Meeting Title:	**Subcommittee (SC) on Antifungal Susceptibility Tests**	**Contact:**	clam@clsi.org
		Secretary	Camille Hamula, PhD, D(ABMM)
Virtual Meeting Dates/Times:	**Friday, 26 August 2022, 10:00 AM – 1:00 PM Eastern (US) time**		
Meeting Purpose:	The purpose of this meeting is to discuss Antifungal SC business.		
Requested Attendee(s):	SC Chairholder, Vice-chairholder, Members, Advisors, and Reviewers; Expert Panel on Microbiology Chairholder and Vice-chairholder; Presenters; Other Interested Parties; CLSI Staff		

Attendee(s):	
Philippe J. Dufresne, PhD, RMCCM **Chairholder**	**Institut national de santé publique du Québec**
Gary W. Procop, MD, MS **Vice-chairholder**	**American Board of Pathology**
Members Present:	
Elizabeth Berkow, PhD	Centers for Disease Control and Prevention
Sharon K. Cullen, BS, RAC	Beckman Coulter, Inc. Microbiology Business
Tanis Dingle, PhD, D(ABMM), FCCM	Alberta Precision Laboratories - Public Health Laboratory
Hari P. Dwivedi, BVSc(DVM), MVSc, PhD	bioMérieux, Inc.
Sixto M. Leal, Jr., MD, PhD	University of Alabama at Birmingham
Audrey N. Schuetz, MD, MPH, D(ABMM)	Mayo Clinic Rochester
Amir Seyedmousavi, VMD, PhD, FECMM	National Institutes of Health
Paul E. Verweij, MD, FECMM	Radboud University Medical Center
Nathan P. Wiederhold, PharmD	University of Texas Health Science Center at San Antonio
Advisors Present:	
Barbara Alexander	Duke University Medical Center
David Andes, MD	University of Wisconsin - Madison Medical School
Andrew M. Borman, BSc, PhD	UK Health Security Agency
Mariana Castanheira, PhD	JMI Laboratories
Anuradha Chowdhary, MD, PhD	Vallabhbhai Patel Chest Institute
Jeff Fuller, PhD, FCCM, D(ABMM)	London Health Sciences Centre
Mahmoud Ghannoum	Case Western Reserve University
Kerian K. Grande Roche, PhD	FDA Center for Drug Evaluation and Research
Camille Hamula, PhD Committee Secretary	Saskatoon Health Region/University of Saskatchewan
Kimberly Hanson, MD, MHS	ARUP Laboratories
Nicole M. Holliday, BA	Thermo Fisher Scientific
Julianne Kus, HONBSc, MSc, PhD, FCCM	Public Health Ontario
Shawn R. Lockhart, PhD, D(ABMM), F(AAM)	Centers for Disease Control and Prevention
Jaques F. Meis, MD, PhD, FIDSA, FRCPath, FAAM	Canisius Wilhelmina Hospital
David S. Perlin, PhD	Hackensack Meridian Health Center for Discovery and Innovation
Ribhi Shawar	FDA Center for Devices and Radiological Health
Adrian M. Zelazny, PhD, D(ABMM)	National Institutes of Health Department of Laboratory Medicine
Sean X. Zhang, MD, PhD, D(ABMM)	Johns Hopkins University

Staff:	
Kathy Castagna	CLSI
Emily Gomez, MS, MLS(ASCP)MB	CLSI
Christine Lam, MT(ASCP)	CLSI

AGENDA (Part 1) Friday, 26 August 2022 10:00 AM – 1:00 PM All times are Eastern (US) time					
#	**Time**	**Length**	**Presenter**	**Description**	**Background**
1.	10:00 AM	5 min.	C. Lam	Zoom meeting instructions	N/A
2.	10:05 AM	5 min.	P. Dufresne	**Opening Remarks** • Agenda review (VOTE) • 2022 Winter Meeting Summary Minutes (VOTE)	2a_Agenda 2b_Winter Meeting Summary Minutes 2c_Roster 2d_DOI Summary
3.	10:10 AM	30 min.	P. Dufresne	**Status of Antifungal Documents** • Overview of newly published M27M44S, M38M51S, M57S • Document status • M27 review recommendations (M. Castanheira and G. Garcia-Effron) • M38 Review recommendations (J. Fuller and S. Zhang)	3_Presentation
4.	10:25 AM	10 min.	A. Schuetz V. Tesic	**Reporting WG – Intrinsic Resistance WG** • Informational Update	4_Presentation
5.	10:35 AM	15 min.	D. Andes A. Borman N. Wiederhold	**Breakpoint WG Update** • *A. fumigatus* isavuconazole and posaconazole work in progress • *A. fumigatus* and voriconazole rationale document	5_Presentation 5a_Voriconazole Rationale Document Draft
6.	10:50 AM	45 min.	P. Dufresne S. Lockhart N. Wiederhold	**ECV WG Update** • Membership, request for MIC data, and publication plan • *Aspergillus* ECV (round 5) – update and request for isolates • Pragmatic approach for MIC interpretation for species with no breakpoints	6_Presentation 6a_Pragmatic Approach Susceptibility
7.	11:00 AM	30 min.	P. Dufresne	**Other Business** • Antifungal mutations: detection protocol and impact on resistance • Next meeting (January 2023)	7_Presentation 7a_Molecular Mechanisms Acquired Resistance
8.	11:50 AM	N/A	P. Dufresne	Adjournment	N/A

Summary of Voting Decisions

Motion Made and Seconded	Voting Results[a]	Page[b]
To approve the agenda for the meeting.	9-0-0-0	5
To approve the 2022 Winter Meeting Summary Minutes.	9-0-0-0	5
To revise the M27 and M38 documents.	9-0-0-0	8

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

[b] Page links can be used to go directly to the related topic presentation and voting discussions.

SUMMARY MINUTES
Friday, 26 August 2022

#	Description
1.	**ZOOM MEETING INSTRUCTIONS (C. LAM)** • Ms. Lam provided the instructions for voting, commenting, and asking questions.
2.	**OPENING REMARKS (P. DUFRESNE)** Dr. Dufresne welcomed everyone to the meeting. He noted that all three working groups (WG) will be presenting updates (Breakpoint WG, ECV WG, and Reporting WG, which includes Intrinsic Resistance WG and Body Site Reporting WG). • **Agenda Review** – Dr. Dufresne reviewed the agenda and requested any changes. – No changes were requested and the agenda was approved (9 for; 0 against; 0 abstain; 0 absent – Pass). **A motion to accept the agenda for the meeting was made and seconded. VOTE: 9 for; 0 against; 0 abstain; 0 absent (Pass).** • **Meeting Summary Review and Vote: Winter 2022 Meeting Summary Minutes** – There were no corrections to the Winter 2022 meeting summary minutes. **A motion to accept the 2022 Winter meeting summary minutes was made and seconded. VOTE: 9 for; 0 against; 0 abstain; 0 absent (Pass).** • **General rules for the SC were reviewed** – Disclosures of interest have been reported. It was requested that any new conflicts be reported during the meeting discussion. – The SC voting rules were reviewed. It was noted that those with leadership roles do not vote.
3.	**STATUS OF ANTIFUNGAL DOCUMENTS (P. DUFRESNE)** • **The category and status of each antifungal document was reviewed.** – General Rules ○ **Active (procedural documents):** Still in the review process and can be revised every 3-5 years ○ **Archived:** Content is static but useful and valid; Are not in the review process ○ **Withdrawn:** Documents are no longer valid or available for sale. ○ **Supplements:** Can be revised yearly or as needed • Antifungal documents M38M51S Ed 3, M27M44S Ed 3, M57S Ed4 supplements were all published and available on ECLIPSE on 5 August. • Main Highlights: – M27M44S changes: ○ BMD tentative S Breakpoints for Rezafungin and *Candida* spp. including *C.auris*

Committee Status	"Pass" Vote
All members present and voting	9-0; 8-1; 7-2; 6-3
One member not present or abstaining	8-0; 7-1; 6-2
Two members not present or abstaining	7-0; 6-1
Three members not present or abstaining	6-0
If more than three members not present	Chairholder's discretion to conduct vote or table until sufficient members are present, or an electronic vote is taken.

#	Description

Table 1: Breakpoint for BMD at 24 hrs *Candida* spp.

Antifungal Agent	Species	MIC Breakpoints and Interpretive Categories, µg/mL			
		S	I	SDD	R
Rezafungin[i,j]	*C. albicans*	≤ 0.25	-	-	-
	C. auris	≤ 0.5	-	-	-
	C. dubliniensis	≤ 0.12	-	-	-
	C. glabrata	≤ 0.5	-	-	-
	C. krusei[b]	≤ 0.25	-	-	-
	C. parapsilosis[c]	≤ 2	-	-	-
	C. tropicalis	≤ 0.25	-	-	-

- Table 3 MIC QC range for Micafungin and *C. krusei* ATCC 6258 has changed and shifted by one dilution (change from 0.12-0.5 µg/mL to 0.06-0.25 µg/mL)
- Body site reporting for *Candida* spp. is now available in Appendix A, will tell labs body sites from which certain antifungals are not appropriate to report or with specific comments.
- Appendix B Intrinsic Resistance for Yeasts Table:

Appendix B. Intrinsic Resistance for Yeasts

Organism	Antifungal Agent				
	Amphotericin B	Anidulafungin	Caspofungin	Fluconazole	Micafungin
Candida krusei[a]	-	-	-	IR[1-12]	-
Candida lusitaniae	-[b]	-	-	-	-
Cryptococcus spp.	-	IR[13-23]	IR[13-23]	-	IR[13-23]
Rhodotorula spp.	-	IR[13-23]	IR[13-23]	IR[24-31]	IR[13-23]
Trichosporon spp.	-	IR[13-23]	IR[13-23]	-	IR[13-23]

Abbreviation: IR, intrinsic resistance.

Footnotes

b. *C. lusitaniae* is not intrinsically resistant to amphotericin B. However, *C. lusitaniae* may develop resistance to amphotericin B *in vivo* during therapy. When phenotypic resistance was noted in studies, the phenotype was observed only when agar gradient strips were used and was not detected by broth microdilution methods.[32]

– M38M51S: Appendix Table Intrinsic Resistance for Molds (*Aspergillus, A.terreus, Lomentospora prolificans, Mucorales, Purpureocillium lilacinum*

#	Description

Appendix table. Intrinsic resistance for Molds

Organism	Antifungal Agent: Amphotericin B	Fluconazole	Flucytosine	Voriconazole
Aspergillus spp.	-	IR[1-8]	-[a]	-
Aspergillus terreus	-[6,9-24,b]	IR[1-8]	-[a]	-
Lomentospora prolificans	IR[25-29]	IR[27,30]	-	-
Mucorales[c]	-	IR[31-56]	-	IR[31-56]
Purpureocillium lilacinum	IR[57-59]	-	-	-

Abbreviation: IR, intrinsic resistance.

Footnotes

a. Do not test flucytosine for *Aspergillus* spp.[60] The activity of flucytosine against *Aspergillus* spp. cannot be appropriately determined by the *in vitro* CLSI broth microdilution method because changes in pH can cause variation in minimal inhibitory concentration (MIC), including lower MIC values under more acidic conditions. There are no robust studies on correlation of MIC to clinical outcome.

b. Low MICs do not correlate with positive clinical outcomes, and testing is not recommended. Use of this antifungal agent is not recommended according to published treatment guidelines.[6,21,61]

c. Eg, *Cunninghamella*, *Lichtheimia*, *Mucor*, *Rhizomucor*, and *Rhizopus* spp.

- M57S Ed 4: 37 new ECVs for 15 species of yeast, *Candida* spp. and Asco Yeasts, Crypto and Basidiomycete yeasts, and some ECVs for Rezafungin

Table 1: ECV for *Candida* spp and Asco Yeasts

Added		
	ECVs for amphotericin B	• *Candida pelliculosa* • *Saccharomyces cerevisiae*
	ECVs for anidulafungin	• *Candida auris* • *Candida haemulonii* • *S. cerevisiae*
	ECVs for caspofungin	• *C. auris* • *S. cerevisiae*
	ECVs for fluconazole	• *C. haemulonii* • *Candida pararugosa* • *C. pelliculosa* • *Candida rugosa* • *S. cerevisiae*
	ECVs for itraconazole	• *C. pelliculosa* • *S. cerevisiae*
	ECVs for micafungin	• *C. auris* • *C. pelliculosa* • *S. cerevisiae*
	ECVs for posaconazole	• *C. haemulonii* • *C. pelliculosa* • *S. cerevisiae*
	ECVs for voriconazole	• *C. haemulonii* • *C. pelliculosa* • *S. cerevisiae*

Table 2: ECV for Crypto and Badisiomycete Yeasts

Added		
	ECVs for amphotericin B	• *Rhodotorula mucilaginosa* • *Trichosporon asahii*
	ECV for fluconazole	*T. asahii*
	ECVs for itraconazole	• *R. mucilaginosa* • *T. asahii*
	ECVs for posaconazole	• *R. mucilaginosa* • *T. asahii*
	ECV for voriconazole	*R. mucilaginosa*

Table 4: ECV for Yeasts with BP

Added		
	ECVs for rezafungin	• *Candida albicans* • *C. auris* • *Candida dubliniensis* • *Candida glabrata* • *Candida krusei* • *Candida parapsilosis* • *Candida tropicalis*

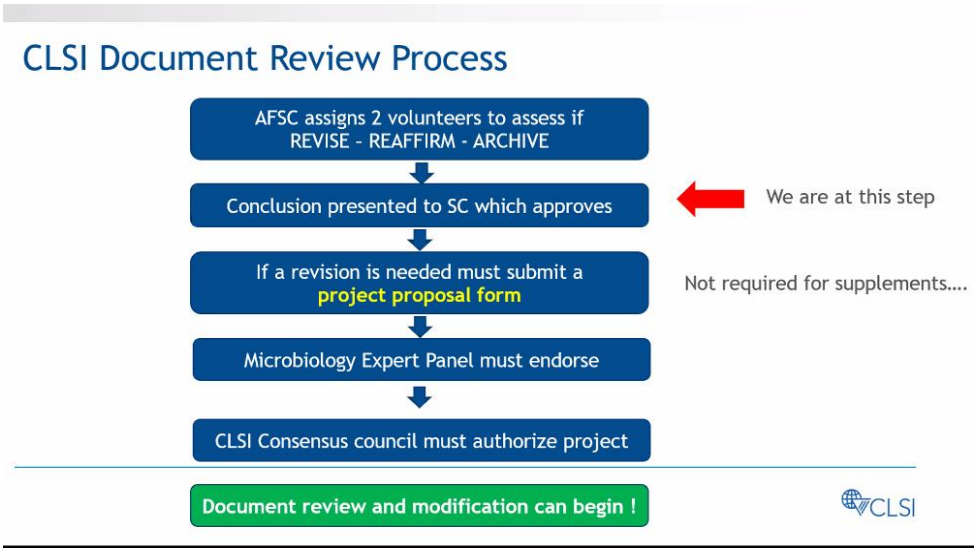
37 new ECVs (15 species / all yeasts)

- Other Document status updates:
  - M27 review recommendation for 2022, if revision needed a project proposal will need to be drafted.
  - M38 review recommendation for 2022, if revision needed a project proposal will need to be drafted.
  - M44 review in 2023, 2 volunteers needed please contact Philippe if interested.

#	Description

Action Item: M44 review volunteers contact Dr. Dufresne if interested.

- Review of CLSI Document Review Process



- Dr. Castanheira: Clarified the term "tentative" for rezafungin, should it be "provisional" as for cefidericol breakpoints? Dr. Dufresne is going to check the criteria, should it be tentative for first year then transition to provisional? Want to keep the same naming convention as bacterial. Dr. Castanheira will check the documents.

Action Item: Dr. Dufresne and Dr. Castanheira will check the bacterial criteria for "tentative" vs "provisional."

- **M27 Review Recommendations:**
  - Dr. Garcia-Effron and Dr. Castanheira think it should be revised. Bacterial committee is revising M07 and adding ways to automate BMD panel production. Would be good to include in antifungal documents as well. We also have new antifungals for which to add the ranges. The interpretation section can also be more elaborate.
  - Vote on M27 revision: will be at same time as M38.

- **M38 Review Recommendations**
  - Dr. Dufresne, Dr. Fuller, Dr. Zhang
  - Supplemental material for M38 is incorporated into M38M51S Ed 3. Supplemental info for M57 is incorporated into M57S Ed 4.
  - Chapter 1 introduction modify text to clinical breakpoints for voriconazole and *A.fumigatus* and ECVs for a number of *Aspergillus* spp.
  - Chapter 2, Preparing for Antifungal Susceptibility Testing: revise that the acceptable test reproducibility is ± 2 fold dilution.
  - Chapter 3:
    - Introduce Broth Microdilution Method title to subchapter 3.2.3.
    - Add *Scedosporium* spp. onto non-dermatophyte mold list for testing.

#	Description
	○ Table 1 Recommended Incubation Times for Determining MICs and MECs: add Manogepix and Olorofim. ○ Subchapter 3.3 Reading MIC and MEC, add Oteseconazole, Rezafungin, Manogepix, Olorofim. ○ Subchapter 3.4: modify text to include clinical breakpoints for voriconazole and *A.fumigatus* and recommend using ECVs in absence of clinical breakpoints. ○ Subchapter 3.4.1 Amphotericin B, add IR of Amphotericin B in *P.lilacinus* and *L.prolificans*. ○ Subchapter 3.4.5 Azoles add *Aspergillus* section Usti for reduced azole Susceptibility, add IR of voriconazole in Mucoralean fungi. ○ 3.4.7. include interpretation results for Manogepix. ○ 3.4.8. include interpretation results for Olorofim. – Chapter 4.4.3 Preparing Strains for Storage: add 10% to 20% glycerol in step 7. – Chapter 5 Conclusion: include clinical breakpoints for voriconazole and *A.fumigatus*, also *Aspergillus* ECVs need to be included for a number of species. – References: need to be updated – **Subcommittee Discussion (Note:** Comments and questions may be paraphrased). ○ Dr. Dingle mentions some of the updates also apply to yeast documents. Yeast documents also need similar updates with new agents. ○ Ms. Cullen mentions we should consider moving breakpoints and QC ranges into Supplements which are updated annually. Reading and methods update every 3 to 5 years. Some of the older documents have the breakpoints and QC ranges in the method documents. ○ Dr. Dufresne thinks this is already done. Only thing that is tricky is when we have new agents. ○ Dr. Castanheira mentions in the bacterial documents we don't specify how to read each of the drugs whereas in antifungal documents we do. Dr. Castanheira suggests putting a note into the documents like M27 referring to M60 where reading parameters would be found. ○ Dr. Schuetz mentions the new CLSI limited review process for microbiology expert panels, to expedite reviews. It is quicker and the information can't change a document's scope or methodology. ○ Ms. Castagna points out that if this process is used, the changes to the document will be made before submitting a proposal to the microbiology expert panel.
	A motion to revise the M27 and M38 documents was made and seconded. VOTE: 9 for; 0 against; 0 abstain; 0 absent (Pass).
4.	**REPORTING WG – INTRINSIC RESISTANCE WG (A. SCHUETZ, V TESIC)** **Reporting WG Co-Chairholders:** Audrey Schuetz, Vera Tesic **Members:** Tanis Dingle, Kim Hanson, Stephanie Mitchell, Natasha Petit, Tom Walsh, Nathan Wiederhold, Matt Wikler, Nancy Zhao **Body site:** Vera Tesic, Kim Hanson, Stephanie Mitchell, Natasha Petit, Matt Wikler **IR:** Audrey Schuetz, Tanis Dingle, Priyanka Uprety, Tom Walsh, Nathan Wiederhold, Nancy Zhao • Update only, nothing to vote on today. • M38M51S, M57S ED4, and M27M44S updated.

#	Description

- Bug-drug combinations being retested from prior publications, awaiting results. Delayed by covid.
- Publication proposed for CMR report of IR decisions (will take a year).
- Further intrinsic resistance assessments.

Table 6 M57S ED4

	Anidulafungin	Caspofungin	Fluconazole	Micafungin
C. krusei			IR	
C. gattii (VGI, VGII, VNI)	IR	IR		IR
R. mucilaginosa	IR	IR	IR	IR
T. asahii	IR	IR		IR

In M27M44S, C. lusitaniae is listed as evaluated against amphotericin B but not intrinsically resistant:

b. ***C. lusitaniae*** **is not intrinsically resistant to amphotericin B. However, *C. lusitaniae* may develop resistance to amphotericin B *in vivo* during therapy. When phenotypic resistance was noted in studies, the phenotype was observed only when agar gradient strips were used and was not detected by broth microdilution methods.[32]**

Appendix M38M51S

	Amphotericin B	Fluconazole	Flucytosine	Voriconazole
Aspergillus spp.		IR	a	
A. terreus	b	IR	a	
L. prolificans	IR	IR		
Mucorales		IR		IR
P. lilacinum	IR			

a. Do not test Aspergillus against flucytosine. Activity cannot be determined by an in vitro BMD method, because changes in pH can cause variations in MICs.
b. Low MICs do not correlate with clinical outcome. Testing is not recommended.

#	Description

- New assessments:

Remaining Assessments

- C. rugosa and anidulafungin
- C. haemulonii and itraconazole
- C. inconspicua and fluconazole
- S. boydii and amphotericin B
- L. prolificans/S. apiospermum/S. boydii and isavuconazole
- L. prolificans and posaconazole
- L. prolificans and voriconazole
- Scedosporium/Lomentospora and 5FC
- L. prolificans and echinocandins (awaiting data)
- Fusarium and echinocandins (awaiting data)
- Mucorales and echinocandins (awaiting data)

- Anticipate that the WG may receive additional work from ECV working group. Above list is current from last meeting. These assignments are being divided this fall. Group will be meeting soon.
- Waiting for data for last 3 bullets above, echinocandins against *L.prolificans, Fusarium* and *Mucorales* from published investigators as they had data that may sway us against intrinsic resistance. Reached out to all of them and got ahold of them. The WG asked them to repeat the testing and confirm that species are correct. In many cases, covid slowed it down. Hopeful to see this data in January.

  - **Subcommittee Discussion:** (**Note:** Comments and questions may be paraphrased).
    - Dr. Dufresne has a suggestion to look at *P.variot~~t~~ii* and voriconazole, as it frequently comes up in the literature. Add to remaining assessment list.
    - Dr. Schuetz agrees this is a good suggestion. Will add to list.
    - *Rasamsonia* and voriconazole: another suggestion by Dr. Zhang.
    - Dr. Schuetz agrees this is also a good one.

Action Item: Dr. Schuetz will add *Paecilomyces varitotii* and Rasamsonia to the IR WG pending assessment list.

5. **BREAKPOINT WG UPDATE (DR. WIEDERHOLD, DR. DUFRESNE)**

Breakpoint WG Co-Chairholders: David Andes, Andy Borman
Secretary/Member: Nathan Wiederhold
Members: Mariana Castanheira, Philippe Dufresne, Kim Hanson, Shawn Lockhart, Gary Procop
***A. fumigatus* BPWG Chairholder:** Nathan Wiederhold
Members: David Andes, Philippe Dufresne, Shawn Lockhart

- Update on Clinical Breakpoints against *Aspergillus fumigatus*.
- Seeking data to bring existing antifungals to the breakpoint step. 2 major *ad hoc* working groups, one for Rezafungin and the other for *A.fumigatus* and azoles.
- June 2020: published Voriconazole breakpoints for *A.fumigatus*. Gathering data currently for isavuconazole and posaconazole.
- Preparing rationale document for voriconazole FDA-WG including Dr. Andes, Dr. Borman, Dr. Dufresne, Dr. Lockhart, Dr. Procop, Dr.Wiederhold, Dr. Zhang. Draft now available for AFST subcommittee. Please provide comments to us. Hope to meet during the month of December and then submit to FDA and publish by January 2023.

#	Description

What is included in the rationale document?

1. Standard Dosage and Pharmacokinetic Data
2. Minimal Inhibitory Concentration Distribution Data
3. Pharmacodynamic Data
4. Clinical Efficacy
5. Committee Rationale for the Breakpoint
6. Final Table Entry
7. Voting Record
8. Supporting References

- Once completed, the document published online on CLSI website as a numbered companion document
- FDA submission independent process - not mandatory but will help with FDA BP approval – done through FDA online submission portal

- Gathering data for isavuconazole, posaconazole breakpoints. Present and propose at January meeting. June 2023 timeline for submission of the *Aspergillus fumigatus* voriconazole rationale document to FDA in January 2023.

Action plan

ACTION ITEM	TIMELINE
VORICONAZOLE BP (rationale document only)	
Produce a draft for *A. fumigatus* voriconazole bp rationale document	August 2022
Submit voriconazole RD document at AFSC meeting	December 2022/January 2023
Edit for publication and submit to FDA	December 2022/January 2023
POSACONAZOLE and ISAVUCONAZOLE BP	
Gather data for CBP proposal	Isavuconazole completed Posaconazole pending
Present and propose BP at annual meeting – submit to vote	January 2023 (Isavuconazole)
Draft BP rationale document	December 2022 to March 2023 Isavuconazole
Edit for publication and submit to FDA	June 2023

- **Subcommittee Discussion** (**Note:** Comments and questions may be paraphrased).
  - Dr. Dingle: has the WG considered including *C.auris* Breakpoints for these drugs? Voriconazole/Isavuconazole/Posaconazole?
  - Dr. Wiederhold says no, but it is a good suggestion. They have considered some Rezafungin BPs for *C.auris*. CDC has a recommendation and guidance document but our WG has not considered, but this is a very good suggestion.

#	Description
	○ Dr. Verweij asked for review of rationale document Table 5: comments about isolates phenotypic R to voriconazole but if you look at the isolates some are not resistant and have WT MICs. Should we have some kind of comment about this? Suggested comment: Difficult to trust phenotype. ○ Cyp51A amino acid changes/mutations cause variable voriconazole resistance in *A.fumigatus*. Animal data shows that the phenotypes in the WT population can be treated but not sure you want to treat. Dr. Shawar suggests to use the term "reduced susceptibility" in these situations.
6.	**ECV WG UPDATE (DR. DUFRESNE)** **ECV WG Chairholder:** Shawn Lockhart **Vice-Chairholder:** Philippe Dufresne **Secretary/Member:** Nathan Wiederhold **Members:** Barbara Alexander, Jeff Fuller, Mahmoud Ghannoum, Kerian Grande Roche, Kim Hanson, John Turnidge, Tom Walsh, Amir Seyedmousavi **Advisors:** Mariana Castanheira, Mike Birch Request for MIC data and/or isolates:

Need for more MICs and isolates (rare yeasts)

Round 2 - Yeast

Species	Minimum number of isolates required
Candida haemulonii	15-25
Lodderomyces elongisporus	45-65
Candida bracarensis	55-75
Candida nivariensis	20-50
Candida (Diutina) rugosa	10-20
Candida (Wickerhamiella) pararugosa	20-30

If no MIC data isolates can be dispatched to M27 BMD labs

Round 3 - Yeast

Species	Minimum number of isolates required
Candida pelliculosa (Wickerhamomyces anomalus ()	5
Candida inconspicua (Pichia cactophila)	20
Trichosporon asahii	15
Magnusiomyces capitatus (Saprochaete capitata / Geotrichum capitatum)	20-30

CLSI

#	Description

Need for more MICs and isolates (Round 4- *Scedosporium*)

	AMB	5FC	AND	CAS	MCF	FLU	ISA	ITR	POS	VRC	TERB	OFM	LUC
L. prolificans	141 (5)	5 (2)	61 (5)	63 (5)	81 (5)	32 (3)	142 (6)	124 (6)	136 (6)	172 (7)	17 (1)	58 (3)	15 (1)
S. apiospermum	282 (8)	23 (3)	58(6)	95 (6)	103 (6)	143 (5)	205 (7)	158 (6)	241 (7)	296 (8)	24 (2)	168 (4)	77 (1)
S. boydii	144 (6)	6 (2)	33 (5)	47 (4)	67 (5)	76 (4)	111 (5)	96 (5)	127 (6)	163 (7)	9 (2)	112 (4)	54 (1)
S. angustum	2 (2)	1 (1)	5 (2)	6 (3)	5 (2)	5 (2)	4 (1)	5 (2)	6 (2)	6 (3)	1 (1)	1 (1)	0
S. aurantiacum	70 (8)	1 (1)	15 (4)	25 (7)	35 (5)	21 (4)	26 (4)	42 (7)	50 (8)	73 (9)	5 (2)	46 (5)	18 (1)
S. dehoogii	19 (5)	0	7 (4)	6 (3)	11 (5)	5 (3)	10 (4)	15 (4)	17 (5)	22 (6)	3 (1)	7 (4)	0
S. ellipsoideum	58 (4)	13 (2)	16 (3)	13 (2)	26 (4)	47 (4)	44 (4)	52 (5)	48 (5)	61 (7)	0	43 (3)	30 (1)
S. minutisporum	4 (3)	1 (1)	3 (2)	3 (2)	3 (4)	1 (1)	1 (1)	3 (2)	4 (4)	3 (2)	0	0	0

Number of participating labs in parentheses
> 100 isolates - 3 labs
>= 50 -99 isolates (or < 3 labs)

L. prolificans MICs for: OFM
S. aurantiacum and *S. ellipsoideum* MICs for: AMB, ISA, POS, VRC, OFM

A.Seyedmousavi at NIH currently testing 175 isolates (9 antifungals)
Chen et al. paper in Mycoses (45 isolates) https://doi.org/10.1111/myc.13507

CLSI

- Green are antifungal/species combos for which we have enough data, yellow is where we are close.
- *L. prolificans* and *Scedosporium* spp. with Olorofim are higher priority.
- Highlights of M57S changes voted on in February 2022, to be included in next version:

Lomentospora /*Scedosporium*: 9 ECVs (voted Feb 2022)

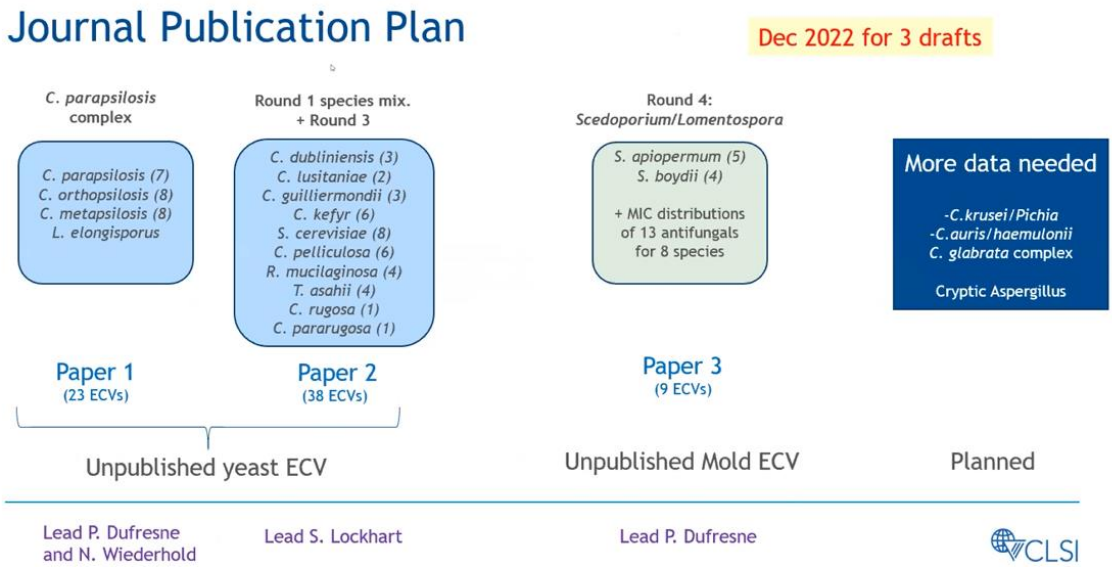
	Species	Antifungal	Comment	ECV
1	*Scedosporium apiospermum*	Amphotericin	Add comment for high modal MIC and not recommended as monotherapy	16
2		Posaconazole		4
3		Voriconazole		4
4		Micafungin		0.5
5		Olorofim		1
6	*Scedosporium boydii*	Isavuconazole	Add comment for high MIC	16
7		Posaconazole	Shifted on right (4?)	8
8		Voriconazole		2
9		Olorofim		0.5

+ 24 antifungal species combinations: Truncated high (TR-H)

- Lots of TR-H combinations with *Scedosporium* in particular.
- Dr. Zhang comment about speciation between *Scedosporium apiospermum* and *boydii*. Many labs can't distinguish with ITS sequencing or MALDI. What molecular methods is ECV WG recommending?

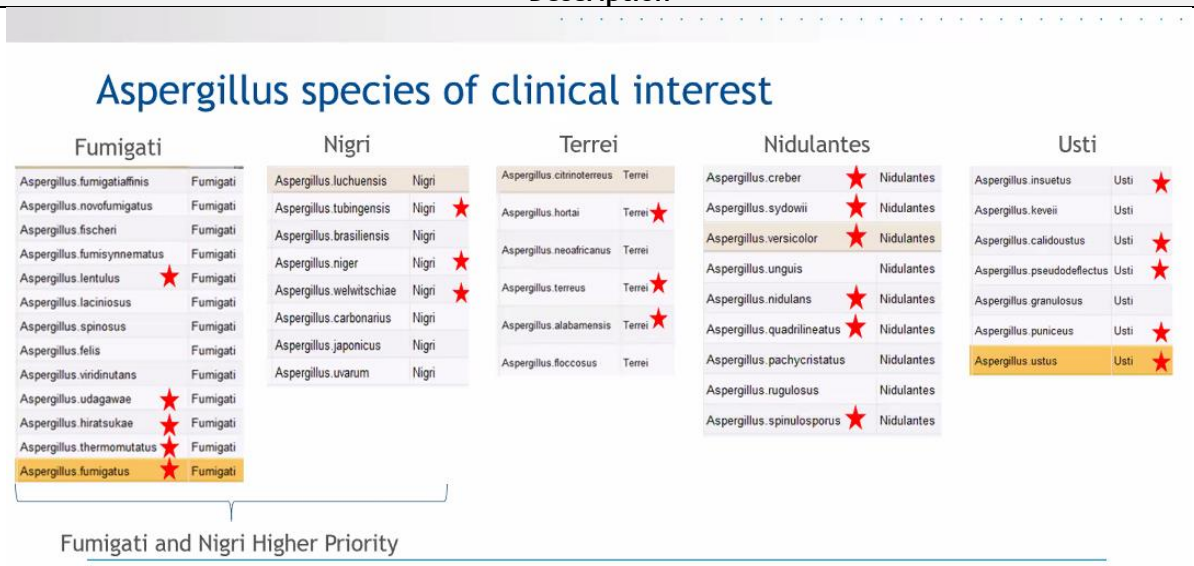
#	Description

- The isolates used in the data are sequenced. Dr. Dufresne says the common species (top 2) work well on VITEK MS MALDI but the rare species do not. Full ITS sequencing works for most species except *S.boydii* and *S. ellipsoideum* where other targets such as ß-tubulin.
- Dr. Wiederhold agrees with Dr. Zhang. Suggests ITS, calmodulin and ß-tubulin to separate all of them. Need multiple loci sequencing.
- Dr. Lockhart: Consider doing a conglomerate ECV: Here is the complex, and here is the ECV if you are unable to distinguish the complex. It may end up that the complex ECV is higher but that would be erring on the side of caution. We need to conform with the groups we are serving. It is not practical to expect all labs can distinguish the complex. Similar to what we do with *C. parapsilosis*. Dr. Schuetz and Dr. Dufresne agree.
- Journal Publication Plan, December 2022 for 3 drafts. More data needed for *C. krusei*/*Pichia*, *C. auris*/*haemulonii*, *C. glabrata* complex, cryptic *Aspergillus*.

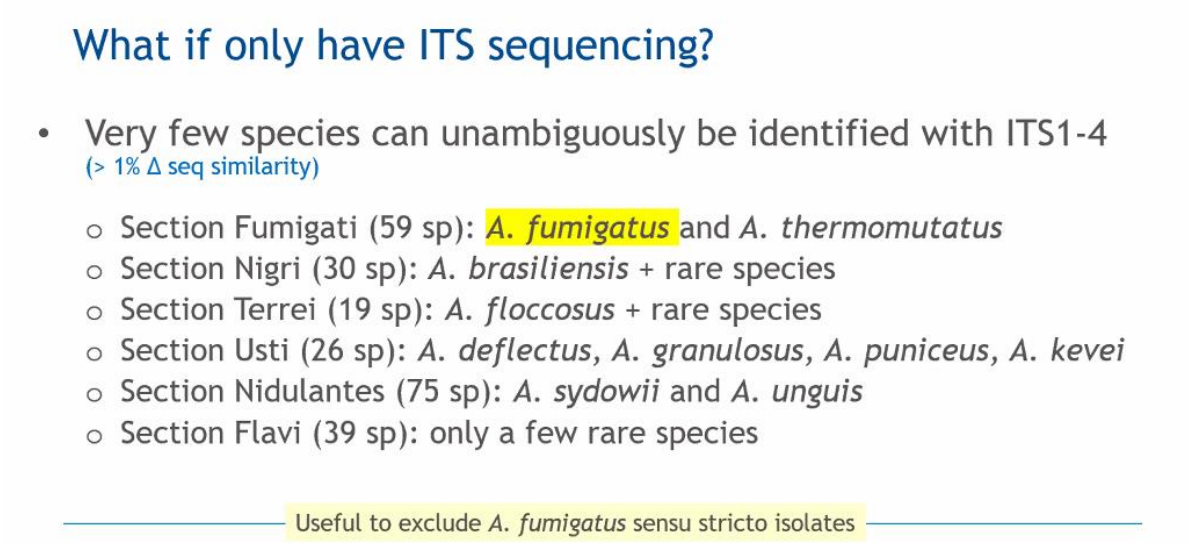


- Update on ECV Round 5-Cryptic *Aspergillus* species.
- Announced Feb 2022. Why? Many cryptic spp. in literature with elevated MIC or claimed to be IR to azoles and amphotericin. Can this be confirmed with multi-lab MIC distributions (n≥100)? Is ECV different from sensu stricto species (is it worth identifying to species level)? Some of our current *Aspergillus* ECVs probably contain a high % of cryptic species (for example *A.niger*).
- Looking at all species. Need ID by BenA and CaM sequencing, CLSI M38 BMD method, isolates also accepted. ECV WG members can test, no M38 BMD MIC data.
- 8 total data contributors (usual ones) contacted: PHE, JMI, UTHSA, CDC, NIH, J.Meis, S. Zhang, LSPQ.

#	Description



- Over 400 species of *Aspergillus* so need to narrow it to above to start. Very few species can be unambiguously identified with ITS alone. *A.fumigatus* can be clearly ID by ITS alone but one of the exceptions.



#	Description

Species that can reliably be identified (delta sequence similarity > 1%)

Section	BenA	CaM	ITS
Fumigati	49/59 (82%)	56/59 (95%)	5/59 (8%)
Nigri	25/30 (83%)	30/30 (100%)	15/30 (50%)
Terrei	19/19 (100%)	18/19 (95%)	7/19 (37%)
Usti	23/26 (89%)	24/26 (92%)	15/26 (58%)
Nidulantes	63/75 (84%)	64/75 (85%)	18/75 (24%)
Flavi	24/35 (68%)	27/39 (69%)	5/39 (13%)

446 recognized *Aspergillus* species !

CaM (and BenA) better than ITS alone (<58% ID)

- CaM alone is a better target.
- What do we have in terms of data for cryptic *Aspergillus* species?

ECV Round 5 – Cryptic Aspergillus species

Isolates only (no MIC yet)

		LSPQ isolates	PHE (A. Borman)	UTHSA (N. Wiederhold)	CDC (S. Lockhart)	JHH (S. Zhang)	JMI	Jacques Meis	LSPQ isolates to test if needed	PHO isolates to test if needed	TOTAL
	Section Fumigati										
1	*A. fumigatus (sensu stricto)*	300	52		222	110	233	819		16	1752
2	*A. lentulus*	6	2	35			10	10	6	4	73
3	*A. hiratsukae*	10	9	21			1		38	6	85
4	*A. undagawae*	3	5	9			1		11		29
5	*A. viridinutans*	1							0		1
6	*A. thermomutatus (syn. A. pseudofischeri)*	10	3	9			2		20	8	52
	Section Nigri										
1	*A. niger (sensu stricto - syn. A. foetidus)*	30	5				1		7	3	46
2	*A. tubingensis*	18		215			1		5	7	246
3	*A. brasiliensis*	1							0		1
4	*A. luchuensis (syn. A. acidus)*	1							0	1	2
5	*A. welwitschiae (syn. A. awamori)*	3		148					0	9	160
6	*A. brunneoviolaceus*	0								1	1
	Section Terrei										
1	*A. terreus (sensu stricto)*	18	5				16	53	14	13	119
2	*A. hortai*	1					1		1	1	4
3	*A. floccosus*	0							1		1
4	*A. neoindicus*	1							0		1
5	*A. alabamensis*	1					4		1	1	7
6	*A. citrinoterreus*	0							0		0

- Will focus on *A. fumigatus* and *A. nigri* sections, as these are majority of clinical isolates. Many of the cases where we have large numbers of isolates are from only one lab.
- Still collecting M38 BMD data, asking labs to contact WG.
- ITS data can be used to filter *A.fumigatus* sensu stricto, need CaM or ß-tubulin sequencing data also for the definitive species identification that will be required after.

#	Description

- **Pragmatic approach for MIC interpretation for species with no breakpoints**
  - **SC Discussion** (**Note:** Comments and questions may be paraphrased).
    - Article included in agenda material. J.Fungi 2022, 8, 141. https://doi.org/10.3390/jof8020141
    - How to interpret MIC when no BP exits?
    - Use ECV to detect NMT isolates (with mechanism of resistance). Some drawbacks: Only 11 ECVs in M57S so not exhaustive. Must validate if not CLSI ref BMD. Some ECV beyond achievable drug levels.
    - Check MIC distribution if no defined ECVs. Some drawbacks: MIC distributions can be difficult to find for rare species. CLSI MIC distribution spreadsheet, CDC, Atlas, EUCAST are not in CLSI documents.
    - Based on in vitro MIC and achievable drug levels in patient and based on susceptibility profile of closely related species. Need more guidance in CLSI documents.
    - Publication to share with group:


Journal of Fungi

J. Fungi 2022, 8, 141. https://doi.org/10.3390/jof8020141

MDPI

Review

A Pragmatic Approach to Susceptibility Classification of Yeasts without EUCAST Clinical Breakpoints

Karen Marie Thyssen Astvad [1], Sevtap Arikan-Akdagli [2,†] and Maiken Cavling Arendrup [1,3,4,*,†]

- Compare MIC to MIC distribution for that species (high vs. low)
- Pragmatic categorization of WT upper limit MIC
  - comparison of mode and range of common species

Critical elements: species ID is correct (MALDI or sequencing) and retest if results appear odd.

    - Approach outlined in above publication starts with ranking different *Candida* species according to modal MIC and range. Table 5 in publication gives example for anidulafungin.
    - Nice rationale paragraph for each antifungal. List ECOFF MIC ranges of susceptible species, PK/PD info and achievable dosage and pathogenicity in rationale paragraph.
    - This approach leads to the development of Pragmatic Breakpoints: Treat if WT. Middle range (consider use if WT), if no better options. Third group: Consider alternative therapy.
    - This publication also issued guidance for interpretation with commercial tests. Labs should perform in house validation to confirm commercial methods. 1) Test QC strains 10X and check that modal MIC on par (± 1 dilution for mode, but not systematically higher or lower and 1 dilution outside range accepted for 1 out of 10). 2) If that works, QC strain passes, then perform test with 10 clinical isolates of most common *Candida* species. Mode should be ± 1 dilution. Remember that some CLSI guidance is found in M52.

#	Description
	○ Dr. Shawar: M52 is under revision. Validation not in the scope of M52, they use the term verification. ○ Ms. Cullen: +/- 1 dil to evaluate commercial method seems more stringent than a reference method comparison might achieve. So, we should be more practical about that. I do support the assessment of bias. This can be done using ISO 20776-2:2021 definition. As Dr. Shawar is indicating, CLSI is using the term verification when the commercial method has regulatory clearance. If not, the lab would need to "validate" since performance characteristics haven't been established or if they are making modifications to a cleared method. Goes beyond ECV cutoff, more comprehensive ○ Genetically related species (will have similar MIC profile) ○ Comparison/ ranking of MIC distribution ○ Known achievable dosage / Guideline recommendations ○ Guidance for using with commercial method ○ Dr. Lockhart: We don't have a lot of BPs. We have BPs for a few combinations, patients with other bugs are still being treated with drugs anyways. We need to serve our customers to give them some guidance about the MIC distributions and ECVs. We have *some* data even if not enough for a BP so we should provide some guidance. We provided a similar guidance document for Caspofungin that is not a CLSI publication but is a manuscript published by the CLSI antifungal committee members. ○ Dr. Palavecino: even though it may not be enough info for us to put into a CLSI document, a publication to give clinical teams guidance would be very important. Is yeast IR going to be put into M60 in the future? Also include info on reading MICs for yeast. ○ Dr. Schuetz points out that M60 is now another number but yes the IR table is now in there. ○ Dr. Procop likes the proposal from Dr. Lockhart and Dr. Dufresne as it includes information about the probability an organism may or may not respond. When probability is high enough we set a BP as we have lots of data. If insufficient data this enables us to still give guidance. Engages clinical laboratory in patient care and treatment. Need to have conversations with clinicians. A good guidance document could come out of this. ○ Dr. Dufresne: Maybe CLSI is not the best vehicle for this. Should we integrate some of these ideas into our documents? Goes beyond ECV cutoff, more comprehensive. Could publish as a comment article to serve as guidance for clinicians. Proposed starting with genetic relatedness of rare yeasts to other more common yeasts, then set pragmatic breakpoints with susceptibility data. Can refer to CLSI Antifungal MIC Master Distribution List. Mostly for species with ECVs, would need to add data for rare yeast species. Draft is available on CLSI website.

#	Description

CLSI antifungal MIC dist. master list

Draft on CLSI website (https://clsi.org/meetings/antifungal/)

Species	BP	ECV	Antifungal	0,004	0,008	0,016	0,03	0,06	0,125	0,25	0,5	1	2	4	8	16	32	64	128	256	>256	No. lab	No. isolates	% Non-WT	Date ECV approved	REFERENCE
Candida orthopsilosis	-	2	AMB				1	3	6	29	76	43	1									4	159	0,0%	January 2020	M59 3rd ED
Candida orthopsilosis	-	TR-L	5FC																			3	58	-	January 2020	M59 3rd ED
Candida orthopsilosis	-	2	AND					1	6	30	48	44	16									5	145	3,4%	January 2019	M59 3rd ED
Candida orthopsilosis	-	1	CAS				5	32	54	50	24	2										4	167	0,0%	January 2020	M59 3rd ED
Candida orthopsilosis	-	1	MCF					1	6	46	69	23										3	145	0,0%	January 2019	M59 3rd ED
Candida orthopsilosis	-	2	FLU						2	22	47	39	16	9	3	3	3	0	1			3	145	13,1%	January 2019	M59 3rd ED
Candida orthopsilosis	-	-	ISA	MORE LAB AND ISOLATES NEEDED																		1	14	-	-	
Candida orthopsilosis	-	0,5	ITR		1	0	5	33	50	34	10	2	0	1								6	136	2,2%	January 2020	M59 3rd ED
Candida orthopsilosis	-	0,25	POS			1	18	57	49	18	2											3	145	1,4%	January 2019	M59 3rd ED
Candida orthopsilosis	-	0,125	VRC		25	42	35	24	8	5	5	1										3	145	3,4%	January 2019	M59 3rd ED
Candida parapsilosis sensu stricto	-	1	AMB				8	36	409	967	2150	477	3	2								7	4052	0,1%	January 2020	M59 3rd ED
Candida parapsilosis sensu stricto	-	TR-L	5FC	TRUNCATED MIC <0,125																		6	2361	-	January 2020	M59 3rd ED
Candida parapsilosis sensu stricto	8	4	AND		4	6	11	8	24	169	681	1031	805	124	1							7	2864	0,0%	January 2020	M59 3rd ED
Candida parapsilosis sensu stricto	8	1	CAS	1	0	7	11	35	361	1108	667	269	70	1								7	2530	2,8%	January 2020	M59 3rd ED
Candida parapsilosis sensu stricto	8	2	MCF	1	6	9	4	4	17	104	578	1465	395	2								7	2585	0,1%	January 2020	M59 3rd ED
Candida parapsilosis sensu stricto	8	2	FLU					7	154	904	2722	1304	428	127	130	113	57	18	11	1		9	5976	7,6%	January 2020	M59 3rd ED
Candida parapsilosis sensu stricto	-	TR-L	ISA	TRUNCATED MIC <0,016																		3	-	-	January 2020	M59 3rd ED
Candida parapsilosis sensu stricto	-	0,5	ITR	1	20	65	249	424	482	245	57	6										7	1549	0,4%	January 2020	M59 3rd ED
Candida parapsilosis sensu stricto	-	0,25	POS	1	2	124	414	805	544	138	13	4										7	2045	0,8%	January 2020	M59 3rd ED
Candida parapsilosis sensu stricto	1	TR-L	VRC	TRUNCATED MIC <0,03																		9	5190	-	January 2020	M59 3rd ED
Candida pelliculosa	-	-	AMB					6	66	59	50	16										6	197	0,0%	TBD Feb 2021	TBD M59 4th
Candida pelliculosa	-	-	5FC	TRUNCATED MIC <0,125																		4	42	-	TBD Feb 2021	TBD M59 4th
Candida pelliculosa	-	-	AND	MORE ISOLATES NEEDED																		6	97	-		
Candida pelliculosa	-	-	CAS	INTERLAB VARIATION 2 modes																		6	179	-		
Candida pelliculosa	-	-	MCF	14	11	38	54	18	4	2	0	0	2	1								5	144	3,5%	TBD Feb 2021	TBD M59 4th
Candida pelliculosa	-	-	FLU							3	6	28	89	55	20	5	0	1				7	207	2,9%	TBD Feb 2021	TBD M59 4th

Proposal for Yeasts (To do list)

First select what antifungals (AMB, FLC, VRC, MCF, AND) and which clinically relevant rare yeast to include.

Element	How (in which document)?	Volunteer
Genetic relatedness and expected susceptibility profile	Appendix M57S: Phylogenetic tree to highlight reduced susceptibility of some group (ex. Pichia FLC)	
MIC distribution per antifungal (ranked/ unranked)	Appendix M57S: **We could finally make those MIC distributions accessible**	
Discussion on **max achievable dosage**, PK/PD, pathogenicity, susceptibility according to genetic group and treatment guidelines	M27 section 3.4 «Interpreting the results» + refer to M57S phylogenetic tree for genetic group Add max achievable dosage table?	
Guidance for validation for use with commercial method	Review M52 recommendations, in M57 and M57S Practical. Also useful for ECV use	
Summary table of recommendations when no BP (pragmatic BP)	To be discussed	
How to report pragmatic BP?	To be discussed How would it co-exist with ECV WT/NWT reporting???	

- Dr. Schuetz thinks this is a fantastic idea, to start with genetic relatedness then talk about susceptibility profile. The genetic information is not easily at our fingertips all the time, would be great to have a table to look at when consulting a treating provider. Dr. Dingle agreed.
- Ms. Cullen: There are rules in M23 about what can be used to establish BPs and QC. Rules are very much centered on reference methods as single point of truth. Need to refer to M23 for guidance about how to ensure there is no bias in using commercial methods. There are also examples within the bacterial docs that have "guidance" not "standards". For example M45 doesn't have enough clinical data, etc to follow all of the M23 rules to be called a standard so it is titled as a guidance. For positive blood culture - a method is now described that have been established as "equivalent" to the standard reference method.

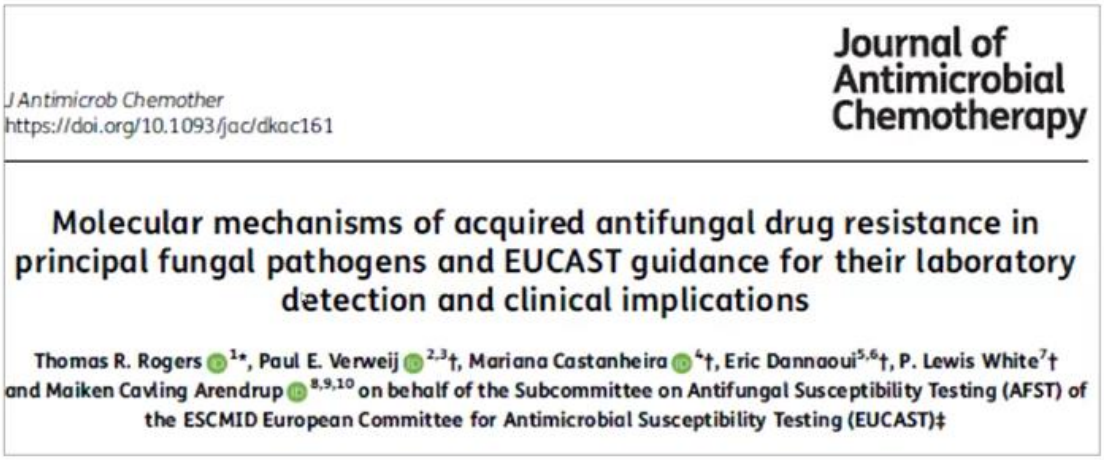
#	Description

- Dr. Dingle is co-chairing M52 revision and suggested including the validation guidance in M52 document. Current scope is verification of FDA approved methods. Will discuss further offline. While this does not fit the current scope, Dr. Dingle is happy to consider work on guidance for validation of commercial methods. May not work in M52 but could be another place for it.
- Ms. Castagna mentions that adding validation back into M52 is under discussion on bacterial side. Proposal has been submitted to CLSI. Need to pump brakes on this until that discussion takes place and the scope of the M52 document is decided. Possibility that the scope will be expanded.
- Ms. Cullen said it is important we recognize there is a void in this area.
- Revision to the Cumitech 31A will include validation for sure. Dr. Schuetz mentions this Cumitech 31A revision document on verification and validation is being updated and written, will be published as a PGCM (practical guidance in clinical microbiology) in Clinical Microbiology Reviews. Potential for synergy between this document and M52. Dr. Shawar cautions that CLSI has certain processes for document revision, M52 focus has been verification not validation. Dr. Dufresne indicated that we will be sure to stay within boundaries of regulations but that guidance is needed.

Action Item: Recruit volunteer and prepare the following 3 annex tables/figures:
1. Yeast genetic relatedness table / phylogenetic tree and susceptibility profile
2. MIC distributions of yeasts
3. Max achievable dosage according to antifungal

Action Item: Dr Dingle and Dr. Schuetz to report if validation section will be included in M52 and Cumitech 31A new drafts.

7. **OTHER BUSINESS**



- EUCAST just published a comprehensive review of molecular mechanisms of antifungal resistance. Example *Candida* and Fks mutations. WGS is becoming more accessible. Need a list of known resistance mutations per species with phenotypic impact on resistance. Recommended sequencing protocols, access to/distribution of reference strains with

SUMMARY MINUTES Friday, 26 August 2022	
#	**Description**
	– known resistance mutations. Antifungal mutations and detection protocol. Is this something that the antifungal group should start working on? A new MM CLSI document? – Dr. Procop mentions when to use ITS, when you need to use different genes like CaM for identification. There is a document about sequence-based identification of microorganisms. Is there a place for a fungal document to deal with molecular identification and detection or resistance? This is a great idea. – Ms. Castagna mentions that MM18 is up for review in 2023 so could be included there. If we want it to be a separate project, submit a proposal to molecular diagnostics if you think it belongs there and not in microbiology. • Winter 2023 meeting January 21 2023 in Orlando at Hyatt Regency Cypress in person with virtual option.
8.	**ADJOURNMENT** Dr. Dufresne thanked the participants for their time. The meeting was adjourned at 1:00 PM Eastern (US) time.

ACTION ITEMS			
#	**Description**	**Responsible**	**Status**
1.	Reviewers needed for M44 review. Contact Dr. Dufresne if interested.	SC Members, Advisors, Reviewers	In Progress
2	Check the bacterial criteria for “tentative” vs “provisional.”	Dr. Dufresne Dr. Castanheira	In Progress
3	Add *Paecilomyces varitotii* and Rasamsonia to the IR WG pending assessment list.	Dr. Schuetz	In Progress
4	Recruit volunteer and prepare the following 3 annex tables/figures: • Yeast genetic relatedness table / phylogenetic tree and susceptibility profile • MIC distributions of yeasts • Max achievable dosage according to antifungal	Dr. Dufresne	In Progress
5	Report if validation section will be included in M52 and Cumitech 31A new drafts.	Dr. Dingle Dr. Schuetz	In Progress

Respectfully submitted,
Christine M. Lam, MT(ASCP)
Camille Hamula, PhD, D(ABMM)

SC Reviewers and Guest Attendees

Amrita Bharat	Michael Huband
Amelia Bhatnagar	Melissa Johnson
Laura Bio	Abdullah Kilic
Michele Burtness	Scott Killian
Darcie Carpenter	Xian-Zhi Li
Cecilia Carvalhaes	Jeffrey Locke
Nydia Castillo-Martinez	Sandra McCurdy
Sukantha Chandrasekaran	Anisha Misra
Ryan Demkowicz	Elizabeth Palavecino
Gina Ewald-Saldana	Mark Redell
Guillermo Garcia-Effron	Josh Shirley
Austin Golia	Jennifer Slaughter
Beth Goldstein	Seyed Mojtaba Seyed Mousavi Tasieh
Armando Gonzalez	Vera Tesic
Carlos Gutierrez	Paula Snippes Vagnone
Rita Hoffard	Tam Van
Denise Holliday	Nancy Wengenack
Heather Holloway	Yanan Zhao