Subcommittee on Antifungal Susceptibility Testing
Meeting
11 January 2014
Hyatt Regency Riverwalk, San Antonio, Texas
8:00 AM Central (US) Time

Summary Minutes

A meeting of the Subcommittee on Antifungal Susceptibility Testing was held Saturday, 11 January 2014, beginning at 8:00 AM Central (US) time. The following were in attendance.

Barbara D. Alexander, MD, MHS **Chairholder**	**Duke University Medical Center**
Mahmoud A. Ghannoum, MSc, PhD, EMBA **Vice-Chairholder**	**Case Western Reserve University**

Voting Members

Ana Espinel-Ingroff, PhD	VCU Medical Center
Laura Kovanda	Astellas Pharma Global Development, Inc.
Michael LaFleur, PhD	Arietis Corp.
Shawn R. Lockhart, PhD, D(ABMM)	Centers for Disease Control and Prevention
David S. Perlin, PhD	New Jersey Medical School-Rutgers
Michael A. Pfaller, MD	University of Iowa College of Medicine
Nancy L. Wengenack, PhD, D(ABMM), FIDSA	Mayo Clinic

Members Absent (with notice)

Sharon K. Cullen, BS, RAC	Siemens Healthcare Diagnostics Inc.
Annette W. Fothergill, MA, MBA, MT(ASCP)	University of Texas Health Science Center
Jacques F. Meis, MD, PhD	Canisius Wilhelmina Hospital
Peter R. Williamson, MD, PhD	National Institutes of Health

Advisors

Mariana Castanheira, PhD	JMI Laboratories
Lynette Y. Berkley, PhD*	FDA CDER
Philippe Dufresne, PhD *	Institut National de Santé Publique
Cynthia C. Knapp, MS	Thermo Fisher Scientific
Gary W. Procop, MD	Cleveland Clinic
Ribhi M. Shawar, PhD, D(ABMM)	FDA Ctr. for Devices/Rad. Health (CDRH)
Maria M. Traczewski, BS, MT(ASCP)	The Clinical Microbiology Institute
Kenneth Van Horn, PhD, D(ABMM)	Focus Diagnostics

*Meeting Recording Secretary

Reviewers

Steven D. Brown, PhD, ABMM	Consultant
Jeff Fuller, PhD, FCCM, ABMM	University of Alberta Hospital
Diane L. Getsinger, MT(ASCP), CLS(NCA)	Stanford University Medical Center
Scott B. Killian	Thermo Fisher Scientific
Maureen Mansfield	Thermo Fisher Scientific
Mary R. Motyl, PhD, D(ABMM)	Merck Sharp & Dohme Corp.
Robert P. Rennie, PhD	University of Alberta Hospital
Dee Shortridge, PhD	bioMérieux, Inc.
Kerry Snow	US FDA/CDER
John D. Turnidge, MD	SA Pathology at Women's and Children's Hospital
Nathan Wiederhold, PharmD	University of Texas Health Science Center
Barbara J. Zimmer, PhD	Siemens Healthcare Diagnostics, Inc.

Guests

April Abbott	University of Washington
Todd Black, PhD	Merck Research Laboratories
Kathy Burtner	Siemens Healthcare Diagnostics
Neil Clancy	University of Pittsburgh
Jenifer Dawson Driscoll	Siemens Healthcare Diagnostics
Eszfer Deak	UCLA
Robert Eusebio, MSHA, MT(ASCP)	Siemens Healthcare Diagnostics, Inc.
Sheila Farnham	bioMérieux, Inc.
Monique M. Fouant	Astella Pharma Global Development, Inc.
Andrea Gaugh	Thermo Fisher Scientific
Nicole Holliday	Thermo Fisher Scientific
Romney M. Humphries, PhD, D(ABMM)	UCLA David Geffen School of Medicine
Seong Jang	FDA/CDER
J. Kristie Johnson	University of Maryland
Brenda Ling	Astellas
Sally Maysent	Thermo Fisher Scientific
Steve Michalik	bioMérieux, Inc.
Shelly Miller	UC Los Angeles
Ross Mulder	bioMérieux, Inc.
Sumathi Nambiar	FDA/CDER
M. Hong Nguyen	University of Pittsburgh
Nilia M. Robles Hdez	bioMérieux, Inc.
Gina Ewald-Saldana, CLS(CA), MT(ASCP)	Siemens Healthcare Diagnostics, Inc.
Audrey N. Schuetz, MD, MPH, D(ABMM)	Weill Cornell Medical College/New York-Presbyterian Hospital
Sharon Shin	Siemens Healthcare Diagnostics, Inc.
Debora Sweeney	Micromyx
Mike Sweeney	Zoetis
Dana Trott	University of Adelaide
Manel Wafa	bioMérieux, Inc.

Peter Warn, PhD	Europrotec
Colette Wehr	Siemens Healthcare Diagnostics, Inc.

CLSI Staff Present

Tracy A. Dooley, BS, MLT(ASCP)
Glen Fine, MS, MBA, CAE
Marcy L. Hackenbrack, MCM, M(ASCP), BA
Luann Ochs, MS

Meeting Materials Provided Prior to Meeting

Note: All background material and revised presentations will be posted on the CLSI website

- Agenda
  - Caspofungin Susceptibility Testing Issues
  - Epidemiologic Cutoff Values (ECVs) for *Candida* and Amphotericin B, Itraconazole, and Flucytosine
  - ECVs for Aspergillus and Azoles, Caspofungin, and Amphotericin B
  - QC ranges and read times for antifungal susceptibility testing
- Presentations, reference materials, and data

Purpose of Meeting

The purpose of the meeting was to review data, discuss issues associated with antifungal susceptibility testing, and to discuss the plan forward for document revision and development.

Opening Remarks

Dr. Alexander opened the meeting at 8:00 AM Central (US) time. She stated that the goals of this meeting were to review the data for caspofungin and ECVs and to vote on any open issues. Since only 7 out of 11 voting members were in attendance, based on the current voting rules, any votes that were unanimous (7-0) would pass. However, any votes that are not unanimous will need to be re-taken electronically. She asked the meeting participants to introduce themselves and provide a brief description of their involvement with the antifungal subcommittee.

Ms. Ochs, senior Vice-President, Operations at CLSI, provided a brief presentation on the status of the finances for the Antifungal Subcommittee. She stated that the policy requiring all volunteers to be members of CLSI was enacted to help offset the cost of the face-to-face meetings (approximately $20,000/day) and document development. She indicated that in recent years, the sale of antifungal documents has not always covered the cost of running the subcommittee and membership fees help to cover those costs.

Dr. Alexander provided a brief introductory presentation and update on the status of the antifungal susceptibility testing documents. She reminded the attendees to note any changes to their disclosure of interests.

Meeting Discussion

The substantive discussion points of the meeting are listed below (see Tables). All presentations, including those that were not available prior to the meeting, will be posted on the subcommittee page on the CLSI website.

Agenda Topic		Committee Discussion Points/ Rational for Decisions Made and/or path Forward
1.	Caspofungin: Why so much variation? (Dr. Pfaller)	Dr. Pfaller summarized the current QC issues and interlaboratory variations experienced with caspofungin. The main points of his presentation are summarized below. • Data on the number of laboratories now reporting antifungal susceptibility results as part of the College of American Pathologists (CAP) proficiency testing program was presented. The number of participants has nearly doubled from 2010 to 2013 (217 to 424). Most of this increase is due to arrival of recently FDA approved Vitek2 platform, which is now most used by US laboratories (Vitek2 40%; YeastOne 35%; gradient diffusion 10%; broth microdilution [BMD] 10%; disk diffusion 5%). • The recent article from Espinel-Ingroff et al (AAC 2013; 57:5836-42) shows that modal caspofungin minimal inhibitory concentrations (MICs) can vary by as much five dilutions between laboratories. The different caspofungin MIC distributions obtained by Pfaller and Castanheira vs. Shields et al. (2013) for *C. glabrata* using fks1 and fks2 mutants also highlights the interlaboratory modal MIC variation problem. The new M27-S4 clinical breakpoints (BP) result in the misclassification of wild type (WT) strains as being intermediate/resistant in laboratories with high caspofungin MICs. Current QC strains are unable to control for reading at low MIC and don't discriminate laboratories with high or low MICs. • Multiple factors have been proposed to explain the interlaboratory variation seen with caspofungin: stability of reagents, plastic ware used, DMSO, endpoint reading etc. The use of polysorbate 80 was also suggested as a way to reduce caspofungin adherence to plastic ware. • These problems may warrant the use of a surrogate marker for caspofungin susceptibility testing. Both anidulafungin and micafungin having been found to provide more reproducible results (Pfaller et al., 2014; JCM, 52:1 108-114). Overall, categorical agreement with caspofungin was 90.9%-97.1% and resulted in only 0.2% major errors (ME) or very major errors (VME). • Present QC strains are insensitive to variations in reagents or methods that may affect the lower end of MIC distribution for caspofungin. • It was noted that better QC strains are needed (eg, *C. albicans* ATCC® 64548 and 64550 or isogenic *C. glabrata* strains (+/- fks mutations). • A look at recent CAP results obtained with BMD vs. other commercial methods (E-test, SYO) points to a lot of variability in caspofungin MIC (spread with a resistant *C. glabrata* isolate, false intermediate wild type strain) with these methods as well. More comparative data is needed and none yet available for the newly released Vitek2 platform.

		• Summary and conclusions – Variability in caspofungin MIC results for *Candida* spp. is a major problem o leads to reporting of false resistance (major error) o impacts commercial and reference methods o caspofungin is the only echinocandin available on commercial systems (FDA cleared) o affects *C. glabrata* the most – This highlights the need for better QC strains (to control low end MIC reads) – Anidulafungin or micafungin may serve as surrogate markers for caspofungin resistance.
2.	Caspofungin Susceptibility Testing (Dr. Motyl)	Dr Motyl presented Merck's perspective on caspofungin susceptibility testing interlaboratory variations • Dr. Motyl provided historical background on the variability issue. – The interlaboratory variability had been observed in the initial multi-national study carried in 2002 using both EUCAST and CLSI methodology. – For this study, plates were produced by one manufacturer (Trek diagnostics) and distributed to 17 participating centers. – Considerable variability (> 3 dilutions) was seen with caspofungin at that time but considerable variability was also seen with itraconazole and fluconazole. – Caspofungin variability was present since the outset but was only revealed with recent changes to CLSI breakpoints. • New M27-S4 breakpoints have led in some laboratory to a dramatic increase in the percentage of resistant *C. glabrata* and *C. krusei*. – Dr. Motyl showed a retrospective study by the University of Texas Health Science Center with *C. glabrata* isolates where they went from 1.9 % resistance to caspofungin with M27-S3 breakpoints to 95.6% with M27-S4, while anidulafungin and micafungin had increased by less than 7% with newly published breakpoints. – As echinocandin resistance is typical, the discrepancy observed indicates that a methodological problem occurs with caspofungin testing using new breakpoints. • Merck has also analyzed caspofungin drug lots used in the recent article by Dr. Espinel-Ingroff (2013; AAC 57[12]: 5836). – The available information on drug lots is fragmentary; however, no correlation could be made with various lots and the interlaboratory variability recorded. It was found that laboratories which have used multiple drug lots generally reported tighter, less

		variable modal MICs. Merck also contacted 9 CLSI investigators; 7/9 provided lot information. No correlation between lot number and a tendency towards high MICs or low MICs could be made. – A number of parameters were proposed as possible root causes of variability during these discussions (eg, plastic adherence or treatment, addition of polysorbate 80, DMSO concentration, reading 50% inhibition). – The general consensus is that current QC strains do not discriminate low MIC vs. high MIC laboratories and cannot elucidate the issue and that no single obvious cause for variability has been identified. • Dr. Motyl (Merck) agreed that the range of modal MICs observed in multiple laboratories is an issue that must be addressed or otherwise misclassification of isolates as "false resistant" or "false susceptible" could seriously impact patient care. She indicated that not testing caspofungin or using a surrogate echinocandin is not an option because, based on feedback from physicians, clinicians will not use a drug that is not tested for resistance in their hospital laboratory. • Although powder lot potency has been proposed as cause of variability (eg, Arendrup et al. AAC 2001; 55(4), 1580), Dr. Motyl believes that no reliable data supports this claim. – Assessment of lot-to-lot potency would require testing with defined variables (drug lot, storage of powder and MIC panels, media, solvent used, timeframe of testing, consumables, personnel reading the panels). – Absence of stringent control of these variables makes it difficult to assess lot potency variability. • Merck has found no evidence of degradation of caspofungin drug powder under recommended storage conditions (-70°C/ ambient humidity) and impurities found to remain stable over a 36 month test period. – Storage at -20ºC or 2-8ºC does lead to an increase in the % impurities (up to 2%). – Caspofungin is subject to stringent requirements for purity and potency. Potency must fall within specifications (96.5-101.5%) and has remained stable for all lots produced by Merck since 2002. • Conclusions – Factors affecting *in vitro* testing of caspofungin need to be assessed and MIC variability concerns resolved.

		– A proposal to not test caspofungin or use a surrogate marker is not acceptable to Merck. – Merck does not agree that MIC issues are related to "potency" or "lot-to-lot" variability and requests that both comments regarding potency be removed from the footnotes in the next version of M27 (Table 2 footnote c; table 7 footnote 2) since data to support these claims have not been provided. – The FDA is aware of issues related to MIC variability of caspofungin and has chosen to retain M27-S3 breakpoints. • Discussion – Dr. Perlin indicated that potency or stability issues have never been observed *in vitro* for glucan synthase inhibition studies from different caspofungin lots that his laboratory tested. – Dr. Lockhart stated that a return to M27-S3 breakpoints was not an acceptable solution. He does not recommend use of previous *Candida* spp. breakpoints as they have less clinical relevance. Dr. Alexander voiced her agreement. Returning to old breakpoints would actually result in isolates with known resistant mutations being called susceptible thus resulting in unacceptable VMEs for patients. – Dr. Pfaller emphasized on the general cross resistance mechanisms of echinocandins. The use of surrogate molecules is not unusual. Surrogates are used in bacterial antimicrobial susceptibility testing and EUCAST has also recommended using other echinocandins as surrogate for caspofungin.
3.	Influence of Treated vs. Untreated Plastic on Caspofungin and Azole MICs against *Candida* (Dr. Wiederhold)	Dr. Wiederhold presented data on the effect of plastics (treated vs untreated microtiter plates) on azole and caspofungin MICs obtained with *Candida* spp. • The first presentation showed the results obtained with caspofungin M27 method with treated/untreated plastic microtiter plates compared to macrodilution method and the E-test strip system. – The University of Texas Health Science Center retrospective analysis of MIC results with the new M27-S4 breakpoints resulted in a dramatic increase in percentage of strains that were resistant to caspofungin for some species of *Candida* (8% increase for *C. albicans*, 24% for *C. tropicalis*, >70% for *C. krusei* and >90% for *C. glabrata*). – A panel of 29 *C. albicans* and 35 *C. glabrata* strains were tested by the three methods. It was shown that the treated plastic tray produced different MIC results. o MIC results obtained with treated polystyrene 96 well culture trays show elevated

		MICs compared to those obtained with untreated microtiter plates or the macrodilution method. o MICs obtained with gradient diffusion were lowest of all with the exception of fks resistant strains. The differences in MICs were more prominent with *C. glabrata* strains than that of *C. albicans*, indicating the effect are also species specific. • The second presentation showed the similar effect of 96 well plate treated plastic on azoles MICs (fluconazole, itraconazole, posaconazole, voriconazole). – In this study, a panel of 21 *C. albicans* and 25 *C. glabrata* strains were tested using treated vs. untreated 96 well cell culture plates from two different manufacturers. – Posaconazole and itraconazole MICs results for *C. parapsilosis* (ATCC® 22019) and *C. krusei* (ATCC® 6258) were found to be 1-2 dilution higher when untreated plates were used. – Similar results were obtained with *C. albicans* and *C. glabrata* clinical isolates where most important differences between treated and untreated plated were seen. The effects were again most pronounced with *C. glabrata*. • Discussion – Based on these results, Dr. Wiederhold recommended that only untreated polystyrene plates be used for the BMD method to minimize interlaboratory variability and that it be specified in the next M27 version. – Dr. Ghannoum suggested that the Caspofungin Ad Hoc Working Group include this parameter in their analysis and that they survey the type of plates that being used in laboratories that perform M27 method. – Dr. Rex noted that issues with binding to plastic are also seen with antibacterial BMD for polymyxin. Polymyxin is a positively charged molecule that binds to polystyrene plates. The effect is more prominent in smaller vessels such as 96 well plates because the surface area of plastic is greater per unit of volume. – Dr. Black (Merck) noted that these results highlight the need to better describe the M27 method in terms of consumables, handling of reagents, solvents, dilutions, and the method for how the plates are read. – Dr. Castanheira reported that her laboratory showed that some bacterial QC strains are more susceptible, for example, to show differences in fresh and not fresh MHB media. According to her experience, *C. glabrata* is generally more sensitive to shifts using the M27, BMD method.

4.	Effect of polysorbate 80 in antifungal susceptibility testing of *Candida* spp. by CLSI reference BMD (Dr. Castenheira)	Dr Castanheira tested the effect of adding polysorbate 80 in antifungal susceptibility testing of *Candida* spp. using the CLSI M27 method. – Polysorbate 80 can be used as a dispersion agent to prevent adherence of antimicrobials known to stick to glass or plastics. – For antibacterial susceptibility testing, the addition of polysorbate 80 leads to better separation of polymyxin resistant and susceptible strains and increased inter-laboratory reproducibility when testing dalbavancin. – She noted out that it must be used in the inoculums as well as in the panel preparation to prevent adherence during storage of plates. – Over 800 *Candida* isolates were tested in presence or absence of polysorbate 80 (0.002%) against echinocandins, azoles, and amphotericin B using the CLSI M27 method. – The addition of polysorbate 80 leads to clumping of cells at the bottom of wells as seen with AST method of bacteria, and changes the way antifungal susceptibility plates must be read. Therefore, some training/experience is required. – Polysorbate 80 supplementation led to an overall reduction in amphotericin B modal MICs by 3-4 dilutions for all *Candida* spp. tested. Modal MIC for echinocandins is also decreased by 1-4 dilutions with the exception of *C. parapsilosis* when tested against anidulafungin. Some MICs were found to be better defined in the presence of polysorbate 80. Fluconazole MICs were mostly unaffected by the addition of polysorbate 80 to broth. • It was also investigated if polysorbate 80 could resolve interlaboratory variations seen with caspofungin by lowering the high MICs obtained in some laboratories. – Parallel testing performed by Dr. Fuller at the University of Alberta showed a reduction in the MICs obtained but not to the level recorded by Dr. Castanheira. – The addition of polysorbate 80 shifted results in both laboratories; therefore, a difference of 2-3 MICs remains. – Polysorbate 80 does not appear to resolve caspofungin interlaboratory variability. • Results obtained on *C. parapsilosis* and *C krusei* QC strains show that the addition of polysorbate 80 lowers their MIC as well, resulting in out-of-range MICs. The use of polysorbate 80 would require that new QC studies be performed and the generation of new ECVs and CBP. • Conclusions

		– Polysorbate 80 improves separation of echinocandins as susceptible and resistant strains – Amphotericin B displayed a broader MIC distribution for all organisms tested in the presence of polysorbate 80. – No significant differences were observed when testing azoles with Tween 80 – The use of polysorbate 80 would require new validation studies to define QC ranges, ECVs, and CBP. – The addition of polysorbate 80 might not be required for all compounds but only those that present challenges. • Discussion – Dr. Alexander questioned if polysorbate 80 may cause foaming problems. Dr. Castanheira stated that she did not observe any foaming at the concentrations used. Dr. Alexander reported that Ms. Cullen noted in an email that she strongly suggests not using polysorbate 80 as it can affect transport of the antifungal molecule across the cell wall membrane. – Dr. Perlin indicated that detergent's micelle will affect binding to cell wall. Echinocandins are known to be hydrophobic molecules that tend to aggregate in solution. He noted that the real issue is whether fks resistant strains can be reasonably separated from wild type ones. – Dr. Ghannoum concurred that polysorbate 80 improves separation of susceptible and resistant strains. The disadvantage is that new QC and CBP would need to be performed. He noted that polysorbate 80 is already used for inoculum preparation of filamentous fungi in the antifungal susceptibility testing method (M38-A2) – Mr. Killian (Thermo Fisher Scientific) confirmed that no polysorbate 80 is used or added to YeastOne plates – Dr. Castanheira confirmed that the polysorbate 80 must be present in the RPMI media to prevent adherence. No effect seen on MICs if it is only added to the water inoculum as binding of the antifungal to plastic can occur during storage. – It was noted that micafungin species specific clinical breakpoints were FDA approved (see FDA, June 2013 update)
5.	Caspofungin Issue: Additional Discussion (All)	• A motion was made and seconded to remove both comments regarding potency of caspofungin from the footnotes in the next version of M27-S (Table 2 footnote c; table 7 footnote 2 (**Vote approved: 7-0**). M27-S4 will be revised and combined with M44-S. Dr. Pfaller will provide revised language.

		• The meeting minutes will include the discussion and data regarding caspofungin antifungal testing variability issue and will be distributed to the attendees and posted on the Antifungal subcommittee page on the CLSI website. No official statement/letter will be distributed to clinical laboratories for the time being.
6.	Presentation of Caspofungin Working Group Testing Proposal (Dr. Pfaller)	Dr Pfaller presented proposed studies to address interlaboratory variability of caspofungin MICs (see attached). • The proposed plan was divided in two phases. – The starting point will be focused on the evaluation of new QC strains to control readings at the low end of MIC distribution. – Follow-up studies will be conducted to assess potential issues with panel preparation that may affect MIC results and to evaluate a set of candidate QC strains in a new Tier 2 QC study. • Initial study – 5-10 laboratories that produce their own frozen panels and who routinely perform M27 method on all three echinocandins will test five *C. glabrata* and two *C. albicans* candidate QC isolates with low end MIC distributions. – The study is to include both high MIC and low MIC laboratories. – Each laboratory will test each candidate QC strains up to three times per day for a total of 10 replicates. – A standardized questionnaire will control for the different variables. o drug and RPMI lot o RPMI sterilization method o panel lot and size o microplate manufacturer and type of treatment o drug solvent and vessel used to produce stocks o inoculum standardization method – The reproducibility of candidate strains within the laboratory and interlab will used to define preliminary MIC ranges from the collected data. – Outlier laboratories will be further investigated to identify other variables that result in interlaboratory variability. • Discussion – It was debated if a detailed protocol should be provided to participating laboratories. Dr. Perlin warned that if all variables are identical, the subcommittee may not be able to identify the factor(s) responsible for caspofungin interlaboratory variability.

		– Dr. Castanheira proposed that a set of standard reagents be sent to different laboratories to discern if there are issues relate to the reading or manufacturing and preparation of the panels. She proposed that a single lot of caspofungin be used for the initial study to exclude that variable. – Dr. Motyl stated, on behalf of Merck, that a single lot could be provided. She noted that it would be wise to include some commercial panels in the protocol design and recommended that laboratories photograph the plates to compare reading criteria. She also pointed out that the laboratories should submit panel and drug storage conditions. • Conclusions – A motion to perform a study to assess lab-to-lab variability and a subsequent Tier 2 QC study was made and seconded. The motion was approved (**Vote approved: 7-0**) – Dr. Alexander requested that suggestions regarding any study design parameters that were missed be sent to Dr Pfaller, Ms. Hackenbrack, or herself.
7.	ECV's for Candida and Echinocandins (Dr. Pfaller)	Dr. Pfaller reviewed data for the development of ECVs for *Candida* and the Echinocandins. • Because of the need for education on ECVs and concern for potential confusion regarding the differences between ECVs and clinical breakpoints, it was proposed that a separate ECV document will be developed. • The document will provide a standardized approach for developing ECVs as well as an educational component. – ECVs will only be developed for those organisms that don't have clinical breakpoints. – The document will clearly state what ECVs do and do not do (allow to differentiate between wild type and non-wild type isolates). • It was suggested that EUCAST should be represented on the working group that will developed the document. Dr. Alexander will contact EUCAST to determine if a representative of EUCAST can be added to the working group. • A motion to develop a document for development of ECVs for yeasts and moulds was made and seconded **(Vote approved: 7-0).** Requirements of the development of ECVs will include: – All laboratories must use the CLSI broth microdilution method. – All results must be read at a specified time. – At least three laboratories must provide data for at least 100 isolates.

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A motion to develop a supplement to present ECVs for yeasts and moulds was made and seconded (Vote approved: 7-0)
Dr. Lockhart and Dr. Espinel-Ingroff will draft a proposal for a new ECV document.

The issue regarding which proportion to use for ECV (95% or 97.5 %) was discussed.

It was suggested that 97.5% agrees best with the MIC value and provides a better statistical value.
A motion to accept the ECVs (95% and 97.5%) listed in Table 3 of Pfaller MA et al (Multicenter Study of Anidulafungin and Micafungin MIC Distributions and Epidemiological Cutoff Values for Eight Species of Candida and the CLSI M27-A3 Broth Microdilution Method) was made and seconded (Vote approved: 7-0). The use of 95% or 97.5 % will be based on what is published in the new ECV document. See below for ECV values.

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<td>8.</td>
<td>ECVs for Candida and Amphotericin B, Itraconazole and 5- flucytosine (Dr. Pfaller)</td>
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The raw data for the designation of ECVs for Candida and amphotericin B, itraconazole, and 5- flucytosine were reviewed.

Amphotericin B

There was not sufficient data collected (<100 organisms tested) for C. lusitaniae, C. dubliniensis, and C. guillerimondii. Data will continue to be collected.
Proposed ECV values for Amp B were as follows:

<table>
<tr><th>Species</th><th>ECV (µg/mL)</th></tr>
<tr><td>C albicans</td><td>2</td></tr>
<tr><td>C glabrata</td><td>2</td></tr>
<tr><td>C parapsilosis</td><td>2</td></tr>
<tr><td>C tropicalis</td><td>2</td></tr>
<tr><td>C krusei</td><td>2</td></tr>
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Itraconazole

Clinical breakpoints will only be considered for C. albicans (≤ 0.12 µg/mL). The ECV is the same as the clinical breakpoint.
Proposed ECVs (24 hr)

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C. glabrata: ≤ 2 µg/mL
C. parapsilosis: ≤ 0.5 µg/mL
C. tropicalis: ≤ 0.5 µg/mL
C. krusei: ≤ 1 µg/mL

- There is insufficient susceptibility data for C. lusitaniae, C. dubliniensis, and C. guillerimondii. Data will continue to be collected.

• Flucytosine
- C. krusei exhibits innate resistance to flucytosine.
- There is insufficient susceptibility data for C. lusitaniae, C. dubliniensis, and C. guillerimondii. Data will continue to be collected.
- Proposed ECVs (24 hr)
<table>
<tr><th>Species</th><th>ECV (µg/mL)</th></tr>
<tr><td>C. albicans</td><td>0.5</td></tr>
<tr><td>C. parapsilosis</td><td>0.5</td></tr>
<tr><td>C. tropicalis</td><td>0.5</td></tr>
<tr><td>C. krusei</td><td>32</td></tr>
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- It was noted that testing at the lower end of MIC range for C. glabrata and 5FC needs to be performed.

A motion to accept the proposed ECVs for Candida (except the uncommon species) and amphotericin B, Itraconazole, and 5- flucytosine was made and seconded (Vote: Approved 7-0).
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<td>9.</td>
<td>ECVs for Aspergillus spp. and Azoles (Itraconazole, Posaconazole, and Voriconazole) (Dr. Espinel-Ingroff)</td>
<td>Dr. Espinel-Ingroff presented the raw data for the designation of ECVs for 6 species of Aspergillus and three azoles.
• The ECVs were based on ≥ 95% of the modal MIC. The values for 97.5% will be reviewed before a final decision on the ECVs is made.</td>
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Proposed ECVs (≥ 95%) for Aspergillus.

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<tr><th>Species</th><th>Antifungal Agent</th><th>ECV = ≥ 95% modeled MIC (µg/mL)</th></tr>
<tr><td>A. fumigatus</td><td>Itraconazole

Voriconazole</td><td>1

1</td></tr>
<tr><td>A. flavus</td><td>Itraconazole
Posaconazole
Voriconazole</td><td>1
0.25
1</td></tr>
<tr><td>A. terreus</td><td>Itraconazole
Posaconazole
Voriconazole</td><td>1
0.5
1</td></tr>
<tr><td>A. niger</td><td>Itraconazole
Posaconazole
Voriconazole</td><td>1
1
1</td></tr>
<tr><td>A. nidulans</td><td>Itraconazole
Posaconazole
Voriconazole</td><td>2
2
0.5</td></tr>
</table>

A motion to accept the 95% values (if there is consensus in the new document) with the 97.5% values being generated and voted upon electronically was made and seconded (Vote approved: 7-0). Regarding A. fumigatus and posaconazole, one laboratory provided most of data and 105 isolates were off on the lower range. Thus, it was decided that more data from additional laboratories is needed and that lower MIC range should be tested.

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<tr>
<td>10.</td>
<td>ECVs for Aspergillus spp. and Isavuconazole (Dr. Espinel-Ingroff)</td>
<td>
Dr. Espinel-Ingroff presented the raw data and ECVs for 5 species of Aspergillus and Isavuconazole.

Proposed ECVs

<table>
<tr><th rowspan="2">Species Complex</th><th colspan="2">ECVs (µg/mL)</th></tr>
<tr><th>≥ 95%</th><th>≥ 97.5%</th></tr>
<tr><td>A. fumigatus</td><td>1</td><td>1</td></tr>
<tr><td>A. flavus</td><td>1</td><td>1</td></tr>
<tr><td>A. niger</td><td>4</td><td>4</td></tr>
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<table>
<tr><td></td><td></td><td>
<table>
<tr><td>A. terreus</td><td>1</td><td>1</td></tr>
</table>

For A. nidulans, ECVs of 0.25 (for both ≥ 95% and ≥ 97.5%) were generated; however, it was noted that the data shows that the distribution of the MICs was skewed and can only be listed as tentative. More data will be collected and analyzed before the ECVs will be voted upon.
A motion to accept the ECVs for A. fumigatus, A. flavus, A. niger, and A. terreus with isavuconazole was made and seconded (Vote approved: 7-0)

</td></tr>
<tr><td>11.</td><td>ECVs for Aspergillus spp. and Caspofugin (Dr. Espinel-Ingroff)</td><td>
Dr. Espinel-Ingroff presented the raw data and ECVs for 5 species of Aspergillus and Caspofungin.

It was noted that polysorbate 80 is used for preparing the inoculum and that the same issues seen with yeasts and caspofungin are not apparent when testing moulds.
Proposed ECVs

<table>
<tr><th rowspan="2">Species</th><th colspan="2">Calculated Statistical ECVs (µg/mL)</th></tr>
<tr><th>≥ 95%</th><th>≥ 97.5%</th></tr>
<tr><td>A. fumigatus</td><td>0.5</td><td>0.5</td></tr>
<tr><td>A. flavus</td><td>0.25</td><td>0.5</td></tr>
<tr><td>A. niger</td><td>0.25</td><td>0.25</td></tr>
<tr><td>A. terreus</td><td>0.25</td><td>0.5</td></tr>
</table>

A motion to accept the ECVs (95%) for A. fumigatus, A. flavus, A. niger, and A. terreus and caspofungin was made and seconded (Vote approved: 7-0). ECVs at 97.5% will be reviewed and voted upon electronically.
More data will be collected for A. nidulans. It was suggested that molecular testing be performed on the isolates to confirm the identifications.

</td></tr>
<tr><td>12.</td><td>ECVs for Aspergillus spp. and Amphotericin B (Dr. Espinel-Ingroff)</td><td>
Dr. Espinel-Ingroff presented the raw data and ECVs for 6 species of Aspergillus and Amphotericin B.

Proposed ECVs

<table>
<tr><th rowspan="2">Species</th><th colspan="2">Calculated Statistical ECVs (µg/mL)</th></tr>
<tr><th>≥ 95%</th><th>≥ 97.5%</th></tr>
<tr><td>A. fumigatus</td><td>2</td><td>2</td></tr>
<tr><td>A. flavus</td><td>2</td><td>4</td></tr>
</table>
</td></tr>
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<table>
<tr><td></td><td></td><td>
<table>
<tr><td>A. nidulans</td><td>4</td><td>4</td></tr>
<tr><td>A. niger</td><td>2</td><td>2</td></tr>
<tr><td>A. terreus</td><td>4</td><td>4</td></tr>
<tr><td>A. versicolor</td><td>2</td><td>2</td></tr>
</table>
A motion to accept the ECVs for both 95% and 97.5% for Aspergillus spp. and amphotericin B was made and seconded (Vote approved: 7-0)
</td></tr>
<tr><td>13.</td><td>Revision of Antifungal documents (Dr. Alexander)</td><td>Dr. Alexander reported that all of the entire antifungal documents should be reviewed for possible revision. She noted that it has already been decided to combine the supplements so that all tables for yeast are in one supplement (M27/M44S) and all tables for filamentous fungi are in another (M38/M51). Volunteers were requested to review each of the documents to determine if revisions are needed. Review of these documents should be completed prior to a Webinar to be scheduled for late May or early June.

M27 – Dr. Lockhart and Ms. Kovanda
M38 – Dr. Espinel-Ingroff and Dr. Dufresne
M44 – Ms. Traczewski and Dr. Motyl
M51 – Ms. Fothergill and Dr. Motyl
M27/M44S – Dr. Pfaller and Dr. Alexander
M38/M51 – Dr. Ghannoum and Dr. Fuller

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<tr><td>14.</td><td>QC Update (Ms. Cullen)</td><td>In Ms. Cullen’s absence, Dr. Alexander reviewed the issues regarding Tier 3 monitoring of antifungal QC ranges and QC ranges for isavuconazole.

A motion to accept the 48 hr QC ranges for C. parapsilosis (0.03-0.12) and C. krusei (0.12-0.5) with isavuconazole was made and seconded (Vote approved: 7-0).
In her submission letter, Ms. Cullen noted that it is not clear in the antifungal documents when or how QC should be read. She suggested that additional guidance should be included in future editions of the document. Possible recommendations include:

Test routinely at 24 hours and provide 48 hour ranges as supplemental information?
Read QC at the same time as clinical results?

Antimicrobial agents whose clinical results can be read at 24 or 48 hours
Antimicrobial agents whose clinical results are read at 48 hours

These issues will be considered by the groups assigned to review the documents for potential revision.

Additional discussion of the Tier 3 data will be tabled until the webinar to be scheduled in

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		late May or early June.

Action Items – Due by Dates listed below

Specific Action Item Descriptions		Responsible Individual/Due Date
1.	Send comments and suggestions to Dr. Alexander, Dr. Pfaller, or Ms. Hackenbrack regarding caspofungin working group testing proposal.	All – Participants should submit any recommendations or comments by February 28, 2014.
2.	Removal of both comments regarding potency of caspofungin to be removed from the footnotes in the next version of M27-S (Table 2 footnote c; table 7 footnote 2).	M27-S/M44-S working group (Dr. Pfaller and Dr. Alexander)
3.	Review and make recommendations for revision of the antifungal documents	M27 – Dr. Lockhart and Ms. Kovanda M38 – Dr. Espinel-Ingroff and Dr. Dufresne M44 – Ms. Traczewski and Dr. Motyl M51 – Ms. Fothergill and Dr. Motyl M27/M44S – Dr. Pfaller and Dr. Alexander M38/M51 – Dr. Ghannoum and Dr. Fuller **By 1 May 2014**
4.	Prepare project proposal for development of an antifungal ECV document	Dr. Lockhart Dr. Espinel-Ingroff **By 1 February 2014 (Note: This proposal has been submitted and is under review by the subcommittee)**

Next Meeting Reminder:

The next Web conference will be scheduled for late May or early June. A poll for availability has been distributed. The main purpose of the Web conference is to:

- Review of raw data for development of ECVs for *Cryptococcus* spp.
- Review of Tier 3 QC data (Ms. Cullen)
- Review of data for a new antifungal agent (Dr. Ghannoum)
- Review recommendations for revision of the antifungal susceptibility testing documents

Specific agenda and reference materials for discussion will be distributed prior to the Web conference.

Adjournment

The meeting was adjourned at 4:00 PM Eastern (US) time.

Respectfully submitted,

Lynette Y. Berkley, PhD
FDA CDER

Philippe Dufresne, PhD
Institut National de Santé Publique

Marcy L. Hackenbrack, MCM, M(ASCP)
CLSI

PROPOSED STUDIES TO ADDRESS LAB-TO-LAB VARIABILITY IN CASPOFUNGIN MICS (Dr. Pfaller)

STARTING POINT WILL BE FOCUSED ON NEW QC STRAINS TO CONTROL READINGS AT THE LOW END OF THE MIC DISTRIBUTION.

INITIAL STUDY

1. **STRAIN SELECTION: 7 STRAINS; 5 CANDIDA GLABRATA AND 2 CANDIDA ALBICANS ISOLATES (QUESTION AS TO WHETHER WE ALSO INCLUDE FKS MUTANT STRAIN OF CANDIDA GLABRATA). ALSO TO INCLUDE THE C.PARAPSILOSIS AND C. KRUSEI QC STRAINS.**
2. **STUDY DESIGN**
   - **A. 5 TO 10 LABS THAT CURRENTLY PERFORM TESTING USING ALL 3 ECHINOCANDINS AND WHO MAKE THEIR OWN FROZEN PANELS**
   - **B. INCLUDE BOTH HIGH MIC LABS AND LOW MIC LABS.**
   - **C. EACH LAB TO TEST EACH CANDIDATE QC STRAINS UP TO 3 REPLICATES PER DAY FOR A TOTAL OF 10. CURRENT QC STRAINS TESTED X1 EACH DAY OF TESTING.**
   - **D. COLONY COUNT FOR AT LEAST 1 REPLICATE FOR EACH CANDIDATE QC STRAIN.**
   - **E. COLLECT INFORMATION ON PANEL MATERIALS USING A STANDARD FORM**
     1) **DRUG LOT**
     2) **RPMI LOT/SOURCE**
     3) **RPMI STERILIZATION METHOD**
     4) **NUMBER OF PANELS MADE IN THE LOT USED**
     5) **PANEL PLASTIC MANUFACTURER AND TYPE OF TREATMENT**
     6) **DRUG SOLVENT AND VESSEL USED TO MAKE STOCK (E.G. PLASTIC VS GLASS)**
     7) **INOCULUM STANDARDIZATION METHOD/COLONY COUNTS.**
   - **F. DATA ANALYSIS**
     1) **INITIAL EVALUATION OF REPRODUCIBILITY OF CANDIDATE STRAINS WITHIN LAB AND BETWEEN LABS AND MANUFACTURERS**
     2) **DEFINE A PRELIMINARY MIC RANGE USING DATA FROM MULTIPLE MANUFACTURERS/LABS**
     3) **OUTLIER LABS SHOULD BE FURTHER INVESTIGATED RE VARIABLES THAT MAY INFORM THE ENTIRE PROCESS.**

FOLLOW-UP STUDIES DRIVEN BY DATA FROM INITIAL STUDY

1. **MANUFACTURABILITY**
   - **A. 3 DIFFERENT MANUFACTURERS TO MAKE FROZEN PANELS**
     1) **SAME LOT OF DRUG POWDERS**
     2) **INCLUDE ANIDULAFUNGIN, CASPOFUNGIN AND MICAFUNGIN**
     3) **SPECIFY LOT OF RPMI USED (FILTER STERILIZE NOT AUTOCLAVE)**
     4) **TEST 10 REPLICATES OF EACH CANDIDATE STRAIN PLUS 10-30 OTHER STRAINS WITH KNOWN S AND R AND WT VS FKS MUTANT STATUS**
     5) **TEST AT 1-3 LABS (UP FOR DISCUSSION)**

6) **HAVE DRUG POTENCY ASSAYED EITHER FROM THE PANEL OR STOCK SOLN USED TO PREPARE PANEL.**

2. **TIER 2 QC STUDY**
   1) **INCLUDE NARROWED DOWN LIST OF CANDIDATE QC STRAINS**
   2) **INCLUDE ANY SPECIFIC INSTRUCTIONS FOR MANUFACTURING**

ECVs for from Table 3 of Pfaller MA et al (Multicenter Study of Anidulafungin and Micafungin MIC Distributions and Epidemiological Cutoff Values for Eight Species of Candida and the CLSI M27-A3 Broth Microdilution Method)

Antifungal agent	Species	No. tested	MIC (µg/mL)		ECV (µg/mL)[a]		
			Range	Mode[b]	≥95%	≥97.5%	≥99%
Anidulafungin	*C. albicans*	8,210	0.008-2	0.03	0.06	0.12	0.12
	C. glabrata	2,680	0.008-4	0.06	0.12	0.12	0.25
	C. parapsilosis	3,976	0.008-8	2	4	8	8
	C. tropicalis	2,042	0.008-2	0.03	0.12	0.12	0.12
	C. krusei	322	0.008-2	0.06	0.12	0.25	0.25
	C. lusitaniae	234	0.008-1	0.25	1	1	1
	C. guilliermondii	222	0.03-4	1	4	8	8
	C. dubliniensis	131	0.015-4	0.03	0.12	0.12	0.12
Micafungin	*C. albicans*	7,874	0.008-4	0.015	0.03	0.03	0.03
	C. glabrata	3,102	0.008-4	0.015	0.03	0.03	0.03
	C. parapsilosis	3,484	0.015-4	1	2	4	4
	C. tropicalis	1,605	0.008-8	0.015	0.06	0.06	0.12
	C. krusei	617	0.015-1	0.06	0.25	0.25	0.25
	C. lusitaniae	258	0.008-≥16	0.25	0.5	0.5	1
	C. guilliermondii	234	0.015-8	0.5	2	2	4
	C. dubliniensis	117	0.008-8	0.06	0.12	0.12	0.12