
In favor: 9; Absent: 1
***Note from subcommittee: Confirm that these agents are truly not available in the US before removing them from Table 1. No Changes at this time.**

WG Motion to remove drug names/ classes and to refer to Glossary (and clean up glossary to remove obsolete agents being removed from Table 1) (later brought to attention of the WG group that Text &Tables WG is already working on this)
WG vote - In favor: 9; 0pposed: 0; Absent: 1

c. Proposed: Table 1 changes:
Add fosfomycin to Group U for Enterobacteriaceae with note "for testing and reporting of *E. coli* urinary tract isolates only." Change to U in Table 2A.
Do the same thing for *Enterococcus* (and change Table 2D)
WG Motion to accept as proposed In favor: 9; Opposed: 0; Absent: 1
Approved by the Subcommittee – 12-0

d. Proposed addition of comment to modification Table 1 comment A, comment B and Tables 2: "Doxycycline and minocycline are not routinely reported on organisms isolated from the urinary tract because of low urine concentrations"
Question: Based on what data? Seems we heard that high doxycycline levels could be achieved in urine from previous discussions.

Recommendation from Methods WG - request more data
Subcommittee also agreed that more information is needed – no change

For Tables 1A, *Acinetobacter* spp., *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, *Haemophilus influenzae/parainfluenzae*: removal of some drugs and rearrangement of others
– **Discussion tabled** by WG due to time constraints

e. Proposed: remove this comment "*MIC testing only; disk diffusion test unreliable" from all Tables 1, as it does not relate to purpose of Tables 1 and is evident in Tables 2.
f. Previously it was approved to remove from Tables 2F and 2G the following fluoroquinolones: Enoxacin, lomeflox, sparflox fleroxacin, and ofloxacin. It was brought to the subcommittees attention by Dr. Hui Wang that these drugs are still used in China so the subcommittee agreed to not remove these at this time (no vote taken).

– **Discussion tabled** for items e and f by WG due to time constraints

6. Report from the Intrinsic Resistance Working Group: Barb Zimmer, Chairholder

WG members: Dyan Luper (Recording Secretary), Jeff Alder, Rafael Canton, German Esparza, Kate Murfitt, Sandy Richter, Susan Sharp, Carole Shubert, Paul Schreckenberger, and Tom Thomson

- Adding *Serratia marcescens* vs. tetracycline (not doxycycline), (not tigecycline nor minocycline) to Appendix B.1 *Enterobacteriaceae*; would state: ***S. marcescens* should be considered resistant to tetracycline, but not intrinsically resistant to doxycycline, minocycline or tigecycline" (wording same as current note for *P. stuartii*).

  a. Reviewed literature: Mahlen, S., 2011 Serratia infections: from military experiments to current practice. Clin. Micro. Rev. 24:755. "All *S. marcescens* …isolates were resistant to tetracycline in the 2003 study by Stock and others…, and most strains were resistant to other tetracyclines, such as doxycycline…"

b. Reviewed current surveillance data

i. For *Serratia marcescens* vs. tetracycline (worldwide) and doxycycline (worldwide) from JMI courtesy of Helio Sader: (Note: Breakpoints for tetracycline, doxycycline, minocycline: 4/8/16)

Tetracycline: *Serratia marcescens*
Validated: sentryMICValidated
2013
Total: 1013

MIC	<= 0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	> 32	MIC_{50}	MIC_{90}
Count	0	0	0	0	0	6	7	98	283	58	192	369	32	>
Percent	0.00	0.00	0.00	0.00	0.00	0.59	0.69	9.67	27.94	5.73	18.95	36.43		
Cum Pct	0.00	0.00	0.00	0.00	0.00	0.59	1.28	10.96	38.89	44.62	63.57	100.00		

Doxycycline: *Serratia marcescens*
Validated: sentryMICValidated
2013
Total: 1013

MIC	<= 0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	> 8	MIC_{50}	MIC_{90}
Count	0	0	0	1	0	0	2	18	227	335	309	121	4	>
Percent	0.00	0.00	0.00	0.10	0.00	0.00	0.20	1.78	22.41	33.07	30.50	11.94		
Cum Pct	0.00	0.00	0.00	0.10	0.10	0.10	0.30	2.07	24.48	57.55	88.06	100.00		

ii. For Serratia marcescens vs. tetracycline from IHMA courtesy of Bob Badal:

IHMA database: 16,574 isolates, only 168 were tested against tetracycline; of those, only 11 were reported as S to tetracycline (and of those 11, 9 were right at the breakpoint of 4 µg/mL, 1 was at 2 µg/mL, and 1 was at 0.25 µg/mL). No doxycycline data at all for *Serratia*.

c. Working Group June 2014: Voted 5-3 to add footnote that *S. marcescens* was resistant to tetracycline, but not minocycline, doxycycline nor tigecycline. Opposing votes thought that some organisms may test susceptible based on data in table, so all would not be R, and that tetracycline may be used for urinary tract infections, where it would be concentrated.
(Note: In full AST Subcommittee vote, it was voted not to add this due to the opposing votes reason - Subcomittee vote 12-0).

- Clarification of text: Appendix B1: insertion of the word "complex" with the name of Enterobacter cloacae. WG Vote 8-0 Yes

- Clarification of text: Appendix B3: insertion of the word "These" in front of Note 1: "These Gram-positive bacteria are also intrinsically resistant to aztreonam…" similar to the note in Appendix B2. WG Vote 8-0 Yes

- Discussion of other intrinsic resistant tables, specifically for those organisms with AST methodology in M45; Initial worksheet of the M45 organisms to initiate this discussion was reviewed, based upon previous edition of M45. This was provided after the meeting to the chairs of the M45 revision. Should Intrinsic Resistance for M45 organisms be in M100 or M45 (or both)? (IR WG thought both). Will also ask Anaerobe AST to develop list as they move forward with developing their revised document.

7. Report on atypical *Staphylococcus aureus* that don't grow in Broth Microdilution (BMD) panels: Romney Humphries

   a. Still waiting to receive more isolates - only about 40 to date from about 3 sources. Not all of them maintain their non-growth phenotype after passage/ freezing. Still collecting - keep sending them in. ***Robin Patel mentioned that she does research in this area and would like to participate in study.***

8. Report on ceftriaxone vs. MSSA issue: Mary Jane Ferraro

   a. A.J. Pickering paper caused much consternation. Pickering suggested that 60% of MSSA were actually resistant to ceftriaxone (as tested by Etest).
   b. Tests with BMD or disk clearly show isolates to be Susceptible, but can test as I or R using ceftriaxone Etest strips.
   c. There is still a need for appropriate dosing of ceftriaxone for Community Acquired Pneumonia and for bone/ joint infections (1g qD is NOT sufficient and should not be used).
   d. Concern was expressed that not reporting an MIC results in treatment with doses lower than those needed
   e. Recommendation - Take this to the subcommittee for discussion.

9. Report on Oritavancin MIC testing: Ron Jones (Informational presentation)
   a. The drug has a very long half-life (days not hours). This allows for a single loading dose for 7-10 days therapy
   b. There are some technical issues with MIC testing, especially skipped wells. Could we use a surrogate?
   c. Oritavancin is many times more potent than vancomycin

d. MICs of ≤0.12 μg/mL for oritavancin could be reliably predicted with vancomycin Susceptibility (MIC ≤ 2 μg/mL) for staphylococci; similar findings for α-hemolytic streptococci.
e. For enterococci: since oritavancin is so much more active, Susceptibility to vancomycin predicts Susceptibility to oritavancin, but Resistance to vancomycin (VRE) does not predict Resistance to oritavancin.
f. For β-hemolytic streptococci would need ≤0.25 breakpoint.
g. Expect to have breakpoints from FDA August 2014, so could bring surrogate issue to CLSI at January meeting. Will likely never have a disk or Etest, and MIC testing doesn't work well with frozen/ dried BMD panels.

10. Call for unmet needs:
    a. Should we address the issue of *mecA*-positive isolates that test as cefoxitin Susceptible? **YES**
    b. Should we address the issue of how to perform direct Antimicrobial Susceptibility Testing (AST) from positive blood cultures? **YES**
    c. Molecular tests for resistance - **YES**
    d. Should we address the issue of how to report molecular tests for specific mechanisms of resistance? **YES**
    e. Develop methods for testing lower potency disks used in Europe (**this was a suggestion added by the executive committee**).

Plenary session disucssion:

1. Molecular tests for antimicrobial resistance mechanisms

Subcommittee discussion:

- CLSI can produce guidance that is agnostic to specific commercial assays but provides laboratorians with useful information about the use of new molecular tests.
- We have AST schizophrenia. We have phenotype-guided criteria for Enterobacteriaceae, but genotype-based criteria for staphylococci. The organism is seeing the phenotype, not the genotype, so we need to focus there.
- No test is going to be 100%, including our phenotypic tests.

This needs to be addressed. We need to form an ad hoc group to address this issue, and this could be conveyed with a new table in the document

2. Is there a need for guidance around direct antimicrobial susceptibility testing?

Subcommittee discussion:

- Most commercial manufacturers have protocols for this and recognize that this as off-label use already. This has important testing caveats and limitations, so a mechanism for guiding laboratories that do this would be very useful.
- Even though molecular tests are coming along as 'the' rapid assay, this will have a role in places where WGS is still not an option. Laboratories will continue to do this, and thus guidance is important
- Hesitant to add this to the document because inclusion in CLSI often means one HAS TO do this, when laboratories will still need to perform the actual test and won't be reimbursed for both.
- This is something that needs to be validated within in each laboratory, and not developed as a reference method.

Suggest that an ad hoc group be formed to address this issue and begin investigating

IX. REPORT OF THE TEXT AND TABLES WORKING GROUP (Electronic Folder 9)

Co - Chairholder – Ms. Jana Swenson

Co - Chairholder – Ms. Maria Traczewski

Members Present: Janet Hindler, Peggy Kohner, Dyan Luper, Linda Mann, Susan Munro, Dale Schwab, Tom Thomson, and Nancy Waltz

Members Absent: Melissa Miller, Flavia Rossi, Jeffrey Schapiro, and Mary York

Working Group (WG) Objectives:

- Complete the revision of M02 and M07 for publication in Jan. 2015
- Discuss comments from WG review of M100-S25 also for publication in Jan. 2015

M100-S25:

- **Refer to Agenda Materials - Tab 9 10_M100 Comment Table 05202014**
- **Few small revisions were made after that:**
  - Instructions for Use, section VII Screening Tests, p. 30, the Screening Test table was simplified for *S. aureus* and CoNS β-lactamase testing
  - Clarified Table 2J (Anaerobe) QC Recommendations box concurrently with the Methods WG.

- Added comment about using manufacturers QC to Table 2J QC box. (see example below) **Approved by Subcommittee 12-0**

- **Table 2J – Anaerobe QC Recommendations (after Methods WG discussion)**

Routine QC Recommendations (See Tables 5D and 5E for acceptable QC ranges.)

Test one or more of the following organisms. The choice and number of QC strains tested should be based on obtaining on-scale endpoints for the antimicrobial agents tested.

Bacteroides fragilis ATCC® 25285

Bacteroides thetaiotaomicron ATCC® 29741

Clostridium difficile ATCC® 700057

Eubacterium lentum ATCC® 43055

NOTE: When a commercial test system is used for anaerobe susceptibility testing refer to the manufacturer's instruction for quality control test recommendations and QC ranges.

- Since instruction to use manufacturers suggested QC if using other than reference method was buried in a footnote in appendix C footnote "g" it was suggested that:

  - We add this footnote to all Table 2C QC boxes.
  - Or we add a statement to the Instruction for Use Section VIII Quality Control and Verification about following manufacturer's recommendation for commercial QC (p. 32)

  Subcommittee voted to add to all Table 2 QC boxes. **Approved 12-0**

M2 and M7 Revisions for publication in January

- Since June 2013, the WG has reviewed both documents extensively.
- The Subcommittee (SC) was asked to review the documents between January and June and all comments raised by the SC were addressed. NOTE: the QC sections that are included in the versions in the agenda book have been updated since the documents were circulated for review by the SC.
- A few changes are still being finalized.
- These 2 documents were not voted on at this meeting, but once all final changes are made a vote will be done electronically.

M2 and M7 Changes to be finalized:

- Revision of Appendixes A1 – A4 (flow charts for QC)
- Reformatting of documents to include:

  - Change to a Path-of-Workflow format which reduces documents from 17 Sections/Chapters down to 5:

- Pictures to illustrate endpoints
- Call out boxes to emphasize certain points
- Formatting some information in tabular form to eliminate repetition
- Note: none of these changes will be in content which was already reviewed, only the way in which the information is displayed.

Example of Path-of Workflow Table (Step/Action Table):

Inoculation of Test Plates

Step	Action	Comment
1a.	Dip a sterile cotton swab into the adjusted suspension.	Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension.
1b.	Rotate the swab several times and press firmly on the inside wall of the tube above the fluid level.	This removes excess fluid from the swab.
2a.	Inoculate the dried surface of an MHA plate by streaking the swab over the entire sterile agar surface.	
2b.	Repeat this procedure by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum.	
2c.	As a final step, swab the rim of the agar.	
3.	Leave the lid ajar for three to five minutes, but no more than 15 minutes.	This allows any excess surface moisture to be Absorbed before applying the drug impregnated disks.

Trailing Endpoints

- Wording to describe how to read trailing endpoints was approved by the SC in January 2012 for inclusion in M07-A12 as follows:

  "For some antimicrobial agents (such as for chloramphenicol, clindamycin, erythromycin, linezolid, tetracycline), trailing growth can make endpoint determination difficult. In such cases, read the MIC at the first well that shows a prominent reduction in growth. Tiny buttons of growth should be ignored (see picture below)."

The WG discussed and decided to modify based on what we know by experience.

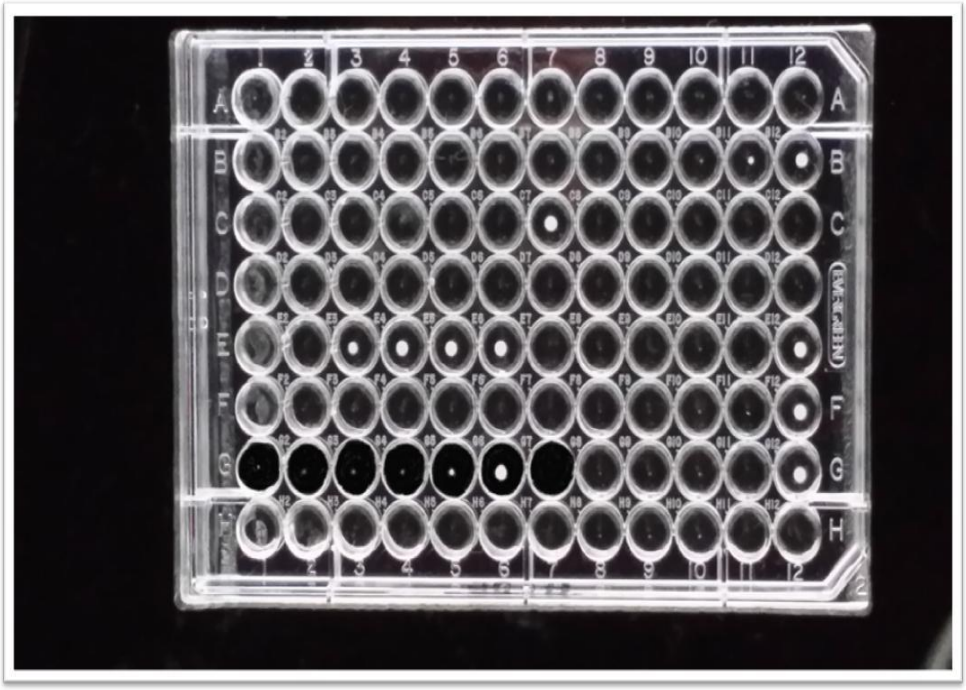
Revised version approved by T&T WG:

> "For Gram positive cocci when testing chloramphenicol, clindamycin, erythromycin, linezolid, and tetracycline, trailing growth can make endpoint determination difficult. In such cases, read the MIC at the first well where the trailing begins. Tiny buttons of growth should be ignored (see pictures below)." WG Vote: 9-0

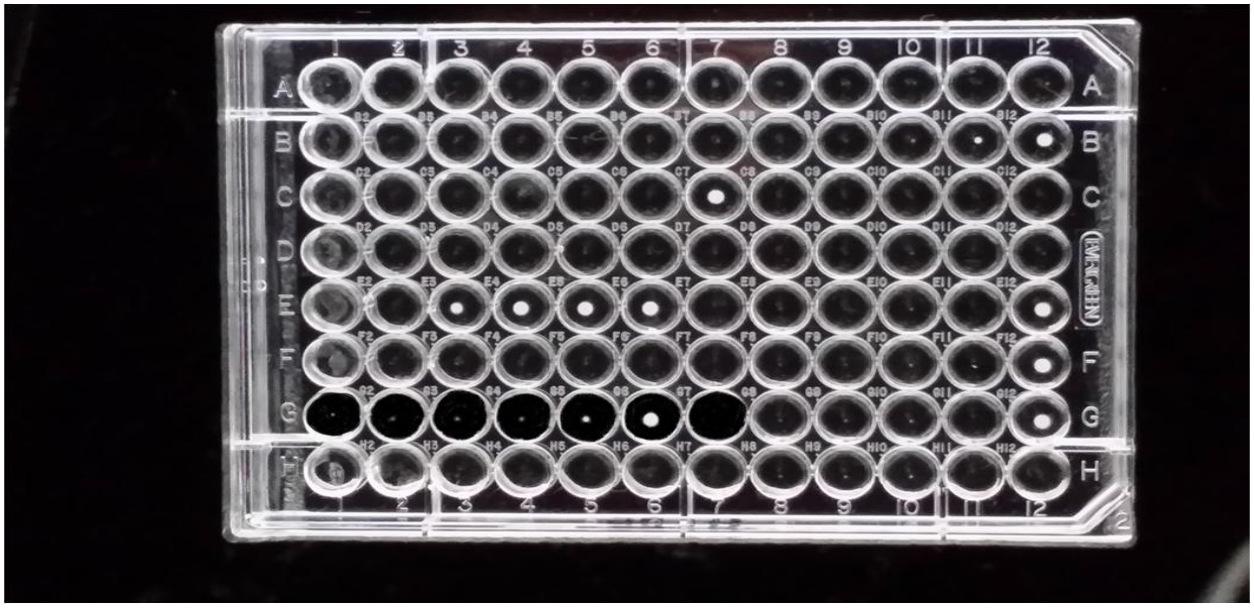
Subcommittee approved 12-0.

Pictures of R and those below for all 3 drugs will be added. We will insert pictures to use as guidelines for reading trailing endpoints and SXT 80% endpoints using the same format used in M11.

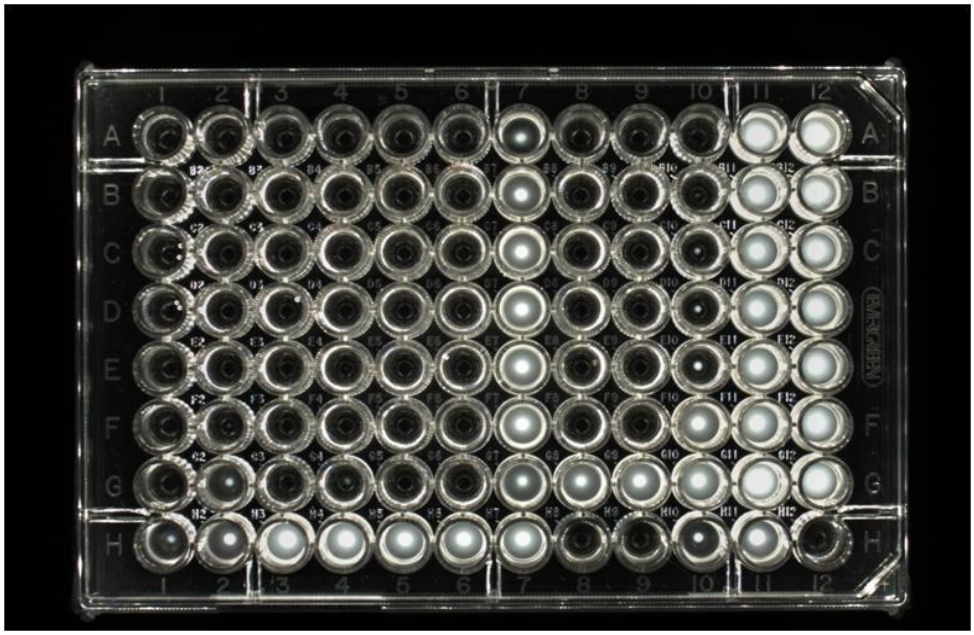
Trailing endpoints
Erythromycin G1-6 (8-0.25 µg/mL)
MIC = G4



Trailing endpoints
Linezolid, B8-12 (16-1 µg/mL)
MIC = B10



Trailing endpoints
SXT, A10-F10, 152/8-9.5/0.5
endpoint at E10



Text & Tables Charter

The suggested addition to the Text &Tables WG Charter is shown in blue below:

- Report standard reference methods and interpretative criteria
- Provide input to CLSI on education ideas and opportunity's (e.g., webinar topics, articles for journal publication).
- Work with various AST Ad hoc WGs to develop educational materials to post to the CLSI AST SC webpage.
- Recruit members to join Ad hoc WG to investigate text & tables issues if needed
- WG requests ideas, volunteers and input on educational materials.

X. M23 WORKING GROUP UPDATE

Co – Chairholders – Dr. Mair Powell and Mr. Kerry Snow

Members Present: Halsey Boyd, Patricia Bradford, Denise Holliday, Sharon Shinn, John Turnidge, Matt Wikler

Members Abent: Sharon K. Cullen, Margaret Ordóñez Smith de Danies, Seong Jang, Ryan OwenJohn Rex, Daniel Rubin, Hala Shamsuddin, Thamban Valappil, Mel Weinstein

Dr. Powell gave an overview on the work to date for the revision of the M23 document. Since the January meeting the WG has held several conference calls including a joint call with the PK/PD Ad Hoc WG. The M23 WG met on Saturday with the last hour of the meeting being a joint session with the PK/PD Ad Hoc WG. Currently there are still some pieces of the M23 draft that are being completed including the final revision of the QC section and the addition of a definition of ECOFFs along with informational text on what is needed to draft an ECOFF. The WG plans to have all final edits in by the end of August. The draft will then be reviewed by Dr. Powell and Mr. Snow prior to circulating the document to the subcommittee for review and comment. All comments received during this review will be addressed and the document revised as appropriate. The M23 document will be be submitted to the CLSI editors to prepare it for vote.

XI. PK/PD AD HOC WORKING GROUP UPDATE (Electronic Folder 10)

Co – Chairholders – Dr. Linda Miller and Dr. Paul Ambrose (absent)
Members Present: Eileen Kim (Recording Secretary), Seong Jang*, Jim Lewis, Ryan Owen*, Elizabeth Palavecchino, John Turnidge

*Participated by conference call for the WG meeting.

Dr. Miller gave an overview on work that the PK/PD WG is doing to provide text for the M23 document that will provide more guidance so that there is consistency in the data packages presented to CLSI. In reviewing the PK/PD section of M23, the WG has been working to define the various 'cut-offs' used to

determine a clinically relevant breakpoint. The 4 'cut-offs' that feed into setting a breakpoint are shown in the table below:

"Cut-Offs" to Use in Determining Clinically Relevant Breakpoints	
Epidemiologic cut-offs	"Wild Type" distribution
Non-clinical PK-PD cut-offs	1. Identify PK/PD target in non-clinical model (e.g. animal models such as neutropenic mouse thigh) 2. Using a population PK/PD model apply Monte Carlo Simulation to assess the probability of target attainment at potential PK-PD cut-off MICs
Clinical cut-offs (MIC vs Outcome Cutoff)	This is a simple observational correlation of response to MIC. In addition, it is helpful to review the overall efficacy of the study.
Clinical PK/PD cut-off (Integrated Patient Exposure Response cut-offs)	Identify **exposure-response relationship from patient clinical trial data** Integrated to include assessment of Clinical Response modifiers

Dr. Miller also discussed the 2 kinds of data packages that the WG updated in M23 as follows:

Non-clinical PK/PD Cut-offs

- Updated information on doing static *in vitro* studies
- Added details on the studies performed to derive non-clinical PK/PD cut-offs and provided examples of acceptable models.
- Addressed the magnitude of the PK/PD index.
- In the Monte Carlo Simulation section the WG has tried to address what is the probability of target attainment and provided language for what % of patients do you want to achieve that target (eg, 90% patient target attainment (PTA) at a given MIC is considered acceptable by CLSI).

Clinical PK/PD Cut-offs (Integrated Patient Exposure Response cut-offs)

- Using patient clinical trial data, clinical PK/PD (aka exposure-response) relationship(s) for efficacy and targets based on such relationship(s) can be identified
- Using a population PK model and the results of the clinical PK/PD analyses, apply Monte Carlo Simulation to assess the following:
  - Model-predicted probability of clinical response by MIC using the clinical PK/PD relationship.
  - Probability of target attainment by MIC using the clinically derived PK/PD target.

XII. SUBCOMMITTEE VOTE ON M100-S25

The subcommittee members voted to accept the M100-S25 supplemental tables with the changes approved at the January and June meetings and recommend the M100-S25 Tables to the Consensus Committee on Microbiology for approval to be published as supplemental tables.

A tally of the votes follows:

Total Subcommittee Members = 12
Votes to Accept = 12 (P. Bradford, G. Eliopoulos, J. Hindler, S. Jenkins, J. Lewis, B. Limbago, L. Miller, D. Nicolau, M. Powell, J. Turnidge, M. Weinstein, B. Zimmer)
Votes to Accept with Comment = 0
Votes to Reject = 0
Votes not Received = 0

XIII. AGENDA SUBMISSIONS FOR 11-13 JANUARY 2015 MEETING IN FT. LAUDERDALE FLORIDA

Materials for the January meeting will be distributed to the subcommittee 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. Please note there will not be internet access in the meeting rooms.

To meet the schedule to have materials available for review a few weeks prior to the meeting, submission due dates and requirements must be met. In order to present at the 11-13 January 2015 meeting please:

1) Submit agenda materials electronically as a PDF file **on or before Monday, 8 December 2014.**

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 6, 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 Jean B. Patel, PhD, D(ABMM) (vzp4@cdc.gov), 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.

XIV. ADJOURNMENT – The meeting adjourned at 10:00 a.m. on Tuesday, 1 July 2014.

Respectfully submitted,

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

Appendix A.

Instructions for Use of Tables, Part 6, new C:

C. **For *Propionibacterium acnes* in Table 2J-2, a clinical breakpoint resulting in interpretive criteria for vancomycin has not been established. In place of interpretive criteria ("breakpoints" or "clinical breakpoints") an epidemiological cutoff value (ECV) is listed (see Appendix G). ECVs and breakpoints are very different. Breakpoints are established using MIC distributions, pharmacokinetic (PK) and pharmacodynamic (PD) data, and clinical outcome data (as described in CLSI document M23). Because they are based on pharmacologically and clinically rich datasets, breakpoints are considered to be robust predictors of likely clinical outcome. By contrast, ECVs are MIC values that separate bacterial populations into those with (non-wild-type [NWT]) and without (wild-type [WT]) acquired and/or mutational resistance mechanisms based on their phenotypes (MICs). They are, therefore, based on *in vitro* data only.**

ECVs are principally used to signal the emergence or evolution of NWT strains. ECVs are <u>not</u> clinical breakpoints, and, thus, proven clinical relevance of ECVs has not yet been identified or approved by CLSI or any regulatory agency. If a strain of *P. acnes* is detected that has an MIC above the CLSI vancomycin ECV, the strain should be retested to confirm results and the laboratory should discuss the findings with relevant clinical services (eg, infectious diseases/pharmacy) including the limitations of using MIC to guide therapy in the absence of interpretive criteria (see Appendix G). The MIC should not be reported with an interpretation.

New Table 2J-2:

Table 2J-2. Minimal Inhibitory Concentration Epidemiological Cutoff Values for *Propionibacterium acnes*

General Comments

(1) Refer to Appendix G for an explanation of epidemiological cutoff values (ECVs). When considering vancomycin therapy for a *Propionibacterium acnes* infection, there are insufficient data available currently to establish clinical breakpoints. Based on ECVs,[1-4] wild-type (WT) *P. acnes* isolates without acquired and/or mutational resistance mechanisms have vancomycin MICs of ≤ 2 µg/mL. ECVs can be used as a measure of the emergence of strains with reduced susceptibility to a given agent. If *P. acnes* strains were to acquire a resistance gene or undergo gene mutation resulting in reduced susceptibility, vancomycin MIC values ≥ 4 µg/mL would be expected. Experience suggests non-wild-type (NWT) *P. acnes* strains are less likely to respond to vancomycin therapy. MIC results must be discussed with appropriate clinical specialists (eg, infectious diseases and pharmacy) when using ECVs for interpretation. The ECVs should not be used as a clinical breakpoint, and the MIC result should be reported with an interpretaion. See Appendix G for more information.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	ECV (µg/mL)		Comments
	WT	NWT	
Vancomycin	≤ 2	≥ 4	

Abbreviations: ECV, epidemiological cutoff value; NWT, non-wild-type; WT, wild-type.

Appendix A. (continued)

References for Table 2J-2:

[1] **Citron DM, Kwok YY, Appleman MD. In vitro activity of oritavancin (LY333328), vancomycin, clindamycin, and metronidazole against Clostridium perfringens, Propionibacterium acnes, and anaerobic Gram-positive cocci. *Anaerobe.* 2005;11(1-2):93-95.**

[2] **Goldstein EJ, Citron DM, Merriam CV, Warren YA, Tyrrell KL, Fernandez HT. In vitro activities of the new semisynthetic glycopeptide telavancin (TD-6424), vancomycin, daptomycin, linezolid, and four comparator agents against anaerobic gram-positive species and Corynebacterium spp. *Antimicrob Agents Chemother.* 2004;48(6):2149-2152.**

[3] **Oprica C, Nord CE; ESCMID Study Group on Antimicrobial Resistance in Anaerobic Bacteria. European surveillance study on the antibiotic susceptibility of Propionibacterium acnes. *Clin Microbiol Infect.* 2005;11(3):204-213.**

[4] **Tyrrell KL, Citron DM, Warren YA, Fernandez HT, Merriam CV, Goldstein EJ. In vitro activities of daptomycin, vancomycin, and penicillin against Clostridium difficile, C. perfringens, Finegoldia magna, and Propionibacterium acnes. *Antimicrob Agents Chemother.* 2006;50(8):2728-2731.**

New Appendix G in M100:

Appendix G. Epidemiological Cutoff Values (ECVs)

What are ECVs?

ECVs are MIC values that separate bacterial populations into those with and without acquired and/or mutational resistance mechanisms based on their phenotypes (MICs). ECVs are based solely on *in vitro* data. The term "Wild-type" (WT) is used to describe strains with MIC values at or below the ECV that are presumed to possess acquired and/or mutational resistance mechanisms, while the term "Non-Wild-type" (NWT) is used to describe strains with MIC values above the ECV that are presumed to possess acquired and/or mutational resistance mechanisms. ECVs are principally used to signal the emergence and evolution of NWT strains. They are not the same as clinical breakpoints. The ECV is defined as the MIC value that best defines the estimated upper end of the WT population.

How are ECVs Determined?

ECVs are determined by collecting and merging MIC distribution data from a range of sources, and then applying techniques for estimating the MIC at the upper end of the WT distribution. In order to be reliable, ECVs are estimated by accounting for both biological (strain to strain) variation and MIC assay variation within and between laboratories. They are based on the assumption that the WT distribution of a particular antimicrobial/organism combination does not vary geographically or over time.

Several conditions must be fulfilled in order to generate reliable ECVs. The most important are:

1) An ECV can only be determined within a single species because of the genetic diversity between species within a genus.

2) All MIC values included in the merged dataset must have been determined using a recognized reference method such as the CLSI MIC broth dilution method (M07)[1] which is also the methodology outlined in international reference standard (ISO-20776-1).[2]

3) Data must be sourced from at least three separate laboratories, and there should be at least 100 unique strains included in the merged dataset.

Appendix A. (continued)

4) As much as possible, the MIC values included in an individual laboratory's dataset must be "on scale". This applies particularly to MICs of the presumptive WT strains. Before merging data for ECV estimation the MIC distribution from each individual laboratory is inspected, and if the lowest concentration tested is also a mode, then these data cannot be included in the merged dataset.

Once acceptable data are merged, there are several methods that can be used to estimate the ECV. The simplest is that of visual inspection. This generally works for MIC distributions when there is clear separation of WT and NWT. When there is obvious overlap between WT and NWT strains, visual inspection becomes too subjective. In general, statistical methods are preferred as they remove any potential observer bias from the estimation. The two most widely referenced methods are those of Turnidge et al.[3] and Kronvall[4].

Estimation of ECVs from MIC distributions may be supplemented with molecular tests for known resistance mechanisms, as a form a validation. The detection of a resistance gene *per se* in strains with MICs at or below the ECV does not necessarily contradict the choice of ECV, unless it can be accompanied by evidence that the gene is being expressed.

How are ECVs used to Set Clinical Breakpoints?

Clinical breakpoints are set using many criteria as detailed in CLSI document M23[5], including MIC distributions for the antimicrobial and relevant populations of bacteria, *in vitro* and *in vivo* PD, human PK and clinical outcome. MIC distributions and ECVs are thus just one component of a whole range of data used to set clinical breakpoints.

How can ECVs be used by the Clinical Microbiology Laboratory?

In rare clinical circumstances, experience may suggest an antimicrobial for use where no clinical breakpoints exist. For example, vancomycin may be considered for treatment of a *P. acnes* infection, but there are insufficient data available to establish clinical breakpoints with interpretive criteria. This is due principally to the absence of strains with acquired resistance and a lack of clinical outcome data.

MIC testing using a reference or approved method and ECVs for the drug/organism combination might then be used to determine if the patient's isolate of *P. acnes* is a WT or NWT strain. If the vancomycin MIC is at or below the ECV (≤2 µg/mL) it can be assumed that the isolate is a WT strain. If the vancomycin MIC is ≥ 4 µg/mL the strain should be re-tested to confirm the NWT result. The confirmed MIC result and the ECV data should be discussed with relevant clinicians/pharmacists. A comment could be added to the report indicating that MIC results were discussed with relevant clinical services (infectious diseases/pharmacists). The MIC result should not be reported with an interpretation.

References for Appendix G:

1. **CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Standard Draft.* CLSI document M07-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.**
2. **ISO. *Clinical laboratory testing and in vitro diagnostic test systems – Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – Part 1: Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.* ISO 20776-1. Geneva, Switzerland: International Organization for Standardization; 2006.**
3. **Turnidge J, Kahlmeter G, Kronvall G. Statistical characterization of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. *Clin Microbiol Infect.* 2006; 12(5):418-425.**
4. **Kronvall G. Normalized resistance interpretation as a tool for establishing epidemiological MIC susceptibility breakpoints. J Clin Microbiol. 2010 Dec; 48(12):4445-52. doi: 10.1128/JCM.01101-10. Epub 2010 Oct 6.**
5. **CLSI. *Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Third Edition*. CLSI document M23-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.**

Appendix B.

Table 3C. Carba NP Confirmatory Test for Suspected Carbapenemase Production in *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp.[1-7]

NOTE: If using FORMER minimal inhibitory concentration (MIC) interpretive criteria for carbapenems described in M100-S20 (January 2010), please refer to modifications in Table 3C-1 below.

Test	Confirmatory Test
When to Do This Test:	**For epidemiological or infection control purposes. No change in the interpretation of carbapenem susceptibility test results is required for Carba NP–positive isolates**
Test Method	**Colorimetric microtube assay**
Test Reagents and Materials	• **Clinical laboratory reagent water** • **Imipenem reference standard powder** • **Commercially available bacterial protein extraction reagent inTris HCl buffer, pH 7.4** • **Zinc sulfate heptahydrate** • **Phenol red powder** • **1N NaOH solution** • **10% HCl solution** • **Microcentrifuge tubes 1.5 mL, clear** • **1 µL inoculation loops** • **Containers to store prepared solutions** **Use reagents above to prepare the following solutions (Instructions for preparation are provided below this table):** • **10mM zinc sulfate heptahydrate solution** • **0.5% phenol red solution** • **0.1 N sodium hydroxide solution** • **Carba NP Solution A** • **Carba NP Solution B (solution A + imipenem)**
Test Procedure	**1. Label two microcentrifuge tubes (one "a" and one "b") for each patient isolate, QC organism, and uninoculated reagent control.** **2. Add 100 µL of bacterial protein extraction reagent to each tube.** **3. For each isolate to be tested, emulsify a 1-µL loopful of bacteria from an overnight blood agar plate in both tubes "a" and "b." Vortex each tube for 5 seconds. (Uninoculated reagent control tubes should contain only bacterial protein extraction reagent, no organism.) NOTE: Do not use growth from selective media or plates containing antibiotics or other agents that select for certain bacteria.** **4. Add 100 µL of Solution A to tube "a."** **5. Add 100 µL of Solution B to tube "b."** **6. Vortex tubes well.** **7. Incubate at 35°C ± 2°C for ≤ 2 hours. Read final results at 2 hours. Isolates that are positive for carbapenemase production may be reported as positive before 2 hours.**

Table 3C. (Continued)

<table>
<tr><th>Test</th><th>Confirmatory Test</th></tr>
<tr><td>Test Interpretation</td><td>
Read color change, comparing color of tubes “a” and “b.” See table and Figure below.

Red-orange or red = Negative
Orange = Invalid
Light orange or dark yellow or yellow = Positive

A slight color change may be observed with the addition of imipenem to solution A. Compare patient tubes to the uninoculated reagent control tubes when interpreting questionable results. Tube “a” must be red-orange to red to be considered a valid test. Interpret as follows:

<table>
<tr><th colspan="3">Results for Patient and QC Tubes</th></tr>
<tr><th>Tube “a”:
Solution A
(serves as internal control)</th><th>Tube “b”:
Solution B</th><th>Interpretation</th></tr>
<tr><td>Red or red-orange</td><td>Red or red-orange</td><td>Negative, no carbapenemase detected</td></tr>
<tr><td>Red or red-orange</td><td>Light-orange, dark yellow, or Yellow</td><td>Positive, carbapenemase producer</td></tr>
<tr><td>Red or red-orange</td><td>Orange</td><td>Invalid</td></tr>
<tr><td>Orange, light-orange, dark yellow, or yellow</td><td>Any color</td><td>Invalid</td></tr>
</table>
Strategy for reading:

1. Read uninoculated reagent control tubes (ie, “blanks”).

• The tubes must be red or red-orange.
• If the tubes are any other color, the test is invalid.

2. Read tube “a.”

• Tube “a” must be red or red-orange. If it is not, the test is invalid.

3. Read tube “b.”

• Red or red-orange = negative
• Yellow, dark yellow, or light orange = positive
• Orange = invalid

For invalid results:

1. Check reagents and QC results (reagent deterioration can be the cause of invalid results).
</td></tr>
</table>

	NOTE: When a reagent control test is invalid, there is a problem with Solution A and/or Solution B. Check the pH of Solution A. If it is < 7.8, prepare new Solution A and Solution B and repeat the tests and reagent control test. **2. Repeat Carba NP test.** **3. If the test invalid, perform molecular assay.**
Reporting:	**Report positive as "Carbapenemase producer."** **Report negative as "No carbapenemase detected."** **Refer to Appendix A for further guidance on testing and reporting.**
QC Recommendations	**Test positive and negative QC strains each day of testing.** ***K. pneumoniae* ATCC® BAA-1705—Carbapenemase Positive** ***K. pneumoniae* ATCC® BAA-1706—Carbapenemase Negative** **Results for uninoculated reagent control tubes tested each day should be negative (ie, red-orange to red) in both tube "a" and tube "b." Tube "b" might appear red-orange because of the addition of imipenem. Any other result invalidates all tests performed that day.**

Abbreviations: ATCC®, American Type Culture Collection; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; QC, quality control; MIC, minimal inhibitory concentration.

NOTE 1: Test recommendations were largely derived following testing of US isolates of *Enterobacteriaceae, Pseudomonas aeruginosa,* and *Acinetobacter* spp., and provide for a high level of sensitivity (> 90%) and specificity (> 90%) in detecting KPC, NDM, VIM, IMP, SPM, and SME-type carbapenemases in these isolates. The sensitivity and specificity of the test for detecting other carbapenemase production can vary. For example, the sensitivity of the Carba NP test for detecting OXA-48-type carbapenemases is low (ie, 11%).

NOTE 2: In CLSI studies, two KPC-positive strains with low carbapenem MICs (one *E. cloacae* susceptible by MIC to all three carbapenems and one *E. coli* that was susceptible to meropenem and intermediate to imipenem and ertapenem) were not detected by this test.

Table 3C-1. Modifications of the Carba NP Confirmatory Test When Using Minimal Inhibitory Concentration Interpretive Criteria for Carbapenems Described in M100-S20 (January 2010)

Test	Confirmatory Test
When to Do This Test:	**Until laboratories can implement the revised carbapenem MIC interpretive criteria, this test (or an alternative confirmatory test for carbapenemases) should be performed when isolates of *Enterobacteriaceae* are suspicious for carbapenemase production based on imipenem or meropenem MICs of 2–4 µg/mL or ertapenem MIC of 2 µg/mL.**
Reporting	**For isolates that are Carba NP positive, report all carbapenems as resistant, regardless of MIC.** **If the Carba NP test is negative, interpret the carbapenem MICs using CLSI interpretive criteria as listed in Table 2A in M100-S20 (January 2010).** **NOTE: Not all carbapenemase-producing isolates of *Enterobacteriaceae* are Carba NP positive and Carba NP–positive results may be encountered in isolates with carbapenem resistance mechanisms other than carbapenemase production.**

Instructions for Preparation of Test Components

10mM Zinc Sulfate Heptahydrate Solution:

1. **Weigh out 1.4 g $ZnSO_4 \cdot 7H_2O$.**
2. **Add to 500 mL clinical laboratory reagent water (CLRW).**
3. **Mix.**
4. **Store at room temperature.**

Expiration: 1 year or not to exceed expiration of individual components

0.5% Phenol Red Solution:

1. **Weigh out 1.25 g phenol red powder.**
2. **Add to 250 mL CLRW.**
3. **Mix.**
4. **Store at room temperature.**

Expiration: 1 year or not to exceed expiration of individual components
NOTE: This solution does not remain in solution. Mix well before use.

0.1 N Sodium Hydroxide Solution:

1. **Add 20 mL 1N NaOH to 180 mL CLRW.**
2. **Store at room temperature.**

Expiration: 1 year or not to exceed expiration of individual components

Carba NP Solution A:

1. **In a 25- to 50-mL beaker, add 2 mL 0.5% phenol red solution to 16.6 mL CLRW.**
2. **Add 180 µL 10 mM zinc sulfate solution.**
3. **Adjust pH to 7.8 ± 0.1 with 0.1 N NaOH solution (or 10% HCl solution if pH is too high).**
4. **Store at 4 to 8°C in a small vial or bottle, and protect from prolonged light exposure.**

Expiration: 2 weeks or not to exceed expiration of individual components (solution should remain red or red-orange; do not use if solution turns any other color)

Carba NP Solution B (Solution A + Imipenem):

1. **Determine the amount of Solution B required, allowing 100 µL per tube for each patient, QC strain, and reagent control.**

   Example: To test 2 patient isolates, positive and negative controls and an uninoculated reagent control, 500 µL of Solution B is needed.

2. **Weigh out approximately 10–20 mg of imipenem powder. NOTE: It is advisable to weigh out at least 10 mg of powder. Divide the actual weight by 6 to determine the amount (in mL) of Solution A to add to the powder.**

   Example: 18 mg of imipenem / 6 = 3 mL of Solution A, which is sufficient for 30 tubes.

3. **Store at 4 to 8°C for up to 3 days.**

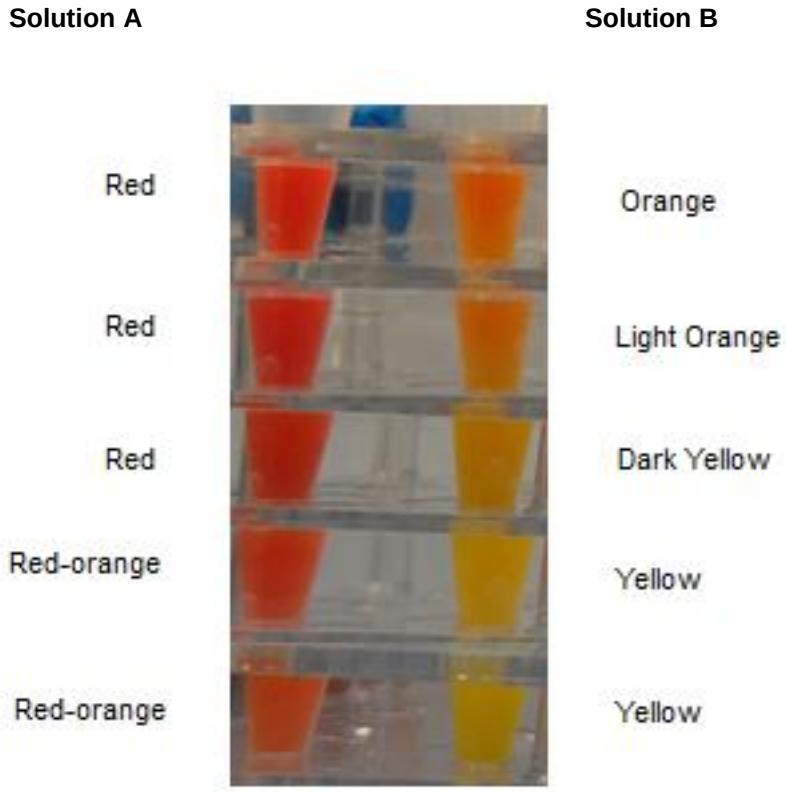


Figure 1. Interpretation of colors comparing tube "a" (Solution A) to tube "b" (Solution B)

References for Tables 3C and 3C-1

[1] **Carvalhaes CG, Picão RC, Nicoletti AG, Xavier DE, Gales AC. Cloverleaf test (modified Hodge test) for detecting carbapenemase production in Klebsiella pneumoniae: be aware of false positive results. *J Antimicrob Chemother.* 2010;65(2):249-251.**

[2] **Girlich D, Poirel L, Nordmann P. Value of the modified Hodge test for detection of emerging carbapenemases in Enterobacteriaceae. *J Clin Microbiol.* 2012;50(2):477-479.**

[3] **Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis.* 2012;18(9):1503-1507.**

[4] **Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing Pseudomonas spp. *J Clin Microbiol.* 2012;50(11):3773-3776.**

[5] **Dortet L, Poirel L, Nordmann P. Rapid identification of carbapenemase types in Enterobacteriaceae and Pseudomonas spp. by using a biochemical test. *Antimicrob Agents Chemother.* 2012;56(12):6437-6440.**

[6] **Cunningham SA, Noorie T, Meunier D, Woodford N, Patel R. Rapid and simultaneous detection of genes encoding Klebsiella pneumoniae carbapenemase (*bla*$_{KPC}$) and New Delhi metallo-β-lactamase (blaNDM) in Gram-negative bacilli. *J Clin Microbiol.* 2013;51(4):1269-1271.**

[7] **Vasoo S, Cunningham SA, Kohner PC, et al. Comparison of a novel, rapid chromogenic biochemical assay, the Carba NP test, with the modified Hodge test for detection of carbapenemase-producing Gram-negative bacilli. *J Clin Microbiol.* 2013;51(9):3097-3101.**