Meeting Title:	**Subcommittee on Antimicrobial Susceptibility Testing (AST)**	**Contact:**	egomez@clsi.org
Meeting Date:	Friday, 23 September 2022 10:00 AM – 1:00 PM US EST (Virtual Only)		
Meeting Purpose:	The purpose of this meeting is to discuss the CFDC disk performance, review and vote on the linezolid transmitted vs reflected light data and wrap up any further discussion needed regarding the aminoglycoside disk diffusion vote in preparation for publication of the 2023 edition of M100 (33rd).		
Requested Attendee(s):	SC Chairholder, Vice-chairholder, Members, and Advisors; MDSWG members; Other related parties; CLSI Staff		

Attendee(s):	
James S. Lewis, PharmD, FIDSA AST Subcommittee Chairholder	**Oregon Health and Science University**
Melvin P. Weinstein, MD AST Subcommittee Vice-Chairholder	**Robert Wood Johnson University Hospital**
Members Present:	
Sharon K. Cullen, BS, RAC	Beckman Coulter, Inc. Microbiology Business
Tanis Dingle, PhD, D(ABMM), FCCM	Alberta Precision Laboratories
Thomas J. Kirn, MD, PhD	Rutgers Robert Wood Johnson Medical School
Brandi Limbago, PhD	Centers for Disease Control and Prevention
Amy J. Mathers, MD, D(ABMM)	University of Virginia Medical Center
Virginia M. Pierce, MD	Massachusetts General Hospital
Sandra S. Richter, MD, D(ABMM), FIDSA	Mayo Clinic (Jacksonville, FL)
Michael Satlin, MD	Weill Cornell Medicine
Audrey N. Schuetz, MD, MPH, D(ABMM)	Mayo Clinic (Rochester, MN)
Susan Sharp, PhD, D(ABMM), F(AAM)	Copan Diagnostics, Inc.
Patricia J. Simner, PhD, D(ABMM)	Johns Hopkins School of Medicine, Department of Pathology
Members Absent:	
Marcelo F. Galas, BSc	Pan American Health Organization
Romney M. Humphries, PhD, D(ABMM), FIDSA	Vanderbilt University Medical Center
Advisors Present:	
April M. Bobenchik, PhD, D(ABMM), MT(ASCP)	Penn State Hershey Medical Center
Carey-Ann Burnham, PhD, D(ABMM)	Washington University School of Medicine
Shelley Campeau, PhD, D(ABMM)	Accelerate Diagnostics, Inc.
Mariana Castanheira, PhD	JMI Laboratories
German Esparza, MSc	Proasecal SAS
Christian G. Giske, MD, PhD	Karolinska University Hospital
Howard Gold, MD, FIDSA	Beth Israel Deaconess Medical Center
Maria Karlsson, PhD	Centers for Disease Control and Prevention
Joseph Kuti, PharmD, FIDP, FCCP	Hartford Hospital
Joseph D. Lutgring, MD	Centers for Disease Control and Prevention
Linda A. Miller, PhD	CMID Pharma Consulting LLC
Stephanie L. Mitchell, PhD, D(ABMM)	Cepheid, Inc.
Greg Moeck, PhD	Venatorx Pharmaceuticals, Inc.
Navaneeth Narayanan, PharmD, MPH	Rutgers University
Samir Patel, PhD, FCCM, D(ABMM)	Public Health Ontario
Ribhi Shawar, PhD, D(ABMM), F(AAM)	FDA Center for Devices and Radiological Health
Eric Wenzler, PharmD, BCPS, AAHIVP	University of Illinois at Chicago
Barbara L. Zimmer, PhD	Beckman Coulter
Reviewers and Guests (Non-SC-roster attendees):	
Kevin Alby	David Lonsway

Jay Bryowsky	Maria-Jose Machado
Susan Butler-Wu	Rod Mendes
Cecilia Carvalhaes	Sean Nguyen
Jennifer Dien Bard	Chris Pillar
Roger Echols	Elizabeth Palavecino
Andrew Fuhrmeister	Helio Sader
Dwight Hardy	Katherine Sei
Mike Huband	Christine Slover
Laura Koeth	Miki Takemura
Frank Kung	Yoshinori Yamano
Chris Longshaw	Hidenori Yamashiro
Staff:	
Kathy Castagna, MS, MT(ASCP)CT, MB	CLSI
Emily Gomez, MS, MLS(ASCP)MB	CLSI
Christine Lam, MT(ASCP)	CLSI

Meeting Agenda

MEETING AGENDA Friday, 23 September 2022 (Virtual) 10:00 AM – 1:00 PM All Times listed are Eastern (US) Time		
Time	**Item**	**Presenter**
10:00 AM – 10:05 AM (5 min)	Meeting Ground Rules	CLSI Staff
10:05 AM – 10:10 AM (5 min)	Opening Remarks	J. Lewis
10:10 AM – 10:40 AM (30 min)	Shionogi CFDC Disk Performance Presentation and Discussion	Shionogi
10:40 AM – 11:40 AM (1 hr)	Methods Development and Standardization WG Linezolid Presentation and Discussion	D. Hardy
11:40 AM – 11:50 AM (10 min)	Break	
11:50 AM – 12:20 PM (30 min)	Quality Control WG Linezolid QC Presentation and Discussion	S. Cullen
12:20 PM – 12:50 PM (30 min)	Breakpoints WG Additional Discussion on Aminoglycoside Disk Diffusion Vote	M. Satlin A. Mathers
12:50 PM – 1:00 PM (10 min)	Closing Remarks	J. Lewis

Summary of Voting Decisions and Action Items

#	Motion Made and Seconded	Results[a]
1.	To add a comment regarding the variable accuracy and reproducibility of cefiderocol testing results and recommend antimicrobial stewardship consultation in M100 in all locations cefiderocol is listed. Also, provide a newsletter explanation.	11-0-0-2
2.	To approve the proposed recommendations of reading linezolid zones of growth inhibition against *S. aureus* 25923 using reflected light with the proposed QC range of 24-30 mm.*	11-0-0-2
3.	To approve amikacin disk BPs for Enterobacterales (S≥20, I 17-19, R≤16).	10-0-0-3

[a] Key for voting: X-X-X-X = For-against-abstention-absent

*Decision made to add linezolid and tedizolid reflected light changes to M100-34th edition after review from the Breakpoints WG.

2022 SEPTEMBER AST MEETING SUMMARY MINUTES Friday, 23 September 2022 10:00 AM – 1:00 PM US EST (Virtual Only)	
#	**Description**
1.	**OPENING REMARKS (J. LEWIS)** Dr. Lewis opened the meeting at 10:00 AM Eastern (US) time by welcoming the participants to the virtual only AST Subcommittee meeting.

2. SHIONOGI CEFIDEROCOL DISK PERFORMANCE PRESENTATION AND DISCUSSION (C. SLOVER)

Topics presented included:

- Initial request from CLSI in February 2021 to further delineate AST correlates for *Acinetobacter* spp. with higher MICs to cefiderocol.
- Background: IHMA and Shionogi *Acinetobacter* spp. Correlation Data (Presented at the January 2022 CLSI meeting).
- Updates on ongoing studies to address AST variations in *Acinetobacter* spp.

IHMA and SHQ *Acinetobacter* spp. Correlation Data

Overview of IHMA and Shionogi *Acinetobacter* spp. Correlation Study

- IHMA conducted MIC vs disk studies using 261 isolates with cefiderocol MIC ≥2 µg/mL (234; ~90% were ≥4 µg/mL)
  - IHMA conducted studies were performed in triplicate
  - Apparent major error were frequent due to the appearance of colonies in the disk zone for many isolates
  - In a sub-group of 112 isolates with original MIC vs disk data available, reproducibility between original and re-test data was poor
- Shionogi also conducted MIC vs disk studies using 197 isolates (selected from 261 isolates due to availability at Shionogi lab)
  - Poor reproducibility between IHMA and Shionogi generated data was observed for some isolates

Summary of trailing and colony observations by Shionogi

- 197 isolates were tested (with MIC ≥2 µg/mL)
  - Trailing phenomenon in broth microdilution MIC study
    - Trailing was observed for 93/197 (47%) isolates
    - 44 isolates in both studies, and 49 isolates in either study
    - Reading guidelines identical between both labs so unlikely cause of variability
  - Colony observations in disk zone study
    - Total of 154 isolates among 197 isolates (78%) exhibited colonies in the disk zone
    - Small colonies appearance or turbid growth in the zone was observed for 130 isolates (70 isolates in both studies and 60 isolates in either study)
    - Large colonies in the zone was observed for 24 colonies
- No strong relationship between trailing and colony observations
  - 63/93 (68%) isolates with trailing growth had microcolonies or turbid growth in zone
  - 84/109 (77%) isolates without trailing, had microcolonies or turbid growth in zone
  - Only 24/197 (12%) isolates showed large colonies in the disk zone

IHMA and Shionogi *Acinetobacter* spp. Correlation: Conclusions

- Poor reproducibility in both disk zone and MIC for 197 *A. baumannii* isolates which showed high cefiderocol MIC (>2 µg/mL)
  - Poor MIC reproducibility between IHMA retest and original data
  - Poor disk reproducibility between Shionogi retest and IHMA retest or previous data
- Many high MIC isolates showed trailing phenomenon in broth and/or the appearance of colonies in the disk zone by disk diffusion method
  - Trailing phenomenon and small colony formation was not consistently reproducible
  - Trailing phenomenon and small colony formation was not correlated
- Based on these results, Shionogi conducted additional studies examining the effect of inoculum size and media on BMD and disk diffusion studies

Updates on Ongoing Studies

BMD Optimization Study Objectives

- Optimize methodology for production of ID-CAMHB
  - Reduce variability in depletion of Fe(III) between base MHB from major media manufacturers and inter-batch variation
  - Further standardize ID-CAMHB reference for AST developers by defining parameters such as iron-binding resin, chelation period, cation supplementation
  - Confirm reproducibility of MIC in optimized ID-CAMHB against isolates for which MIC has been validated through *in vivo* PK/PD studies
- Identify additional QC isolates to verify low iron levels in media
  - Identify strains with a large difference in MIC values determined in ID-CAMHB versus CAMHB

Preliminary Conclusions

- The use of BBL ID-CAMHB provides reproducible MIC which have been associated with *in vivo* efficacy
- Cefiderocol MIC variation detected against only *A. baumannii* isolates with high MIC to cefiderocol due to a small variation (3-fold) of inoculum
- The appearance of micro colonies was often observed for *A. baumannii* isolates with high MIC to cefiderocol but this phenomenon was not reproducible between MHA from different manufacturers

Actions for January 2023 CLSI Meeting

- MIC vs disk studies will be conducted using A. baumannii isolates which were evaluated for *in vivo* studies and were applied to MIC vs disk studies
- BMD MIC will be determined by using BBL ID-CAMHB
- Disk zone will be determined by using BBL pre-made MHA. Disk zone will be determined by both including or ignoring micro colonies
- Some guidance to solve issues will be discussed in January
- AST validation panel is under preparation which could be supplied to various sites

SC DISCUSSION (MAIN POINTS)

- The question was asked if there were other factors in the Mueller Hinton broth that could affect variability in results from different lots. Shionogi could not identify the factors affecting the variation.
- The question was asked if the inoculum studies were done with six hour chelated BBL media. The answer was yes.
- Suggestion to use a readily available organism to look at iron concentrations across all manufacturers. The isolates presented are not readily available to labs. Currently available ATCC strains would be ideal. Thought is to have a supplemental QC strain that is used on each shipment and lot. Add comment on how to prepare the inoculum as well. Need QC strain to properly quality control the media being used by the lab.
- Concerns regarding recommending a single brand of media to use.
- Interest in seeing colony counts with the inoculum data presented.
- The question was asked as to reasons why inside colonies were seen with the lab prepared agar versus the BD prepared agar. Shionogi did not know the reasoning.
- The question was asked if studies will be conducted on additional organisms besides *Acinetobacter*. Shionogi has plans to present data on other organisms in the future.
- Suggestion was made to test with the AR Bank isolates. Shionogi plans to use AR Bank isolates in future studies.
- Suggestion was made to confer with the Methods Development and Standardization WG.
- EUCAST provides a warning to make sure QC strains are working for cefiderocol.
- Suggestion was made to provide a newsletter update or M100 supplement regarding potential issues and help with the QC.

	• Guidance on a QC strain to use and how to read the disk diffusion results is needed. • Suggested comment to add to M100: The accuracy of cefiderocol testing results are strongly impacted by iron concentration, inoculum preparation and may vary by disk and media manufacturer. See Appendix (insert ID-CAMHB appendix) for further recommendations. • Concerns were raised with only including the comment with *Acinetobacter*. Stating the comment with all organisms is important because variability is being seen with other organisms. It is a general warning and not specific to *Acinetobacter*. EUCAST has not limited their warning to *Acinetobacter*.
	A motion to add a comment regarding the variable accuracy and reproducibility of cefiderocol testing results and recommend antimicrobial stewardship consultation in M100 in all locations cefiderocol is listed. Also, provide a newsletter explanation was made and seconded. Vote: 11 for, 0 against, 0 abstain, 2 absent (Pass)

3. **METHODS DEVELOPMENT AND STANDARDIZATION WG (D.HARDY)**

Linezolid Disk Diffusion Testing-Reading Zones against *S. aureus* with Reflected or Transmitted Light

Background – Tedizolid and Linezolid Disk Diffusion Testing and CLSI/EUCAST Harmonization

- In June 2022, the MDSWG presented data from a "4 Lab Study" of Disk Diffusion Testing with tedizolid against Staphylococcus aureus to the AST Subcommittee. Following this presentation, the AST SC approved the recommendation to read tedizolid zones of growth inhibition against *S. aureus* using reflected light.
- As part of the same presentation, the MDSWG recommended that data with linezolid be evaluated to determine whether zones of growth inhibition against *S. aureus* should be read with reflected light rather than transmitted light.

Four Lab Study with Tedizolid and Linezolid against *S. aureus*
Conclusions Against QC Strains

- With respect to tedizolid zones of growth inhibition against QC strains (*S. aureus* ATCC 25923 and *S. aureus* ATCC 29213):
  - No observed media effect
  - No observed disk effect
  - A small difference observed between labs
  - Zone diameters read with transmitted light were smaller than zone diameters read with reflected light
- With respect to linezolid zones of growth of inhibition against QC strains (*S. aureus* ATCC 25923 and *S. aureus* ATCC 29213):
  - No or small observed media effect
  - A small observed difference between laboratories
  - Zone diameters read with transmitted light were smaller than zone diameters read with reflected light

Conclusions Against Clinical Isolates

- Tedizolid zones of growth inhibition against clinical isolates of *S. aureus* were smaller when read with transmitted light versus reflected light
- Linezolid zones of growth inhibition against clinical isolates of *S. aureus* were smaller when read with transmitted light versus reflected light

Impact of Reading with Reflected Light on Current QC Range for Linezolid

- Data from the four lab study with linezolid was separately reviewed to determine the impact of reading zones with reflected light on current CLSI QC ranges for ATCC 25923 (25-32 mm)

Proposal to the AST Subcommittee

- Based on data presented with linezolid, the proposal is to recommend reading linezolid zones of growth inhibition against *S. aureus* using reflected light.
  - Reading with reflected light is consistent with CLSI M02 recommendations for all other drugs including tedizolid
  - Reading with reflected light is easier to read
  - Reading with reflected light harmonizes with EUCAST recommendation for reading zones
  - Reading with reflected light correlated better to current CLSI QC range for *S. aureus* ATCC 25923 (25-32 mm) and demonstrated better inter-lab reproducibility than did reading with transmitted light

Summary of Linezolid and Tedizolid QC with *S. aureus* ATCC 25923 – Reflected Light (S. Cullen)

Tedizolid: 2 mcg disk

Study	Media	Disk	Labs	Gavin	Range Finder	Comments
Tier 2: Slide 10 2019 QCWG_6A_Tedizolid_Tier 2_2mcg	21, 21, 22	22, 22	20,3@21,3 @22,23	18-25mm 99.6% 8mm,	19-25mm, 99.0% 7mm,	Lab variability, some media variability
JMI 4 lab: Linezolid Disk Diffusion Testing September 2022 Presentation	Stats not available	NA	NA	NA	NA	Disks: MAST and Liofilchem, no effect Media: Remel, Hardy, BD, no effect Labs: Small variability Zones smaller with transmitted light compared to reflected light 100% of results within range 18-24 (very few at 18 and 19)

Current Ranges

- CLSI range with transmitted light: 18-24, footnote h: read using transmitted light
- EUCAST range (2 mcg disk): NA for S. aureus 25923, for S. aureus 29213 19-25, target 22

Linezolid: 30 mcg disk

Study	Media	Disk	Labs	Gavin	Range Finder	Comments
Tier 2: Linezolid 30 mcg disk results from tedizolid Tier 2 QC Study	26, 27, 29	27 (BD)	25, 3@26, 2@27, 28, 29	24-30mm, 7 mm, 97.5%	24-31mm, 8mm, 100%	Lab and media variability Few results >30mm (6/240@31, 0@32)
4 Lab: JMI Lzd 30 disk for CLSI_LKoeth_September 2022	27, 28, 28	28 (BD)	27, 3@28	25-32mm, 8mm, 100%		Disks: no comments/not shown Media: Remel, Hardy, BD, some effect Labs: Small variability Zones smaller with transmitted light compared to reflected light 563/570, 98.8% using 24-30mm range 567/570, 99.5% using 24-31mm range Few results >30mm (4@31, 3@32)

Current ranges

- CLSI range with transmitted light: 25-32, footnote h: read using transmitted light
- Current EUCAST range (10 mcg disk): NA for S. aureus 25923, for S. aureus 29213 21-27, target 24
- Note: Very few results >30mm with either study.

Recommendations

- Tedizolid: 19-25 (7) 99.0%
- Linezolid: 24-30 (7) 97.5%

SC DISCUSSION (MAIN POINTS)

- Question was asked regarding how the linezolid data presented varies from the data that was used to originally set the breakpoint. If the reading guidance is changed, is there a need to change the breakpoint as well? MDSWG was tasked to decide on how to read the zones and then the Breakpoint WG can reevaluate the linezolid and tedizolid breakpoints later.

- There is a comment in M100 stating that organisms with resistant results by disk should be confirmed using an MIC method. Question was asked if this comment would still apply when reading with reflected light. MDSWG concluded that overall, for reproducibility it is better for labs to read with reflected light.
- Question was asked if other organisms will be reviewed. The data the MDSWG has is only on S. *aureus*. Reflected reading for *E. faecalis* for linezolid has always been used.
- Question was asked if the linezolid 31 and 32 mm results were seen with one lab or a few labs. Answer was one lab and very few results.
- Concerns with including linezolid and tedizolid reflected light method and QC ranges in M100 33rd edition without the Breakpoint WG evaluation. Changes will be postponed to the 34th edition after the Breakpoint WG review.

A motion to approve the proposed recommendations of reading linezolid zones of growth inhibition against *S. aureus* 25923 using reflected light with the proposed QC range of 24-30 mm was made and seconded.* Vote: 11 for, 0 against, 0 abstain, 2 absent (Pass)

*Decision made to add linezolid and tedizolid reflected light changes to M100-34th edition after review from the Breakpoint WG.

4. **BREAKPOINT WG (M. SATLIN)**

Aminoglycosides Disk Breakpoints

- Breakpoints passed (vote: 11-0-1-1) at the June 2022 AST Subcommittee Meeting. Request was made to review again prior to M100 publication.

Organism	Antimicrobial	S	I	R	Errors (VME/ME/mE)
Enterobacterales	Gentamicin	≥18	15-17	≤14	0% / 0% / 2.7%
Enterobacterales	Tobramycin	≥17	13-16	≤12	0% / 0% / 4.5%
Enterobacterales	Amikacin	≥19	16-18	≤15	0% / 0% / 6.1%
Ps. aeruginosa	Tobramycin	≥19	13-18	≤12	0% / 0% / 2.8%

SC DISCUSSION (MAIN POINTS)

- Suggestion to use 17-19 mm intermediate range for amikacin and Enterobacterales. The passed range makes minor errors weighted in one direction of the disk under calling resistance. More even split with minor errors with the 17-19 mm range.
- Concerns with tobramycin and *P. aeruginosa* intermediate zone. Few isolates at 4 and 8 in the data set. Might be too wide of an intermediate zone. Suggestion to look for isolates for future studies.

A motion to approve amikacin disk BPs for Enterobacterales (S≥20, I 17-19, R≤16) was made and seconded. Vote: 10 for, 0 against, 0 abstain, 3 absent (Pass)

5. **ADJOURNMENT**

Dr. Lewis thanked the participants for their attention. The meeting was adjourned at 12:30 PM Eastern (US) time.