Meeting Title:	**Subcommittee (SC) on Antifungal Susceptibility Tests**	**Contact:**	clam@clsi.org
		Secretary	Camille Hamula, PhD, D(ABMM)
Hybrid Meeting Dates/Times:	**Saturday, 21 January 2023,** in Orlando, FL, from 7:30 – 11:30 AM US EST and 12:30 – 4:30 PM US EST		
Meeting Purpose:	The purpose of this meeting is to discuss Antifungal SC business.		
Requested Attendee(s):	SC Chairholder, Vice-chairholder, Members, Advisors, and Reviewers; 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:	
David Andes, MD	University of Wisconsin - Madison Medical School
Elizabeth Berkow, PhD	Centers for Disease Control and Prevention
Tanis Dingle, PhD, D(ABMM), FCCM	Alberta Precision Laboratories - Public Health Laboratory
Hari P. Dwivedi, BVSc(DVM), MVSc, PhD	bioMérieux, Inc.
Stephanie Mitchell, PhD, D(ABMM)	Cepheid
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, MD, MHS	Duke University Medical Center
Marwan Azar, MD	Yale University
Andrew M. Borman, BSc, PhD	UK Health Security Agency
Mariana Castanheira, PhD	JMI Laboratories
Anuradha Chowdhary, MD, PhD	Vallabhbhai Patel Chest Institute
Sharon K. Cullen, BS, RAC	Beckman Coulter, Inc. Microbiology Business
Ryan Demkowicz, MD	West Virginia University
Jeff Fuller, PhD, FCCM, D(ABMM)	London Health Sciences Centre
Mahmoud Ghannoum, PhD, FIDSA, MBA	Case Western Reserve University
Kerian K. Grande Roche, PhD	FDA Center for Drug Evaluation and Research
Natasha Griffin, PhD	FDA Center for Devices and Radiological Health
Camille Hamula, PhD, D(ABMM) 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
Sixto M. Leal, Jr., MD, PhD	University of Alabama at Birmingham
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
Vera Tesic, MD, MS, D(ABMM)	University of Chicago Hospital
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 Emily Gomez, MS, MLS(ASCP)MB Barbara Jones, PhD	CLSI CLSI CLSI
Christine Lam, MT(ASCP)	CLSI

AGENDA (Part 1) Saturday, 21 January 2023: 7:30 AM – 11:30 AM All times are Eastern (US) time Room Location: Regency 1 -4					
#	**Time**	**Length**	**Presenter**	**Description**	**Background**
1.	7:30 AM	5 min.	C. Lam	**Zoom meeting instructions**	N/A
2.	7:35 AM	5 min.	P. Dufresne	**Opening Remarks**	N/A
3.	7:50 AM	10 min.	B. Jones	**CLSI Update**	N/A
4.	8:10 AM	40 min.	P. Dufresne	**Subcommittee Status Presentation** • Agenda review (VOTE) • Summary minutes from 2022 August meeting (VOTE) • SC Roster rotations / new participants • Process review • Document status update • Announcement of next Vice-Chairholder (2024)	4a_Meeting Agenda Letter 4b_Agenda 4c_August 2022 Meeting Summary Minutes 4d_Subcommittee Roster 4e_Working Group Roster 4f_DOI Summary 4g_Voting Rules 4h_Subcommittee Status Presentation
5.	8:50 AM	15 min.	P. Dufresne	**M27 and M38 Review** • Process and highlight of some of the proposed changes	5a_M27 M38 Review
6.	9:05 AM	10 min.	N. Wiederhold D. Andes P. Dufresne A. Borman	**Breakpoint Working Group Update** • Rationale document for voriconazole (to be published) • Ongoing work on posaconazole and isavuconazole breakpoints for *A. fumigatus*	6a_*A.fumigatus* voriconazole rationale document draft 6b_Breakpoint working group update presentation
7.	9:15 AM	45 min.	N. Wiederhold	**Isavuconazole Breakpoint Proposal (VOTE)**	7a_Isavuconazole MIC breakpoint vs *A. fumigatus* presentation
8.	10:00 AM	20 min.		**Break**	N/A
9.	10:20 AM	5 min.	N. Wiederhold P. Dufresne	**Rationale Document for Isavuconazole (draft)**	9a_*Aspergillus fumigatus* isavuconazole rationale document draft
10.	10:25 AM	20 min.	P. Dufresne N. Wiederhold	**Posaconazole Breakpoint /ECV Data – Interlab Variation Issues**	10a_Posaconazole BP ECV Interlab Issues
11.	10:45 AM	30 min.	M. Ghannoum	**Olorofim Data on *A. fumigatus* and *A. flavus***	16a_Olorofim Data on *A. fumigatus* and *A. flavus* presentation

AGENDA (Part 1) Saturday, 21 January 2023: 7:30 AM – 11:30 AM All times are Eastern (US) time Room Location: Regency 1 -4					
#	**Time**	**Length**	**Presenter**	**Description**	**Background**
12.	11:15 AM	15 min.	P. Dufresne S. Lockhart N. Wiederhold	**ECV Working Group Update (Part 1)** • Ongoing projects • **ECVs to be published and corrections** • **M57S – ECV guidance annex tables** o Yeast taxonomy/expected susceptibility profile o Yeast MIC distribution table o Expected reduced susceptibility cutoff	11a_ECV WG Update Presentation 11b_M57S-ECV Annex Tables 11c_High MIC MEC Threshold
13.	11:30 AM	60 min.		**Lunch Break**	N/A

AGENDA (Part 2) Saturday, 21 January 2023: 12:30 PM – 4:30 PM All times are Eastern (US) time Room Location: Regency 1 -4					
#	**Time**	**Length**	**Presenter**	**Description**	**Background**
14.	12:30 PM	30 min.	P. Dufresne S. Lockhart N. Wiederhold	**ECV Working Group Update (Part 2)** • See Part 1 Listing	See Part 1 Listing
15.	1:00 PM	90 min.	A. Schuetz	**Intrinsic Resistance Working Group Updates**	13a_Reporting WG Intrinsic Resistance Updates 13b_*Scedosporium* and *Lomentospora* vs Flucytosine Summary 13c_*Mucorales* vs Echinocandins Summary 13d_*S. boydii* vs Amphotericin B Summary 13e_*C. rugosa* vs Anidulafungin Summary 13f_*C. inconspicua* vs Fluconazole 13g_*L. prolificans* and *Scedosporium* spp. vs Isavuconazole Summary 13h_*C. haemulonii* vs Itraconazole Summary
16.	2:30 PM	20 min.		**Break**	

AGENDA (Part 2) Saturday, 21 January 2023: 12:30 PM – 4:30 PM All times are Eastern (US) time Room Location: Regency 1 -4					
#	Time	Length	Presenter	Description	Background
17.	2:50 PM	30 min.	J. Oliver	**DHODH Inhibitor Fungicide/Herbicide and Potential for Resistance Development to Olorofim**	14a_ DHODH Inhibitor Presentation
18.	3:20 PM	15 min.	P. Dufresne	**Other Business**	TBD
19.	3:35 PM	5 min.	P. Dufresne	**Plans for Next Virtual Meeting**	N/A
20.	3:40 PM	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.	8-0-0-1	7
To approve the 2022 August Meeting Summary Minutes.	8-0-0-1	7
To approve the proposed isavuconazole breakpoints for *A. fumigatus* sensu stricto.	9-0-0-0	11
Based on the variability data presented for Olorofim and *Aspergillus fumigatus* at 48h at 100% inhibition, the results presented are consistent and reproducible in agreement with what we have already approved for the QC ranges.	8-0-0-1	16
To create a WG for antifungal reading and interpretation with audiovisual support from CLSI leadership about mold susceptibility reading.	8-0-0-1	16
To correct ECVs (originals were from February 2022) for *Scedosporium*/*Lomentospora*/rare yeast. The corrected ones will go into next version of M57S.	9-0-0-0	18
To approve for Intrinsic Resistance: *Scedosporium boydii* vs amphotericin B, *Lomentospora prolificans* vs isavuconazole. Voted against Intrinsic Resistance: *Candida rugosa* vs anidulafungin, *Scedosporium apiospermum* and *S. boydii* vs isavuconazole, *Candida haemulonii* vs itraconazole, Mucorales vs echinocandins.	9-0-0-0	31

[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
Saturday, 21 January 2023

#	Description
1.	**Zoom Meeting Instructions (Ms. Lam)** Ms. Lam provided the instructions for voting, commenting, and asking questions.
2.	**Opening Remarks (Dr. 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).
3.	**CLSI Update (Dr. Jones)** Dr. Jones shared a career story about the impact CLSI has on the medical community. She thanked the CLSI volunteers for the work completed for the mission of CLSI.
4.	**Subcommittee Status Presentation (Dr. Dufresne)** • Agenda Review – Dr. Dufresne reviewed the agenda and requested any changes. – No changes were requested and the agenda was approved.
	A motion to accept the agenda for the meeting was made and seconded. VOTE: 8 for; 0 against; 0 abstain; 1 absent (Pass).
	• **Meeting Summary Review and Vote: August 2022 Meeting Summary Minutes** – There were no corrections to the August 2022 meeting summary minutes.
	A motion to accept the 2022 August meeting summary minutes was made and seconded. VOTE: 8 for; 0 against; 0 abstain; 1 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.

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.

- Subcommittee Roster Rotations/New Participants
  - Ribhi Shawar replaced by Natasha Griffin (Advisor)
  - David Andes rotating from Advisor to Voting Member
  - Sharon Cullen rotating from Member to Advisor
  - Ryan Demkowicz rotating from Reviewer to Advisor
  - Camille Hamula continuing as Committee Secretary
  - Sixto Leal rotating from Member to Advisor
  - Stephanie Mitchell rotating from Reviewer to Voting Member
  - Vera Tesic rotating from Reviewer to Advisor
  - Zoe Freeman Weiss joined as a Reviewer

- This is Dr. Procop's last year as Vice-Chairholder; he will rotate to an Advisor role, and Dr. Wiederhold will assume the Vice-Chairholder role.

#	Description

- Document Status Update
- 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

- Procedural documents: M27, M38, M44, M57, M51 (archived)
- Supplements: M27M44S, M38M51S, M57S

Antifungal Document Status (01/05/2023)

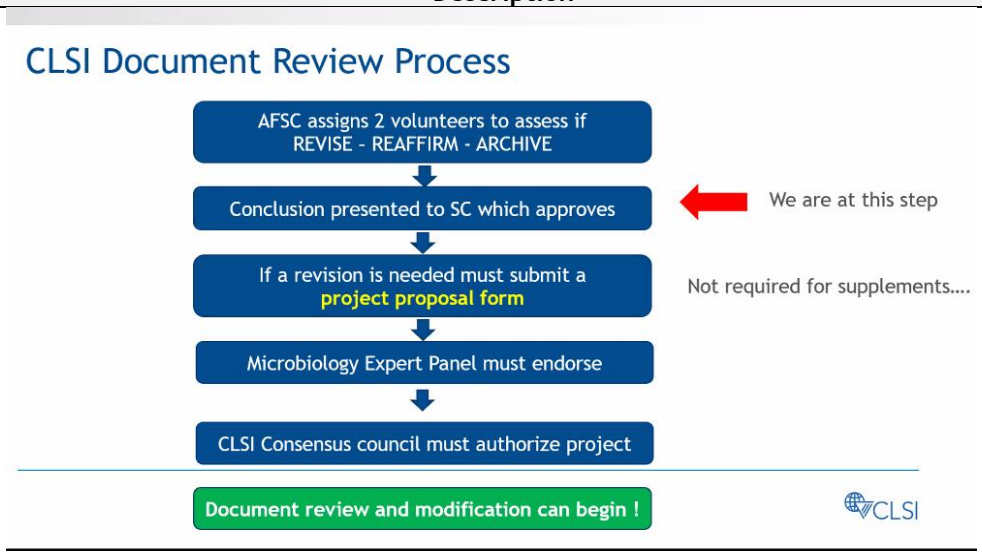
Document #	Document Type	Edition	Publication Date	Final Due Date for Next Review	Reaffirm/ Revise/ Archive	Comments
M27, *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*	Standard	4th	11/2017	2022	To be Revised	• Reviewers recommend revision • Project proposal draft in progress
M38, *Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*	Standard	3rd	11/2017	2022	To be revised	• Reviewers recommend revision • Project proposal draft in progress
M44, *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts*	Guideline	4th	12/2018	2023	N/A	• Two volunteers needed for review

- Two volunteers needed for M44 review
  - Dr. Hanson volunteered to be senior "chaperone" along with Dr. Griffin. Will submit review findings for summer meeting.
- Timeline for documents is 14 months once project is approved, project proposal form must be submitted (CLSI document review process) for new or revised documents. Does not apply to supplements.

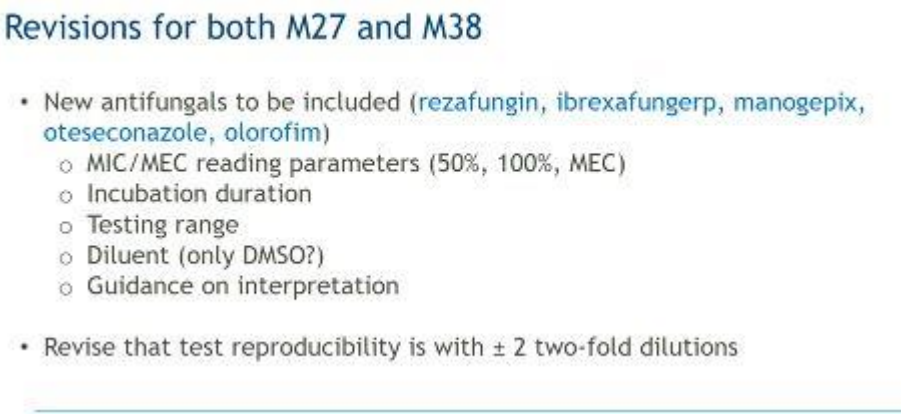
5. **M27 and M38 Review (Dr. Dufresne)**

- Review of CLSI Document Review Process

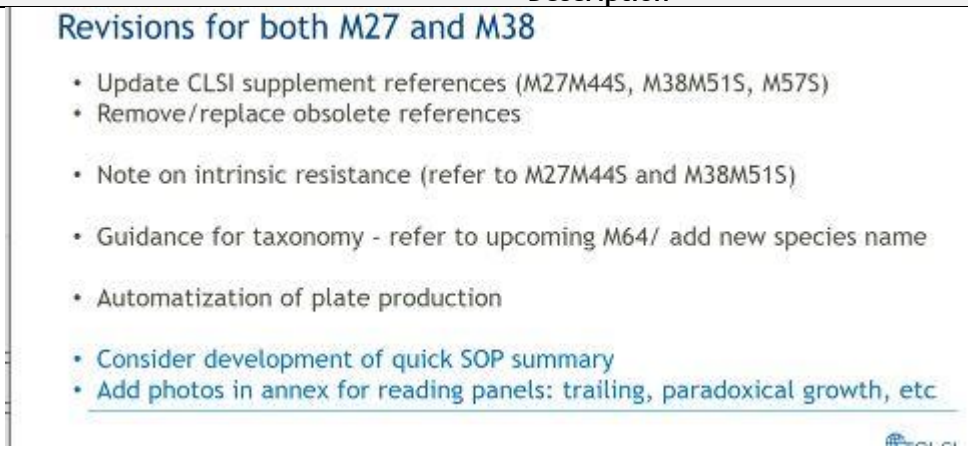
#	Description



- M27 reviewers: Dr. Castanheira and Dr. Garcia-Effron
- M38 reviewers: Dr. Fuller and Dr. Zhang
- We just completed project proposal forms, to submit and get expert panel endorsement for M27/M38. Timeline is 14 months once approved.



#	Description



- **M27 specific recommendations for revision**
  - Chapter 2.1: Add a few more mechanisms of resistance as not all mechanisms listed. Add a note to indicate correct species identification is critical.
  - Chapter 2.2: Include reference to IDSA recommendations for echinocandin testing (only mention the recommendations in reference to azoles in current version).
  - Chapter 3: Add other yeast that we test like *S. cerevisiae*, and basidiomycetous yeasts like *Rhodoturula* and *Trichosporon*. Add a warning for paradoxical growth (Eagle effect) for *C.auris* and related species with caspofungin.
  - Chapter 4: Remove *C.neoformans* (48h) as no QC or reference strains of that species.

- **M38 specific recommendations for revision**
  - Chapter 4: Quality System Essential. In subchapter 4.4.3, Preparing Stains for Storage, add 10% and 20% glycerol.
  - Appendix G if someone has better resolution photos for MEC reading examples, please provide. Would like to replace. Microscopic check of hyphal growth?

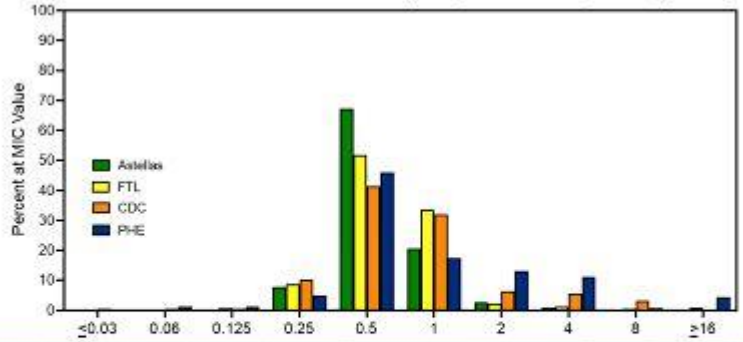
Discussion:

Ms.Cullen: *Cryptococcus* QC does not have to be same species, but QC strains need to be adequate to test materials and method. Do we remove *Cryptococcus* testing just because there is not a safe genus/species for QC? Not sure that we should. Dr. Dufresne clarifies it is just wording that needs changing not that we should remove *Cryptococcus* testing from the document. Dr. Alexander: So if we are reading *Cryptococcus* at 72h does QC need to be read at 72h? Ms. Cullen says practically no, but it is best practice. You can do shorter duration QC if you have shown it is sufficient. There is potential to justify reading QC at 24h and 48h. Dr. Dufresne mentions his lab does 24h and 48h reads. Dr. Castanheira says they read at 72h but the data shows that there is no change for QC at 72h so data supports not having it but theoretically we should have 72h reads. Most *Cryptococcus* isolates grow at 48h. Dr. Wiederhold mentions that there are some *Cryptococcus* isolates that are barely able to be read at 48h and for consistency better to do 72h. Dr. Dufresne suggests that results can be released at 48h but wait the whole 72h if you can't read them at 48h. Ms. Cullen suggests we should have a standard for this. It sounds like we need it. Dr. Dufresne thinks the current QC bugs are doing a fair job for QC and is extra work. You are testing whether or not the plate works. Many labs indicate that they read the plates at 72h for clinical strains and the QC at 48h. No difference in QC result between 48 and 72h.

#	Description
6.	**Breakpoint Working Group Update** **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 • 2 major projects: Rezafungin and Azole breakpoint for *Aspergillus fumigatus*. • Rezafungin Ad hoc BP WG tentative breakpoints: tentative or proposed? • Voriconazole/*Aspergillus fumigatus* BP published in M61Ed2. Rationale document presented this summer. CLSI will publish this in Jan/Feb and will be submitted to FDA. Draft included in meeting materials. • Isavuconazole: Dr. Kovanda (Astellas) provided data. Proposed BP presented today. Rationale document drafted. • Posaconazole: Dr. Motyl (Merck) presented in November. BP and ECV data interlab variation issues. Even the ECV may be difficult. Data to be presented today. • New antifungals in clinical usage: New agents for resistant or difficult to treat IFIs Antifungals / Class (target) / PO-IV / *Candida spp. C. auris* / *Cryptococcus* / *Aspergillus* / *Fusarium* / *Scedoporium Lometospora* / Mucorales / Dimorphics / Pneumocystis Rezafungin / B-glucan synthase (echinocandin) / IV (weekly) / X / - / X / ? / ? / ? / ? / X Ibrexafungerp / Triterpenoid (B-glucan synthase) / PO and IV / X / ? / X / - / +/- / - / X / X Manogepix / GPI inhibitor (GWT1) / PO and IV / X / X / X / X / X / +/- / X / X Olorofim / Orotomide (Pyrimidine synth.) / PO / - / - / X / +/- / X / - / X / - Ref: N. Wiederhold J Fungi 2022; M. Hoenigl et al. 2021 Drugs Potent activity No activity 3 new classes of antifungals with oral administration
7.	**Isavuconazole Breakpoint Proposal (Dr. Wiederhold) (Vote)** • **Isavuconazole data** – MIC distributions, PK/PD parameters, gathered from literature and provided by Astellas – Pharmacokinetic data from package insert based on FDA-approved dose – Half-life is very long (130-135h) leading to very extensive AUC – AUC varies depending on population a bit, but $AUC_{0\text{-}24h}$ of 97 is often quoted – WG gathered MIC distributions from multiple labs plus Astellas for *Aspergillus fumigatus* – PH England data a bit higher MICs but data pretty consistent overall. Higher MICs at PHE likely resultant of more environmental resistance seen in the UK.

#	Description

Isavuconazole MIC Distributions vs. *Aspergillus fumigatus* (CLSI Method)

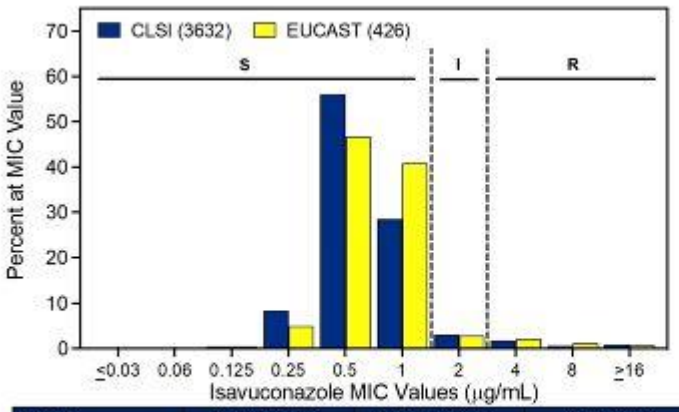


MIC	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥16
Astellas	0.00%	0.00%	0.17%	7.85%	67.40%	20.61%	2.62%	0.78%	0.34%	0.25%
FTL	0.05%	0.19%	0.68%	8.72%	51.91%	33.61%	2.23%	1.16%	0.53%	0.92%
CDC	0.45%	0.45%	0.45%	10.38%	41.44%	31.98%	6.31%	5.41%	3.15%	0.00%
PHE	0.00%	1.24%	1.24%	4.97%	45.96%	17.39%	13.04%	11.18%	0.62%	4.35%

[redacted]

- Comparing CLSI and EUCAST methods, CLSI methods are about 1 dilution lower
- EUCAST has published BPS. S at 1 µg/mL and R at >2 µg/mL.
- MICs in range of 2 are “area of technical uncertainty” and recommend those instances go with what you are reporting for voriconazole.
- WG decided to recommend similar based on their data:

Isavuconazole vs. *A. fumigatus*



Method	% Susceptible	% Intermediate	% Resistant
CLSI	93.75%	3.08%	3.17%
EUCAST	93.19%	2.82%	3.99%

CLSI ISA BP (proposed)	
Susceptible	≤1 µg/ml
Intermediate	2 µg/ml
Resistant	≥4 µg/ml
EUCAST ISA BP	
Susceptible	≤1 µg/ml
Resistant	>2 µg/ml
ATU	2 µg/ml

CLSI ECV for ISA vs. *A. fumigatus* = 1 µg/ml

#	Description

- Published PK/PD data: 10 different *A. fumigatus* isolates. Median AUC/MIC stasis at total Isavuconazole concentration of 503. Statis was not achieved for isolates MIC 2 or higher. For cidal activity, not achieved at 1 µg/mL or higher
- Lepak et al. 2013 and Seyedmousavi et al. 2015. 2015 publication used both CLSI method and EUCAST method. They looked at probability to survival. Listed CLSI and EUCAST MICs alongside each other. With MIC being denominator, there are some differences between CLSI and EUCAST AST methods.
- Kovanda et al. 2016: rabbit model. Endpoint is AUC/MIC associated with reductions in serum galactomannan. 50% reduction in serum GM in rabbits with AUC/MIC just under 80, 80% reduction when value goes up to 130.
- Box et al. 2016: model simulating human alveolus. Endpoint is AUC/MIC associated with GM < 1. Probability at 50% is seen with AUC/MIC of 7, 90% probability if AUC/MIC at 11.
- A lot of variability seen with AUC/MIC target reported as efficacious in these different studies. Isavuconazole in different animal models varies quite a bit. Challenging drug to study.
- Review of what EUCAST used to make their recommendations: They looked at 2015 Amir Seyedmousavi paper and what probability you have of target attainment of AUC/MIC of 33 (about 59 in CLSI method)
- Did Monte Carlo simulations. AUC/MIC of 33 obtained more than 95% of the time when AUC/MIC was 1 µg/mL. Dropped when raised to 2 µg/mL.

Proposed Isavuconazole Breakpoints vs. *Aspergillus fumigatus*

MIC Breakpoint and Interpretive Criteria (µg/mL)		
Susceptible (S)	Intermediate (I)	Resistant (R)
≤1	2	≥4

Discussion:

Dr. Schuetz: Why did EUCAST decide to refer back to voriconazole? Voriconazole and Isavuconazole MICs seem to parallel each other for *Aspergillus*, usually same or within 1 dilution. Cyp51 mutations affect both drugs similarly. Why did they not just stick to the ATU for isavuconazole why decide to parallel? Should we do the same? Dr. Castanheira and Dr. Alexander mention to be cautious as this may encourage use of voriconazole as a surrogate for isavuconazole, or may result in clinicians choosing the one with the lowest MIC and going with it. Also these should always be tested together and resulted together but is this practical? Dr. Procop: If we pull *S. aureus* out of a normally sterile site and didn't do susceptibility testing, it would be malpractice. If we pull out an *Aspergillus fumigatus*, we don't do susceptibility testing

#	Description
	and make them call and request and delineate the ones they want. Should we push through CLSI in partnership with IDSA for more routine mold susceptibility testing? Dr. Castanheira says IDSA does not recommend testing for *Aspergillus* currently and wonders how much labs will consider testing in the absence of an IDSA guideline change and only a CLSI change. IDSA guidelines are currently being worked on/updated. Dr. Dufresne: motion to go forward with the proposed isavuconazole breakpoints for *A. fumigatus* sensu stricto. Breakpoints will have accompanying comments and subcommittee will craft a statement at summer meeting about how we need to be cautious for the intermediate category and comment to refer to voriconazole. Also need a comment about how to report when isavuconazole/voriconazole don't agree.
	A motion to accept the proposed isavuconazole breakpoints for *A. fumigatus* sensu stricto was made and seconded. VOTE: 9 for; 0 against; 0 abstain; 0 absent (Pass).
	Dr. Schuetz questions as to why we are treating this differently from other breakpoints or are we doing this because of EUCAST? Dr. Wiederhold mentions one reason is that we accept +/- 2 dilutions and not all our data shows agreement between labs and we need to take this into account.
8.	**Morning Break**
9.	**Rationale Document for Isavuconazole (Draft) (Dr. Dufresne, Dr. Wiederhold)** • A brief presentation of the Isavuconazole rationale document draft was made. • The authors of the draft were asked to draft a statement regarding isavuconazole and voriconazole MICs and the use of voriconazole results as a surrogate marker for isavuconazole against *A. fumigatus*. The statement will be presented at the next Antifungal Subcommittee meeting.
10.	**Posaconazole Breakpoint/ECV Data – Interlab Variation Issues (Dr. Dufresne, Dr. Wiederhold)** • Merck team data • In vitro susceptibility surveillance data and recently completed double blind clinical trial • SENTRY surveillance data from JMI 2 data sets 2011-2017, 2018 – Mode, MIC50 0.25 µg/mL – MIC90 at 0.5 µg/mL – ECV 0.5 µg/mL – No difference seen according to region • MERCK PN069 double blind clinical trial: Posaconazole effective primary treatment for invasive Aspergillosis • 288 patients/26 countries/91 sites, phase 3 randomized controlled non-inferiority trial. • Divided group into quartiles according to exposure, some have no exposure data. Q1-4 mortality vs. clinical response no significance difference. • Posaconazole vs voriconazole. Posaconazole effective and not inferior to Voriconazole. • No relationship was found for the exposure-response • MIC distribution. Out of 288 isolates only 76 grew. 75/76 were WT. All isolates with MIC below or equal to 1 µg/mL (almost no resistant isolate)

#	Description

- No resistant isolates so could not correlate R with treatment failure.
  - Merck proposed BPs: S at 0.5 µg/mL, I at 1 µg/mL, R at 2 µg/mL
  - The BP distribution relies heavily on MIC distribution since no trend with mortality or clinical success.
- Additional data BPWG:
  - Lepak et al 2013, Howard SJ 2011. PSC efficacy for IA in neutropenic mouse model. Highest MIC with favorable outcome is 0.5 µg/mL. PSC less effective for Cyp51 mutants (they never cross the threshold).
  - EUCAST current BPs published in 2012 (!). Revised in 2020. S at 0.125, 0.25 is ATU, R is 0.5. Twofold dilution difference between EUCAST and Proposed BP.
  - EUCAST distribution is however very similar to CLSI method distribution. EUCAST ECOFF is 0.25 µg/mL. Mode for EUCAST is 0.12 or 0.06, CLSI is 0.06. Lots of Cyp51 mutants with low MICs in the published study.
  - Data from JMI published, ECV is 0.5 and also a few other studies. If you remove Cyp51 mutants, value is 0.25.
  - CLSI received recent MIC data from 7 labs for *A.fumigatus*. 9 Datasets.
  - Seem to have 2 groups of labs – with low and high mode.

CLSI MIC DISTRIBUTION CHECK

7 labs - 9 datasets

MIC	LAB 4	LAB 5 (2017-2020)	LAB5 (2015-2020)	LAB6 (2017-2020)	LAB6 (2008-2017)	LAB16	LAB17	LAB 18	LAB19	ALL	CLSI ECV (2014-15)	
0.016							2		0	2	105	
0.03	**146**	240	241	1	1	45	15		3	450	422	Low mode
0,06	51	**274**	277	5	6	**288**	**46**	3	9	673	**430**	MIC: 0.06-0.12
0,125	5	165	195	**56**	17	141	30	12	166	563	351	
0,25	12	137	**300**	41	52	90	15	38	**860**	**1155**	236	High mode
0,5	4	19	66	21	**63**	70	2	**40**	414	530	103	MIC: 0.25-0.5
1	1	18	45	0	14	21		8	29	69	28	
2	1	9	16	2		9		1	0	21	7	
4	0	3	4	0		1		1	2	6	2	
8	0	1	2	0		1			0	2	0	
≥16	2	5	9	3		9	2	1	0	21	0	

Important interlab variation seen in CLSI MIC distributions

- 5/9 datasets with low mode (0.06-0.12)
- 4/9 datasets with high mode (0.25-0.5)
- Including 2 labs with a shift ↓2 dil. when their PSC panels changed (Lab 5 and 6)

BP WG EXPERIENCE: - isolates with MIC ≥ 1 µg/mL are normally resistant (CYP51 mutants)
- gray zone at MIC of 0.5 µg/mL

  - Something is going on with the method/prep that is making the mode jump around.
  - ECV for individual labs also varied quite a bit.
  - When you see values above 0.5 the agreement is a lot better. Lots of MIC variability for S range but R range may be feasible as less variability.
  - Some labs changed panels and saw a difference but what was it? Should compile the differences. Similar situation as Caspofungin, ultimately put in comment about Micafungin and surrogate markers.

#	Description

Action Plan – Posaconazole CLSI BP Determination

- **Analyse FTL MIC azole MIC data**
  - What proportion of CYP51 mutants with MICs < 1 µg/mL ?
  - Can other azoles (ex. ITR) be used to confirm borderline PSC resistance ?
- **Constitute and send CYP51 mutant panel to a few high and low mode PSC CLSI labs** (~25 isolates - many with MICs 0.5 to 2 range - collect MICs for all triazoles)
  - Evaluate in interlab variation impact R detection
  - Find BP that could work for most CLSI labs
- Long run: animal studies with isolates with MICs in 0.25 to 2 µg/mL range

Ms. Cullen: Suggests comment about what to do with different QC ranges. To ensure that R reporting is on target. Good comments in M45 about "this breakpoint set based on PK/PD distributions and limited clinical data" then if better data shows up later you can modify.

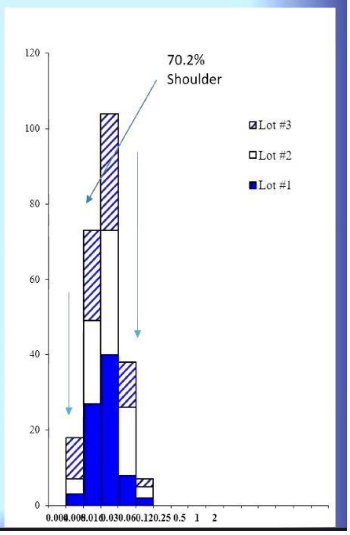
11. **Olorofim Data on *A. fumigatus* and *A. flavus* (Dr. Ghannoum)**

- 6/10 isolates showed >95% interlab agreement at 100% inhibition
- No ranges identified for 50% or 100% inhibition at 24h
- The ranges for all 100% inhibition/48h endpoints were bimodal.
- Olorofim MIC ranges. No proposed range for any of the species (*A. fumigatus, A. flavus, A. nidulans, A. niger, A. terreus*)
- Variation by species, interlab agreement was good for each of the pairs of the same species.

OLOROFIM Against Isolate 1 - *Aspergillus fumigatus* MRL 43309
100% Inhibition at 48 hrs
Proposed Bi-modal range 0.008-0.06 ug/ml , 97.1% included.

MIC (ug/ml)	Lot 1	Lot 2	Lot 3	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	All Labs
0.004												
0.008	3	4	11		8					8	2	18
0.016	27	22	24		21	2	2	13		22	13	73
0.03	40	33	31	21		22	25	13	9		14	104
0.06	8	18	12	6	1	5	3	4	18		1	38
0.12	2	3	2	3		1			3			7
0.25												
0.5												
1												
2												
N	80	80	80	30	30	30	30	30	30	30	30	240
GEOMEAN	0.026	0.029	0.024	0.040	0.014	0.034	0.031	0.025	0.052	0.013	0.021	0.026
MODE	0.030	0.030	0.030	0.030	0.016	0.030	0.030	0.016	0.060	0.016	0.030	0.030
MIN	0.008	0.008	0.008	0.030	0.008	0.016	0.016	0.016	0.030	0.008	0.008	0.008
MAX	0.120	0.120	0.120	0.120	0.060	0.120	0.060	0.060	0.120	0.016	0.060	0.120
RANGE	4.907	4.907	4.907	3.000	3.907	3.907	2.907	2.907	3.000	2.000	3.907	4.907

Calculated Range	0.008-0.12
Dilution Range	5
% Observations Captured	100



- Similar data for *A. flavus, A. nidulans, A. niger* 100% inhibition at 48h.
- Voriconazole done also as a control with same species and timeframes.
- For several species you see a different mode for some labs. Due to difficulty of reading.
- Dr. Castanheira suggests pictures for M38 for this compound may be helpful to assist in reading.

#	Description
	Ms. Cullen: If you see a bimodal distribution (M23 definition) if you see a shoulder > 60% and if you define it as bimodal you treat it like a mode and take +/- 1 from that result. For antifungals you generally have the precision +/-2 so in lieu of 3 you have 4 dilution ranges. Nothing to vote on. Suggest that we go back and focus on 10 strains of *A. fumigatus* only. To lump them all together is not helpful. • **The CLSI M23 standard calls for testing 10 clinical strains for reproducibility, without specifying whether they need to be a variety of species within a certain genus.** • **In this study, we tested 2 strains each of 5 different *Aspergillus* species, which failed to give us the required >95% interlab agreement. The issue with this is that these *Aspergillus* MICs fell into 3 different bi-modal ranges, dependent on the species.** • **Regarding the 2 *Aspergillus* strains previously identified as QC strains for Olorofim, it is possible that inclusion of the missing values not reported by labs 6 and 8 may have provided data that would result in >95% agreement.** **Discussion:** Dr. Procop suggestion that CLSI provide photos for mold susceptibility testing training. Nothing exists but does for cytopathology. CAP should also have a proficiency testing challenge for mold susceptibility. Training needed is extensive and reading is subjective. Dr. Schuetz and Dr. Lockhart will write about this for the website. Photos will be put on website. Recommendations for going forward: General process improvements needed for reading and competency. Ms. Cullen: Related to Olorofim, is the reproducibility study adequate/sufficient for decisions? There are at least 2 open questions were enough strains tested for each species? M23 suggests 20 but can do fewer when there are additional species. Were the results reproducible? Where there any questions we need to tease out? There are some issues with the reading especially for the one strain with a 2-dilution difference. Are there some reading issues? It is reproducible and it is probably a couple of dilutions but the one strain needs some follow up but we probably don't need additional testing. This bug/drug compound seems reproducible ±1 or 2 dilutions. Is there any follow up action needed about the reading differences? 100% inhibition at 48h for all *Aspergillus* species. Can we accept the presented ranges?
	A motion was made and seconded that based on the variability data presented here for Olorofim and *Aspergillus fumigatus* at 48h at 100% inhibition, these results are consistent and reproducible in agreement with what we have already approved for the QC ranges. VOTE: 8 for; 0 against; 0 abstain; 1 absent (Pass).

#	Description
	A motion was made and seconded to create a WG for antifungal reading and interpretation with audiovisual support from CLSI leadership about mold susceptibility reading. VOTE: 8 for; 0 against; 0 abstain; 1 absent (Pass).
	• Antifungal reading and interpretation to work with M27 and M38 revisions. M02 and M07 revisions will also have a reading guide so may want to align. Fungal guidelines should be in own document.
12.	**ECV Working Group Update (part 1) (Dr. Dufresne, Dr. Lockhart, Dr. Wiederhold)** **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 • More isolates needed. 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 /* 20-30 • Accept isolates for testing at reference labs. Also accept data with CLSI method and molecular confirmation.

#	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)

New set of MIC data

L. prolificans	15
S. apiospermum	102
S. boydii	21
S. angustum	10
S. aurantiacum	8
S. dehoogii	9
S. ellipsoideum	10

Data request for:

L. prolificans MICs for: OFM

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

Amir Seyedmousavi (NIH) - 175 isolates
AMB, ITC, VRC, POSA, ISA, TRB, MFG, Olorofim

- *Scedosporium*. Green is enough isolates, yellow is close.
- Manuscript publication plan for unpublished yeast ECVs summer 2023 (3 planned papers)

Manuscript publication plan

Paper 1-2-3 timeline: spring-summer 2023

Round 2 + Round 3 Yeast species mix.
C. dubliniensis (3)
C. lusitaniae (2)
C. guilliermondii (3)
C. kefyr (6)
S. cerevisiae (8)
C. pelliculosa (6)
R. mucilaginosa (4)
T. asahii (4)
C. rugosa (1)
C. pararugosa (1)

Paper 1
(38 ECVs)

C. parapsilosis complex
C. parapsilosis (7)
C. orthopsilosis (8)
C. metapsilosis (8)
L. elongisporus

Paper 2
(23 ECVs)

Unpublished yeast ECV

Lead S. Lockhart

Round 4: *Scedoporium/Lomentospora*
S. apiopermum (5)
S. boydii (4)
+ MIC distributions of 13 antifungals for 8 species

Paper 3
(9 ECVs)

Unpublished Mold ECV

Lead P. Dufresne

Ongoing or more data needed
-C.krusei/Pichia
-C.auris/haemulonii
C. glabrata complex
Cryptic Aspergillus (round 5)

Planned

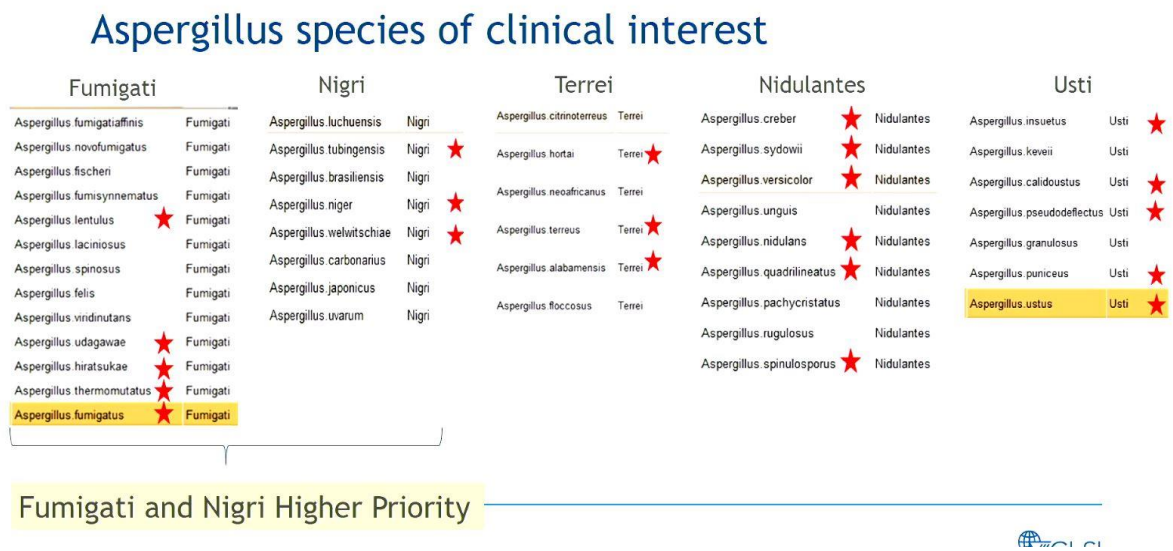
Lead P. Dufresne and N. Wiederhold

CLSI

- CDC has interest in ECV development for *Sporothrix schenckii* and *Sporothrix braziliensis*. Round 6?
- Data published in 2017 not submitted to CLSI (Espinel-Ingroff et al. 2017 AAC). Will contact all labs for data and submit to CLSI officially.

SUMMARY MINUTES Saturday, 21 January 2023	
#	Description

- Update on Round 5 ECVs for cryptic *Aspergillus spp.*



- Fumigati and Nigri higher priority because we have more isolates and are closer.
- Summary of current isolates:

ECV Round 5 – Cryptic Aspergillus species

Isolates only (no MIC yet)

		LSPQ isolates with MIC	PHE (A. Borman)	UTHSA (N. Wiederhold)	CDC (S. Lockhart)	JHH (S. Zhang)	JMI	Jacques Meis	NIH (A. Seyedmousavi)	LSPQ isolate to test if needed	PHO isolates to test if needed	TOTAL
	Section Fumigati											
1	*A. fumigatus (sensu stricto)*	300	52		222	110	233	819	275		16	2027
2	*A. lentulus*	6	2	35			10	10	15	6	4	88
3	*A. hiratsukae*	10	9	21			1		2	38	6	87
4	*A. undagawae*	3	5	9			1		4	11		33
5	*A. viridinutans*	1								0		1
6	*A. thermomutatus*	10	3	9			2		5	20	8	57

	Section Nigri											
1	*A. niger*	30	5				1		18	7	3	64
2	*A. tubingensis*	18		215			1		8	5	7	254
3	*A. brasiliensis*	1								0		1
4	*A. luchuensis (syn. A. acidus)*	1								0	1	2
5	*A. welwitschiae*	3		148						0	9	160
6	*A. brunneoviolaceus*	0									1	1

Majority of isolates from section Nigri are non *A. niger*

	Section Terrei											
1	*A. terreus (sensu stricto)*	18	5				16	53	14	14	13	133
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

#	Description

Ready for the next version (M57S Ed5)!

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 declared as Truncated high (TR-H)

- Corrigendum-step omitted for ECOFFinder (refer to presentation).
- *S. boydii*-Posaconazole (refer to presentation)
- Green fitting curve was shifted, when corrected with ECOFFinder ECV moves from 8 to 4. Makes more sense.
- Isavuconazole, curve fitting was good but stretched at end. ECV now >16 instead of 16.
- *S. apiospermum* and amphotericin B, Olorofim.
- Summary of corrections:

Round 2/3 ECV (Feb 2022- Corrected)

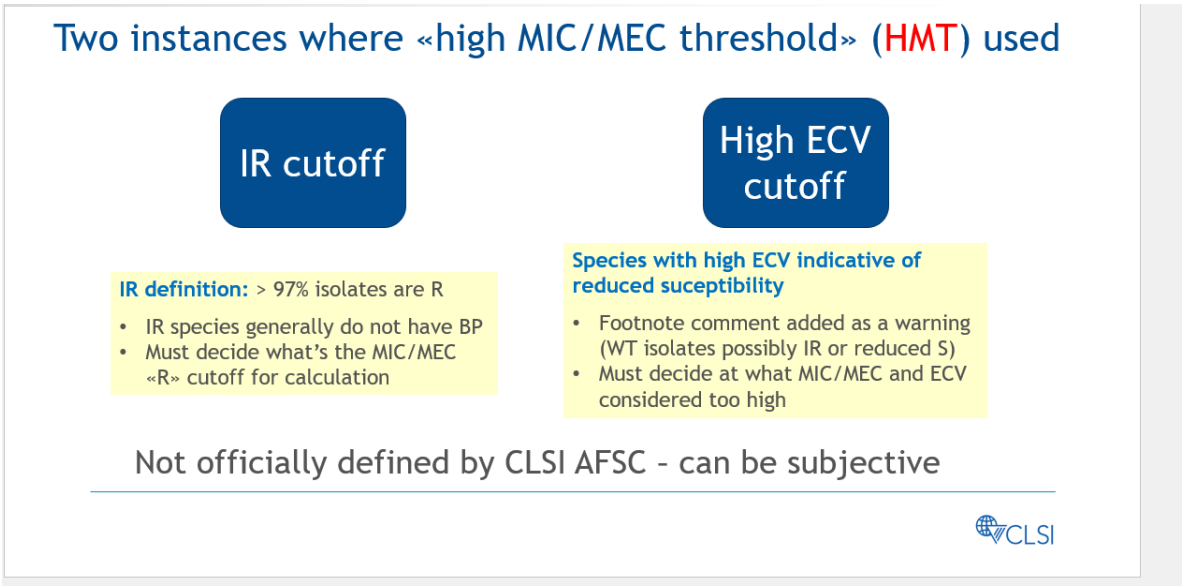
	Species	Antifungal	Comment	Voted ECV	Corrected ECV
1	*Candida rugosa*	Amphotericin		2	-
2		Anidulafungin	Add comment for high MIC	8	-
3		Caspofungin		2	-
4		Micafungin		1	-
5		Itraconazole		1	-
6		Posaconazole	Shoulder at 0.125	0.125	0.25
7		Voriconazole	Shoulder at 0.06	0.06	0.125
8	*Candida haemulonii*	Itraconazole	MIC spread Add comment for high MIC	8	4
9	*Candida inconspicua*	Amphotericin		2	-
10		Fluconazole	Add comment for high MIC	64	-
11		Itraconazole		1	-
12		Voriconazole		0.5	-
13	*Candida pelliculosa*	Anidulafungin	21% NWT at 0.016 Manually adjusted (ECV 99%)	0.03	0.03 (97.5%)
14	*Trichosporon asahii*	Voriconazole		0.25	-

ECOFFinder reanalysis for all 3/14 corrected

#	Description
	• For rare yeast also are corrections.
	A motion was made and seconded to vote on corrected ECVs (originals were from February 2022) for *Scedosporium/Lomentospora*/rare yeast. The corrected ones will go into next version of M57. Audrey asks to look at *L.prolificans* vs. Isavuconazole. Phillipe says this one, the rerun of the analysis gave the same result and did not need to be corrected. VOTE: 9 for; 0 against; 0 abstain; 0 absent (Pass).
	Discussion: Dr. Zhang: speciation between *S. apiospermum* and *S. boydii*, for clinical labs this is difficult to separate based on phenotypic and not possible by MALDI. ECVs are separately but most of the time labs cannot distinguish them. Often even mixed IDs in sequencing. How can clinical labs use these ECVs if they cannot be reliably separated? Dr. Dufresne agrees many labs cannot differentiate (MALDI works but not perfect, ITS sufficient except for *S. boydii* and *S. ellipsoidea* differentiation). What he sees is that the ECVs are pretty similar within the complex. We could eventually propose an ECV for the complex, but we are not there yet. ECVs still species specific. Dr. Zhang proposes grouping them together if they are similar and do it as a slash call. Dr. Procop suggests to leave it for a molecular mycology workgroup to decide which targets are best for which species. Dr. Lockhart says there is not this info in a MM document. Dr. Lockhart thinks it is appropriate to put this info into M57S.
13.	**Lunch Break**
14.	**ECV Working Group Update (part 2) (Dr. Dufresne, Dr. Lockhart, Dr. Wiederhold)** • ECV guidance-Annex Tables Dr. Dufresne – Discussion Summer 2022 about putting more ECV guidance. – Max achievable serum concentration (Cmax) table. – Expected susceptibility profile linked to genetic relatedness. – Preliminary ideas from summer 2022 meeting: max achievable serum concentration (Cmax), link susceptibility profile according to yeast genetic group, provide MIC distributions, guidance for validation with commercial method to implement ECVs (currently on hold). – Not at approval step. • Max achievable Concentration (Cmax) Table – Cmax data is highly variable, depends on dosage and patient population, drug formulation. – If MIC exceeds Cmax likely nonsusceptible. – Intended usage is to define the High MIC/MEC threshold. To flag ECVs where WT isolate may be IR, R or with reduced susceptibility. Flag MIC/MEC in “danger zone” or “proceed with treatment with caution.” – Use this to flag when ECV is so high that even for WT isolates, it makes no sense to call them WT.

#	Description

– If MIC/MEC is high: "Danger Zone" or "will not respond to treatment."



– *S. cerevisiae* and *C. haemulonii* are 2 examples with Fluconazole, where we could see the ECV was so high we could flag it.

- **<u>*S. cerevisiae* and *C. duobushaemulonii* - FLC comment:</u>**

Antifungal Agent	Species	ECV, µg/mL[b,c,d]	
Fluconazole	*C. dubliniensis*	0.5	
	C. duobushaemulonii	32	
	C. guilliermondii[e]	8	
	C. haemulonii	**128**[g]	←
	C. kefyr[e]	1	
	C. lusitaniae[e]	1	
	C. metapsilosis	4	High MIC comment
	C. orthopsilosis	2	
	C. pararugosa[e]	**16**	
	C. pelliculosa[e]	**8**	
	C. rugosa[e]	**8**	
	S. cerevisiae	**32**[h]	←

g. The fluconazole ECV for *C. haemulonii* is very high and may be an indication of intrinsic resistance or of limited susceptibility to this agent. A minimal inhibitory concentration (MIC) value lower than the ECV does not imply that the isolate is susceptible to fluconazole.

h. The fluconazole ECV for *S. cerevisiae* is high and may be an indication of intrinsic resistance or of limited susceptibility to this agent. An MIC value lower than the ECV does not imply that the isolate is susceptible to fluconazole.

– Dr. Dufresne asked Dr. Andes, Dr. Walsh, and Dr. Wiederhold for feedback and these would be the values below if we decide to go with the threshold

#	Description

- Tentative High MEC/MIC threshold values proposed below:

Tentative «High MIC/MEC Threshold» (HMT) values

Antifungal	High MIC/MEC Treshold (µg/mL)
Amphotericin	>1-2 (?)
Anidulafungin	>2
Caspofungin	>2
Rezafungin	>2
Micafungin	>2
Fluconazole	>16
Flucytosine	>16
Isavuconazole	>2
Itraconazole	>0.5
Posaconazole	>0.5
Voriconazole	>1
Terbinafine	>0.5

Talking: Live Video Feed

STD Threshold High MIC/MEC value :

- To caculate % R for IR determination
- To decide if »high ECV» comment needed (when WT might be at high risk of not beeing susceptible)

Also informs users what CLSI AFSC experts consider is a high MIC.

Published in as M57S annex with proper explanation and langage **(HMT not a BP)**

- Gives users of the document what experts on the committee consider to be a high ECV
- What current ECVs exceed those HMT values? Summary table.
- We need to decide whether to put a comment for the examples below where the ECV>>>HMT

What current ECVs exceed those HMT values?

Amphotericin B ECVs

Antifungal Agent	Species (genotype)	ECV, µg/mL[b,c]
Amphotericin B	*C. gattii* (VGI)	0.5
	C. deuterogattii (VGII)	1
	C. neoformans (VNI)	0.5
	Rhodotorula mucilaginosa	**2**
	Trichosporon asahii	**1**

Antifungal Agent	Species	ECV, µg/mL[b,c,d]
Amphotericin B	*C. albicans*	2
	C. dubliniensis	0.5
	C. glabrata[e]	2
	C. guilliermondii[e]	2
	C. kefyr[e]	2
	C. krusei[e]	2
	C. lusitaniae[e,f]	2
	C. metapsilosis	1
	C. orthopsilosis	2
	C. parapsilosis	1
	C. pelliculosa[e]	**1**
	C. tropicalis	2
	Saccharomyces cerevisiae	**2**

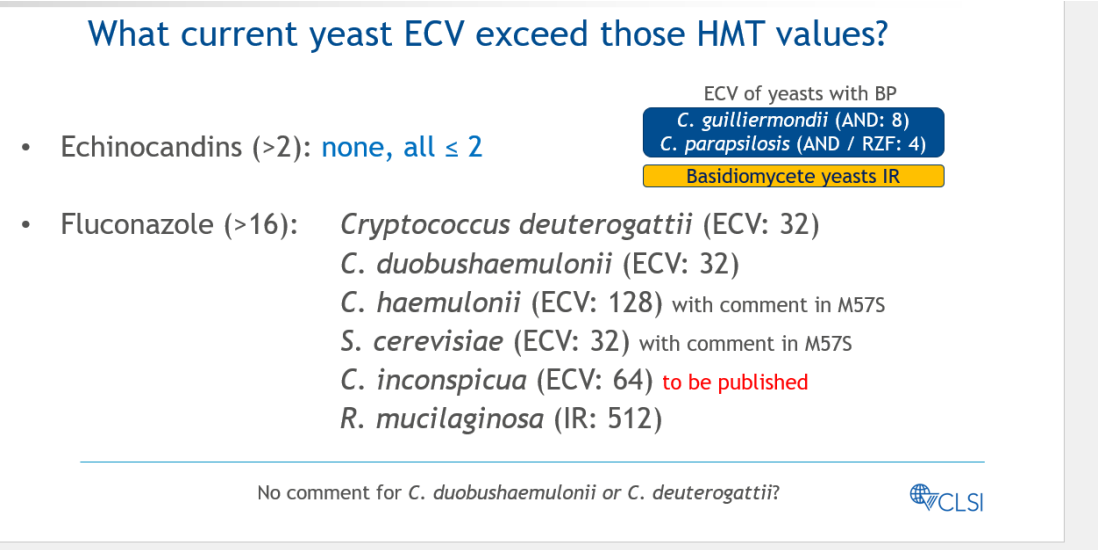
Antifungal Agent	Species	ECV, µg/mL[b,c]
Amphotericin B	*A. flavus*	4
	A. fumigatus	2
	A. niger	2
	A. terreus[d]	4
	A. versicolor	2

13 ECVs > HMT at EVC of 2

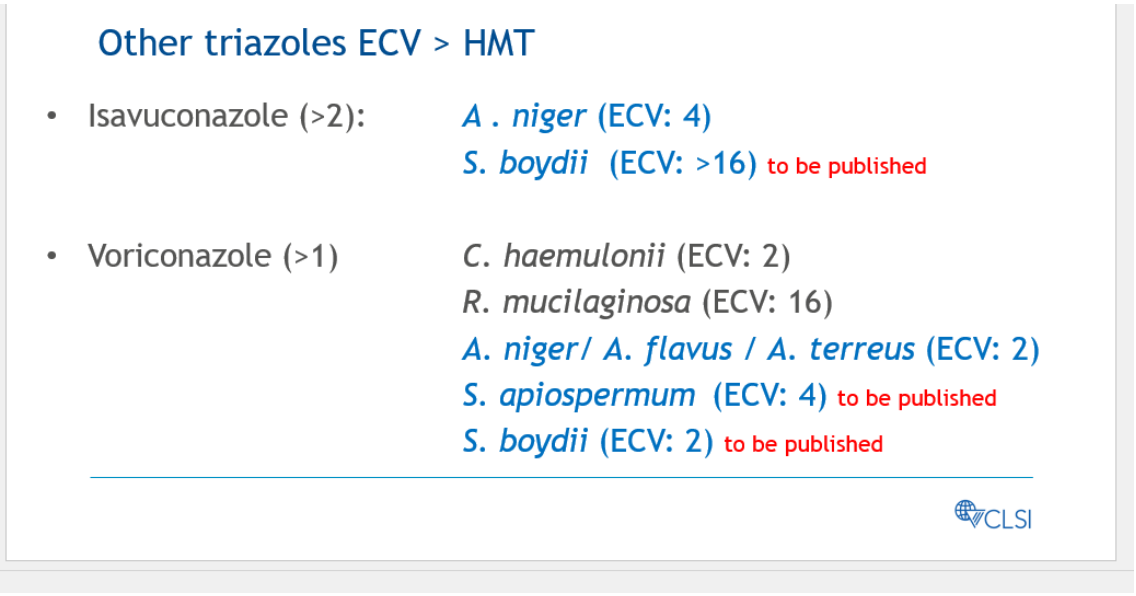
Only *A. flavus* and *A. terreus* with ECV at 4 (>2)

Upcoming ECV
S. apiospermum: >16

#	Description



- At the moment we do not have any comments for *C. deuterogattii* and *C. duobushaemulonii*
- Did this exercise for other combinations also. Voriconazole, Isavuconazole and Posaconazole. Both yeasts and *Aspergillus*.



#	Description

Other triazoles ECV > HMT (Yeasts)

Itraconazole (>0.5):

C. duobushaemulonii (ECV: 1)
C. glabrata (ECV: 4)
C. guilliermondii (ECV: 2)
C. krusei (ECV: 1)
C. lusitaniae (ECV: 1)
C. metapsilosis (ECV: 1)
C. pelliculosa (ECV: 1)
S. cerevisiae (ECV: 2)
C. rugosa (ECV: 1) to be published
C. haemulonii (ECV : 4) to be published
C. insconpicua (ECV : 1) to be published

C. deuterogattii (ECV: 1)
R. mucilaginosa (ECV: 4)
T. asahii (ECV: 1)

A. flavus / *A. fumigatus* / *A. terreus* (ECV: 1 / 1 / 2)

- Submitted to IR WG for feedback
- Can we use this as a trigger to add a "high MIC" comment or IR determination? Many ECV to be published exceed HMT but comment not *de facto*.
- Publish in M57S as an annex?
- Discussion is needed as well as a publication plan.
- Discussion needed with IR WG.

Discussion:
Dr. Castanheira mentions that EUCAST put this out for PK/PD distributions for bacteria. They did something similar in their breakpoint working group meeting presented by Dr. Giske. What dosage can you use to achieve these MICS?
Dr. Andes says this looks like "poor mans' PK/PD" and is only relevant if you don't have PK/PD data. Also, *in vivo* it is almost without exception the non-protein bound drug available for activity and that is not taken into consideration in ECV values. Propose to take that into consideration when talking about Cmax. Dr. Dufresne agrees, but indicates we need to flag those high ECVs to prevent high MIC WT confusion. Would be good to have standardized approach to do so.
PK/PD breakpoints are not the same as Cmax. PK/PD driver not taken into account in Cmax. This is useful when you don't have PK/PD data.
Dr. Hanson: You should provide a couple examples with voriconazole. You want to guide treatment not restrict it.

- Yeast susceptibility according to genetic group/clade
  - *Candida* genus is highly polyphyletic.
  - Large group of species, some are vastly unrelated, yeasts of clinical importance found in at least 14 clades.
  - Big push mainly in Europe for major reclassification/renaming of *Candida* spp. within new genera, since susceptibility profile generally similar between related species.

#	Description

- Whether or not we agree with the reclassification and use of the new names in a clinical setting, knowing closest well known relative and to which clade/genetic group they belong is clinically relevant and useful.
- Susceptibility profiles and treatment likely to be similar within a clade/genetic group.
- This info can be presented as phylogenetic tree or summary table of clinically relevant species.
- Yeast classification is not often up to date, difficult task
- Not a new concept, published in *FEMS Yeast Research* 19 2019 Stavrou et al. and also Kidd et al. OFID 2023 Jan 7.
- How to create a list of clinically relevant species? Includes current names and *Candida* names, family clade/genus, type strain and Mycobank accession number, Genbank DID2 and ITS sequences.

- **Which includes:**
  - Current / anamorph *Candida* names
  - Family and Clade/Genus
  - Type strain and Mycobank accession number (MB)
  - Associated Genbank D1D2 and ITS sequences

- **Data sources**
  - Atlas of Clinical fungi (https://www.clinicalfungi.org/)
  - Manual of clinical microbiology 12th Ed (Chapter 120)
  - The Yeasts (https://theyeasts.org/)
  - MycoBank https://www.mycobank.org/
  - Westerdjik Institute strain db https://wi.knaw.nl/page/Collection
  - NCBI Taxanomy browser

- Yeast classification info is often not up to date. *Candida parapsilosis* example.
- D1/D2 sequences were difficult to obtain.
- Many simply classified Saccharomycetales order on MycoBank.
- 78 species so far, created a database of 78 species.
- Only 8 families have clinically relevant species of Candida spp.

#	Description

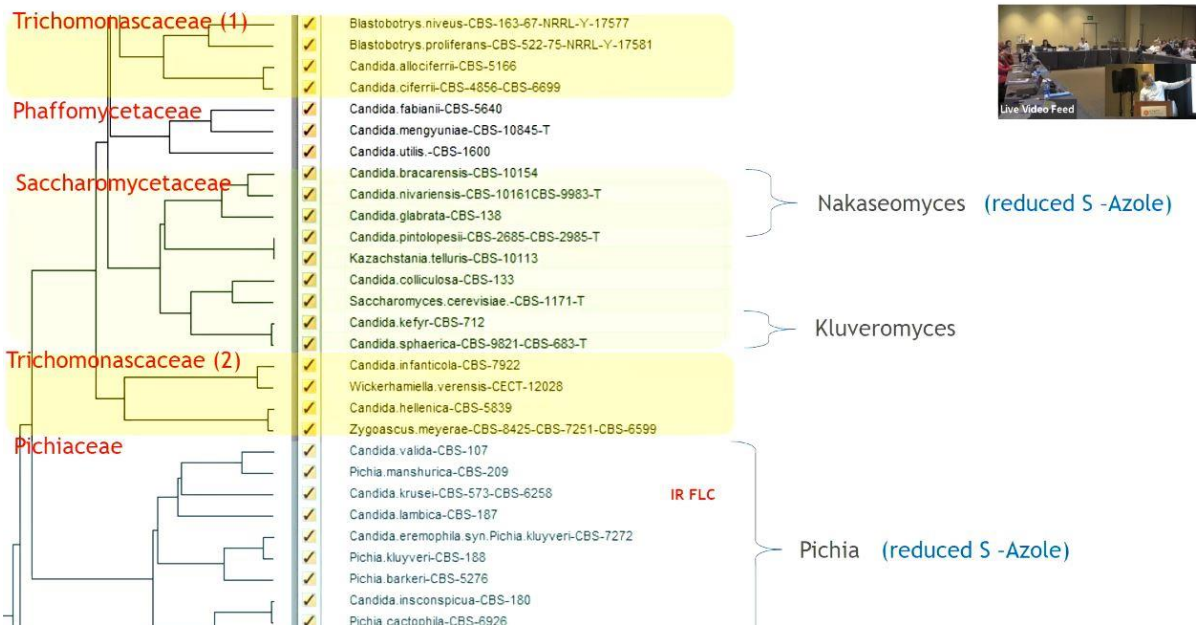
Order Saccharomycetales (78 spp.)

- **Debaryomycetaceae (23)**
- **Metschnikowiaceae (8)** — CUG-Ser1 Clade
- **Pichiaceae (11)**
- **Saccharomycetaceae (9)**
- **Trichomonascaceae (9)**
- Phaffomycetaceae (5)
- Dipodascaceae (4)
- Saccharomycodaceae (2)

- Saccharomycetales incertae sedis (7)
  - Ex. Diutina, Starmerella and *C. blankii*

Candida species of clinical importance in 8 families

(most in 5)



- Dr. Dufresne proposes making phylogenetic trees, then clarify which groups have reduced susceptibility to azoles based on data (see above).
- Alternately, could present them in a table and list the clades, family, current “clinical” name and teleomorph name (see below).

#	Description

DEBARYOMYCETACAE

***Lodderomyces* clade**	**Susc. to all antifungals**		
Clinical name	Taxonomic name	Group/complex	IR
Candida albicans	-	*C. albicans*	
Candida africana (var.)	-	*C. albicans*	
Candida dubliniensis	-	*C. albicans*	
Candida tropicalis	-	*C. tropicalis*	
Candida viswanathii	-	*C. tropicalis*	
Candida sojae	-	*C. tropicalis*	
Candida parapsilosis	-	*C. parapsilosis*	
Candida orthopsilosis	-	*C. parapsilosis*	
Candida metapsilosis	-	*C. parapsilosis*	
Lodderomyces elongisporus	-	*C. parapsilosis*	
Meyerozyma clade	**Reduced Susc. to Azole**		
Candida guilliermondii	*Meyerozyma guilliermondii*	*C. guilliermondii*	
Candida fermentati	*Meyerozyma caribbica*	*C. guilliermondii*	
Candida carpophila	*Meyerozyma carpophila*	*C. guilliermondii*	
Other clade	**Reduced Susc. to Azole**		
Candida palmioleophila	-		
Candida famata	*Debaryomyces hansenii*		
Candida sake			

Decisions to be made:

a) Which species to include? Probably exclude those that are rare.
b) Which output format table or tree?
c) Also decide on a definition of reduced susceptibility designation.

Reviewed by ECV and IR WG then submit to subcommittee for approval.

Discussion: Dr. Lockhart agrees this is a good idea. M64 suggests *Candida* name and providing the teleomorph. When you have a taxonomically valid name whether or not it is phylogenetically valid is not important you need to meet the needs of the clients/clinicians. Clinicians are the clients we need to keep in mind. Suggest use M64 guidance. This is complicated when designing studies and using data from academic sources that use the teleomorph names or where teleomorph was found first and they never got a *Candida* name. It becomes a nightmare so sticking with *Candida* in the naming but using a table like this to see what they are dealing with is ideal. Clinicians don't have time to look at all these research articles. Also, teleomorph names are what taxonomists care about so these will be changing all the time, anamorph names will not be changing as much.

Dr. Dufresne proposes organizing by antifungal to start, with species listed alphabetically then include modal MIC, MIC_{50}, geometric mean (GM), %NWT, %R.

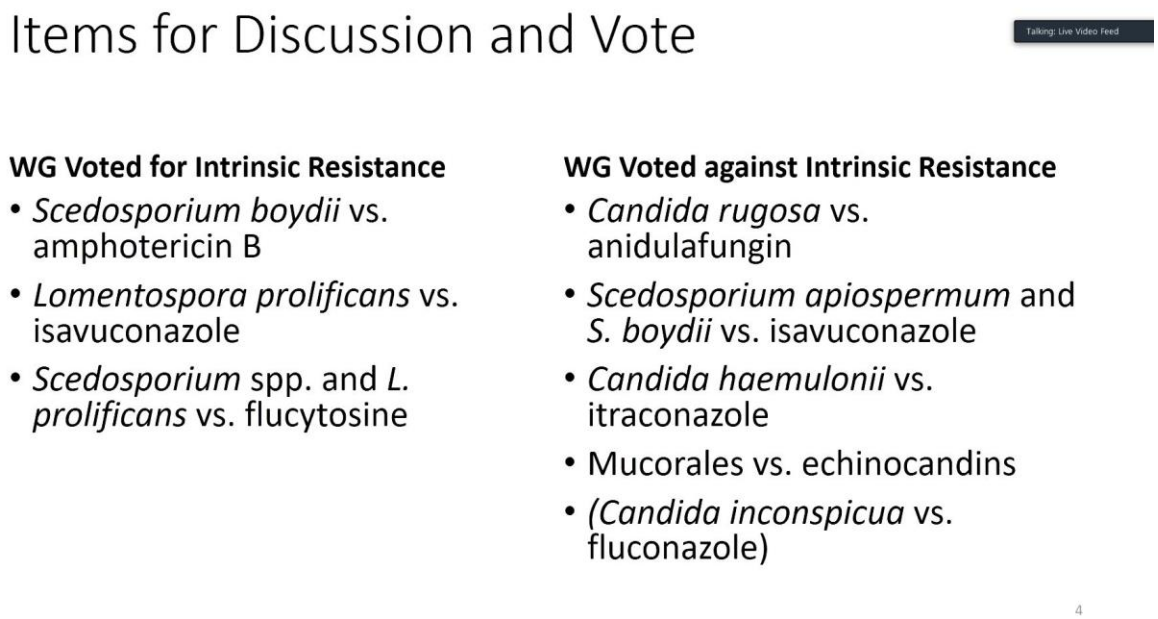
Yeast MIC distribution table proposed example:

#	Description

Table 1. Fluconazole MIC distribution for *Candida* and other yeast species (28)

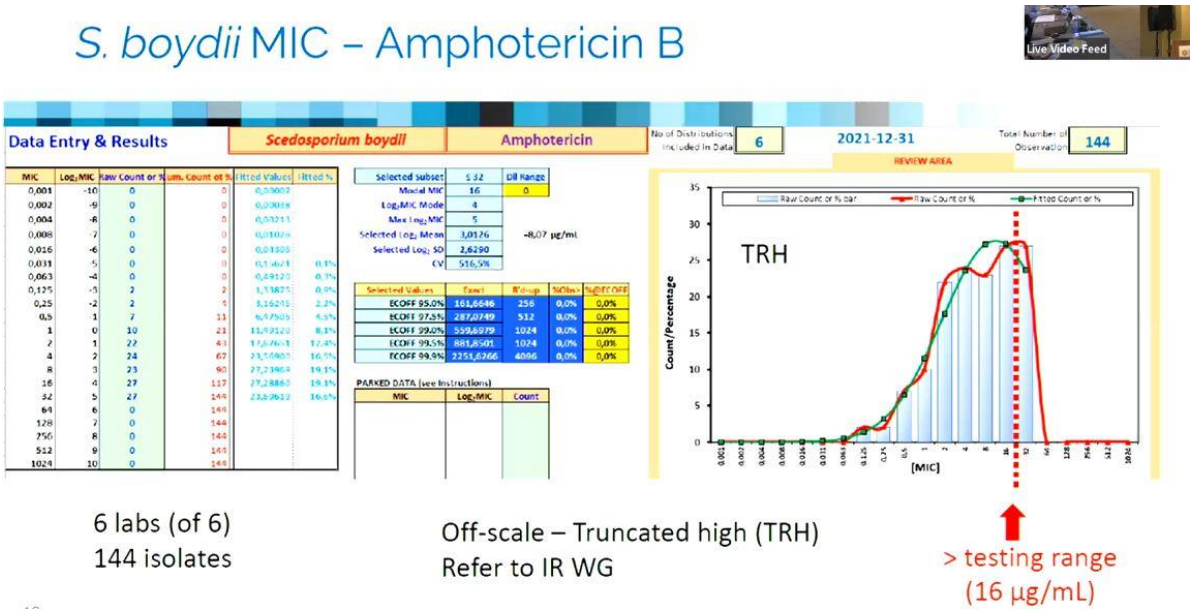
Species	Labs	Isolates	MIC values in µg/mL																
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	MIC_{50}	MIC_{90}	GM	% R/ NWT
C. albicans	9	5274	7	247	**1729**	1647	855	370	137	91	59	48	37	26	12		0.25	1	0.29	3.5%
C. auris	7	540	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. bracarensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. dubliniensis	8	180		18	**71**	54	23	4	2		2	3		2	1		0.12	0.5	0.23	7.8%
C. duobushaemulonii	8	143				2	1	1	3	35	**46**	38	8	2	5	2	8	32	9.34	6.0%
C. glabrata	8	7548		0	29	78	189	474	2065	**2676**	773	343	322	441	144		2	32	4.33	7.8%
C. guilliermondii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. haemulonii	11	111				1	1	3	16	13	**26**	19	12	13	3	4	8	64	6.87	RS
C. inconspicua	7	147	0	0	1	2	0	0	0	3	9	**59**	47	14	11	1	32	64	23.11	8.2%
C. kefyr	4	129	1	1	23	**52**	46	4	0	0	1	1					0.25	0.5	0.30	1.6%
C. krusei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IR
C. lusitaniae	10	574		1	76	181	**197**	66	12	4	8	8	15	6			0.5	1	0.48	8.7%
C. metapsilosis	11	193			1	16	40	**70**	35	19	6	4	2				1	4	1.16	6.2%
C. nivariensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. orthopsilosis	3	145			2	22	**47**	39	16	9	3	3	3	0	1		0.5	4	0.91	13.1%
C. parapsilosis	9	5976		7	154	904	**2722**	1304	428	127	130	113	57	18	11	1	0.5	2	0.70	7.6%
C. pelliculosa	7	207				3	6	28	**89**	55	20	5	0	1			2	8	2.49	2.9%
C. pararugosa	6	100				1	1	3	13	**37**	29	8	2	1	5		4	16	4.81	8.0%
C. rugosa	12	112			3	1	9	**39**	19	32	8	0	0	0	1		2	4	1.79	0.9%
C. tropicalis	13	3732	**2042***	962	445	144	57	34	25	8	13	2					0.03	0.25	0.05	0.4%
L. elongisporus	6	66*			**38**	19	7	0	2								0.12	0.5	0.19	TR-L
G. capitatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. cerevisiae	4	318				5	16	39	65	**86**	62	33	9	3			4	16	3.63	0.9%
C. neoformans	6	1137			4	12	40	127	376	**456**	89	20	10	3			4	8	2.70	2.9%
C. gattii	7	260				1	18	39	69	**101**	29	3					4	8	2.54	0%
C. deuterogattii	5	457					4	8	44	147	**167**	73	9	5			8	16	6.19	1.1%
R. mucilaginosa	7	298				1	1	0	0	1	3	9	17	**237**	OR	OR	>64	>64	54.41	IR
T. asahii	5	143				2	5	26	42	**48**	6	8	0	3	3	0	2	16	2.88	9.8%

Discussion: Dr. Borman agrees is a good idea. Ms. Cullen: We need to be careful in describing the action steps for conclusions that will be drawn from this. Is the objective to somehow say here are the ECVs and when the MIC you get is greater than the ECV, here are one or more tables you can use to evaluate how to interpret? Dr. Dufresne says that it is for cases where there is No ECV at all, and it will help just knowing which bug you have and what family it is in to predict the susceptibility pattern (MIC distribution). Gives broader context to ECV data also when we have an ECV. Dr. Lockhart mentions there will be 2 talks at ASM Microbe in June about fungal ECVs. It would be helpful if people posed questions they would like to be addressed during those talks to the people giving them. We should think about what questions those talks should address. Dr. Procop asked what clinicians present think. Dr. Hanson says that the distribution is useful, and if the species distribution is published and the results are different/unusual, she may ask the lab to repeat it. There is value in knowing that the MIC is within the ECV or way outside the ECV/resistance range. If there is clinical data that supports then it trumps this type of data. Dr. Lockhart states that this information will be included in M57S and their publication. For the rare yeasts there is zero data available, other than general MIC ranges. For some species it will take 2-3 years to get enough isolates to get an ECV but we can publish a range of MIC values for each bug-drug combo in a table for reference. Use it as preliminary data on the way to an ECV. Dr. Schuetz suggests placing this into the VET09 document. It should also be in a document geared towards clinicians. The lab will struggle if it is only in lab documents like M57S. Needs an appropriate home. A lot of labs are sending isolates out and not testing the weird ones in house, so they may not have access to this. How can we make this information available to those not super involved with CLSI or mycology experts?
Dr. Borman says they used a similar format in their rare yeast MIC paper and the taxonomy plan is good, compromise between taxonomists and clinicians. They currently report all rare species with a comment explaining lack of BPs and interpreting loosely with *C. albicans* breakpoints.
Dr. Zhang added comment about ECVs. Recent CAP survey since less than 30% of labs are using ECVs. One of the big reason is that the majority of labs are using commercial products which prevent them from using ECVs since ECVs are for BMD CLSI method. One thing that CAP is trying to do is look at all MIC results from commercial products to compare to BMD and see the variations. Maybe the ECV group can send validation panels to clinical labs and compare what is the performance between commercial and CLSI methods. A YeastOne-CLSI conversion factor would be

#	Description
	nice. Dr. Schuetz suggest CLSI come up with a plan on how to validate the ECVs using commercial methods. Not in Cumitech. Dr. Dingle comments that validation guidance will not be M52, M52 will only have verification but a separate CLSI document will be created to deal with validation and this new document would be a good place for it. A project proposal is being submitted now.
15.	**Reporting WG – Intrinsic Resistance WG (Dr. Schuetz, Dr. 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 • Current roster now includes Philippe who has been joining recent meetings with ECV data. • Role: To develop guidelines for reporting of certain antifungal agents from specific body sites (and conversely, those body sites from which antifungals would not be appropriate to report). Not talking about this today. • Role: Intrinsic Resistance • Review Intrinsic Resistance Definition. "Intrinsic resistance is defined as inherent or innate (not acquired) antimicrobial resistance, which is reflected in wild-type antimicrobial patterns of all or almost all representatives of a species. Intrinsic resistance is so common that susceptibility testing is unnecessary"…"A small percentage (1-3%) may appear susceptible due to method variation, mutation, or low levels of resistance expression."  • Review of 2022 ECV data. Refer to PDF write ups in agenda for detail. • Important points: • WG concluded that *S. boydii* is IR to Amphotericin B, ECV was recalculated for this one to >16 (change from 16) – 130 isolates, high MIC50s and MIC90s

#	Description

  - Discouraged for monotherapy for *S. boydii* (ECMM guidelines)
- *L. prolificans*/*S. apiospermum*/*S. boydii* Ampho B MIC truncated high
- *L. prolificans and S. boydii* truncated higher mode and MIC50 than S. *apiospermum*, MIC90 is similar.



***Lomentospora prolificans*, *Scedosporium apiospermum*, and *Scedosporium boydii* vs. Isavuconazole**

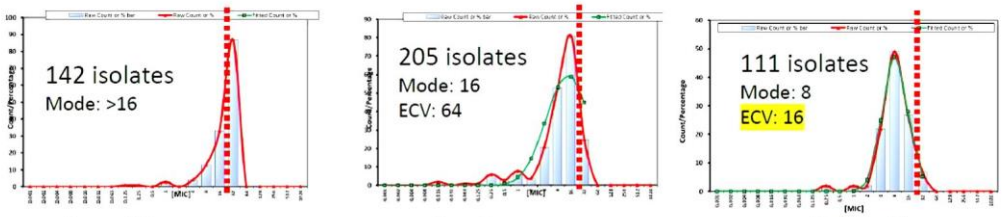
- *L. prolificans* and Isavuconazole. Overall MICs are very high. The results suggest that *L. prolificans* is IR to Isavuconazole.
  - Comment for consideration: despite the recommendation of isavuconazole monotherapy as a second-line option by some organizations (ECMM reference), the published literature supports an intrinsic resistance designation per CLSI criteria.
- *S. apiospermum*/*S.boydii* and isavuconazole in vitro data does not fit criteria for IR. WG concluded they are not IR.
  - Comment for consideration: While MIC50 and MIC90 values to this antifungal agent will be high for the majority of isolates, >3% of isolates demonstrate low MICs. Therefore, intrinsic resistance criteria as defined by CLSI have not been met.

- See PDF write up from agenda.
- These comments should probably go into M38?

#	Description

L. prolificans / S. apiospermum / S. boydii Isavuconazole -ECOFFinder

Species	No. lab	No. isolates	0.016	0.03	0.06	0.125	0.25	0,5	1	2	4	8	16	>16	MIC90	MIC50	MODE
Lomentospora prolificans	8	142	0	0	0	1	1	0	3	0	4	13	33	87	>16	>16	>16
Scedosporium apiospermum	7	205	2	0	1	1	6	3	8	4	21	53	81	25	>16	16	16
Scedosporium boydii	5	111	0	0	0	0	2	0	2	2	22	49	27	7	16	8	8



L. prolificans *S. apiospermum* *S. boydii*

- All off-scale, truncated high (except *S. boydii*?)
- Surprising as it is second line treatment. Refer to IR WG

19

- *Scedosporium* spp. and *L. prolificans* are intrinsically resistant to Flucytosine (5-FC) based on data summary from Dr. Wiederhold. High MIC90s and MIC50s.

All species – 5FC and Fluconazole

5FC

Species	No. lab	No. isolates	1	2	4	8	16	32	64 (>32)	>64	MIC90	MIC50	MODE
Lomentospora prolificans	2	5	0	0	0	0	0	0	2	3	>64	>64	>64
Scedosporium angustum	1	1	0	0	0	0	0	0	1	0	64	64	64
Scedosporium apiospermum	3	23	0	0	0	0	0	0	9	14	>64	>64	>64
Scedosporium aurantiacum	1	1	0	0	0	0	0	0	1	0	>32	>32	>32
Scedosporium boydii	2	6	0	0	0	0	0	0	6	0	64	64	64
Scedosporium ellipsoideum	2	13	0	0	0	0	0	0	9	4	128	64	64

Fluconazole

Species	No. lab	No. isolates	0,125	0,25	0,5	1	2	4	8	16	32	64	≥128	MIC90	MIC50	MODE
Lomentospora prolificans	3	IR 32	0	0	0	0	0	0	0	0	0	0	32	≥128	≥128	≥128
Scedosporium angustum	2	5	0	0	0	0	0	0	1	0	1	1	2	≥128	64	≥128
Scedosporium apiospermum	5	143	0	0	0	0	4	5	10	26	7	12	79	≥128	≥128	≥128
Scedosporium aurantiacum	4	21	0	0	0	0	1	0	1	1	1	2	15	≥128	≥128	≥128
Scedosporium boydii	4	76	0	0	0	0	0	4	6	4	10	32	20	≥128	64	64
Scedosporium dehoogii	3	5	0	0	0	0	0	0	1	0	1	1	2	≥128	64	≥128
Scedosporium ellipsoideum	4	47	0	0	0	0	0	0	5	4	4	8	26			

65 Most truncated high - *S.apio* FLU (1 lab 72 isolates at 128) *S.boydii* (1 lab 52 isolates at 64+)

#	Description

TRH – *Scedosporium/Lomentospora*

Species (n/labs)	Agent	ECV	Mode	Comment
S. apiospermum	5FC	-	>64	Truncated high
L. prolificans	Fluconazole	-	>128	Already designated IR
S. apiospermum	Fluconazole	-	>128	Truncated high *
S. aurantiacum	Fluconazole	-	>128	Truncated high
S. boydii	Fluconazole	-	64	Truncated high *
S. ellipsoideum	Fluconazole		>128	Truncated high

Limited MIC data for 5FC, but not recommended antifungal agent (seems truncated for all)
Similar for fluconazole but a few lab with lower MICs for *S. apiospermum* and *S. boydii*

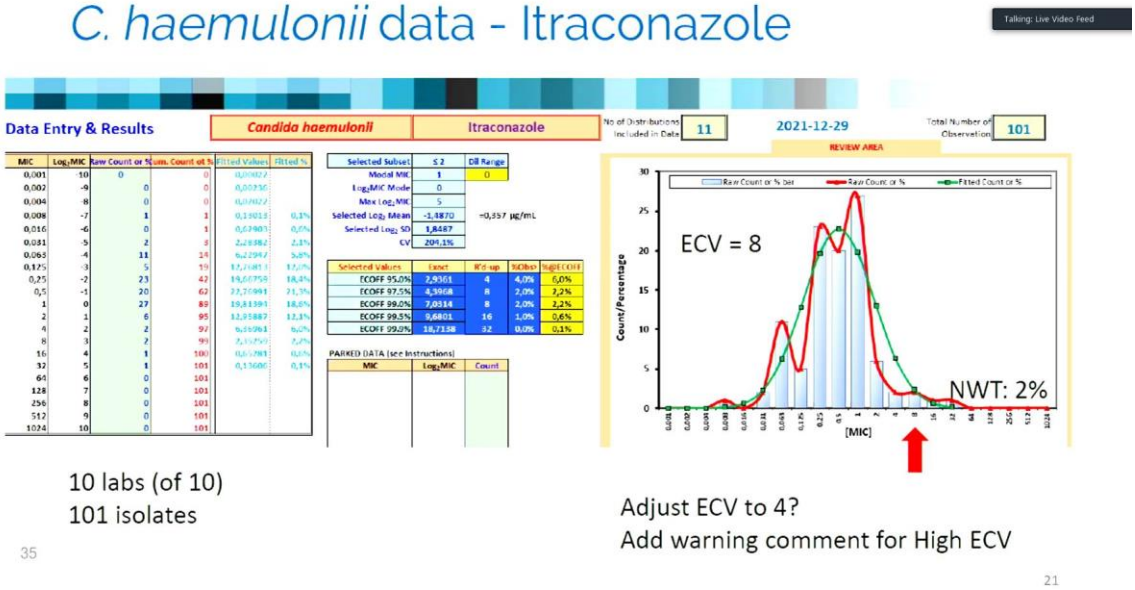
***Candida rugosa* and anidulafungin**

- WG concluded that *C. rugosa* complex is NOT IR to anidulafungin. Refer to PDF summary for literature review. A few studies with 10-30 isolates each. Studies with CLSI M27-A3 method have low MICs down to 0.5. *In-vivo* mice studies looking at isolates with low MICs. Some improved survival and decrease in kidney burden in mice when treated and isolates had low MICs. Given the fact that there are significant low MIC isolates and the animal data we concluded this does not meet IR definition.
- *C. rugosa* ECV data, new ECV is 4 not 8 (recalculated). Fits better with IR WG literature review. 106 isolates provided by biomerieux for ECV studies *C. rugosa* complex.

***Candida haemulonii* and Itraconazole**

- *C. haemulonii* vs itraconazole is NOT intrinsically resistant as per WG definition. ECV has been recalculated. About 170 isolates examined using CLSI method, a few with EUCAST methods. MIC50s were sometimes quite low, down at 0.5. Some were higher greater than 16 seems to be variability. This ECV also recalculated, decreased from 8 to 4.

#	Description



Echinocandins and Mucorales

- Refer to PDF summary from agenda
- Have started seeing some studies with lower MECs. Reached out to authors for some of these studies and had difficulty with COVID and with finding the published isolates. We were unable to finish retesting these. Had a second look at the data and group has reached the conclusion that they just cannot say as a blanket statement that the Mucorales are IR to echinocandins. Most mucorales do appear IR but some species just don't fit IR definition. *R. pusillus* for caspofungin, *L. corymbifera* and anidulafungin, *A. elegans* for both caspofungin and anidulafungin. Too large of an overarching assessment to make.
- Is *Candida inconspicua* IR to fluconazole by CLSI criteria?
- *C. inconspicua* is an emerging pathogen in immunocompromised hosts possessing inherently decreased susceptibility to fluconazole. Whether this is IR is not well understood. MIC50s and 90s are pretty high. Refer to Tom Walsh presentation. Data in Bourgeois et al. 2010 show the MIC50 and MIC90 range going very low down to 0.125. Great amount of MIC variability but only 5 isolates, but points away from IR.
- Dr. Dufresne mentions that *C. inconspicua* that incubation at 48h seems to have very high MIC as opposed to 24h. Dr. Schuetz says they did not review this. Table 1 in Bourgeois paper. Maybe it is slow growing? Many years ago CLSI went to reading yeast at 24h from 48h because there is better intra-laboratory agreement. Maybe more trailing growth at 48h, could be an explanation? Should stick with 24h and stick with it being NOT IR to fluconazole and voriconazole.
- Ongoing work and Future IR assessments: *L. prolificans* against some other azoles voriconazole and posaconazole, echinocandins. Group has decided not to pursue *Fusarium* and echinocandins as at the genus level there is not a lot of evidence supporting IR. Working on 2 manuscripts one for yeast and one for molds, may include body site reporting as a separate manuscript or inside the 2 not sure yet. Goal for submission is Q2 for this year.

#	Description
	Discussion: Dr. Zhang: Is there data for *S. apiospermum* and amphotericin B IR since most labs can't separate this species from *S. boydii*? There is data for *S. boydii* only. Dr. Schuetz states they did not look at this data. Dr. Zhang thinks this is useful as they can perhaps be resulted as a complex if similar. Need to look at that ECV data. Kausik Datta: Once an isolate is designated as IR, further susceptibility testing is not necessary is this correct? Yes. Since the lack of efficacy of antifungal treatment in a patient, if there is no PK/PD data or ECV wouldn't AST give useful information to the clinician? Dr. Schuetz explains that PK/PD and clinical data is considered when developing the IR determinations. We don't want to report out a false susceptible for these IR species due to method variation and erroneously mislead the clinician. Designating them as IR gives fair warning to clinicians not to use them as monotherapy. We are not saying don't report it, we actually suggest to report it out as resistant. Dr. Verweij is concerned about the number of isolates for 5FC and *Lomentospora*. There is only one study with 2 isolates on the pH effect. Dr. Borman comment: How were the *C. inconspicua* isolates identified in this 2010 paper? It was notoriously difficult to ID pre-Maldi without rDNA sequencing. Dr. Schuetz did not look at how in paper as it was not included in presentation. Not sure? Dr. Procop suggests taking this off the list and bringing it back. Concern with the ID method and also the incubation times. Dr. Schuetz agreed to take *C. inconspicua* and fluconazole off the list for now. Also, number of isolates is quite low for 5FC and *Lomentospora*. This should also be taken off. Vote to accept the list Dr. Schuetz has proposed with the amendments above, which means both right and left hand bottom on the below list will be removed. Items for Discussion and Vote Talking: Live Video Feed **WG Voted for Intrinsic Resistance** • *Scedosporium boydii* vs. amphotericin B • *Lomentospora prolificans* vs. isavuconazole • *Scedosporium* spp. and *L. prolificans* vs. flucytosine **WG Voted against Intrinsic Resistance** • *Candida rugosa* vs. anidulafungin • *Scedosporium apiospermum* and *S. boydii* vs. isavuconazole • *Candida haemulonii* vs. itraconazole • Mucorales vs. echinocandins • *(Candida inconspicua* vs. fluconazole)

#	Description
	A motion was made and seconded to approve all of the above list EXCEPT *Lomentospora/Scedosporium* and 5FC (not enough data) and *C. inconspicua* vs fluconazole due to ambiguous IDs. (Approved for Intrinsic Resistance: *Scedosporium boydii* vs amphotericin B, *Lomentospora prolificans* vs isavuconazole. Voted against Intrinsic Resistance: *Candida rugosa* vs anidulafungin, *Scedosporium apiospermum* and *S. boydii* vs isavuconazole, *Candida haemulonii* vs itraconazole, Mucorales vs echinocandins. VOTE: 9 for; 0 against; 0 abstain; 0 absent (Pass).

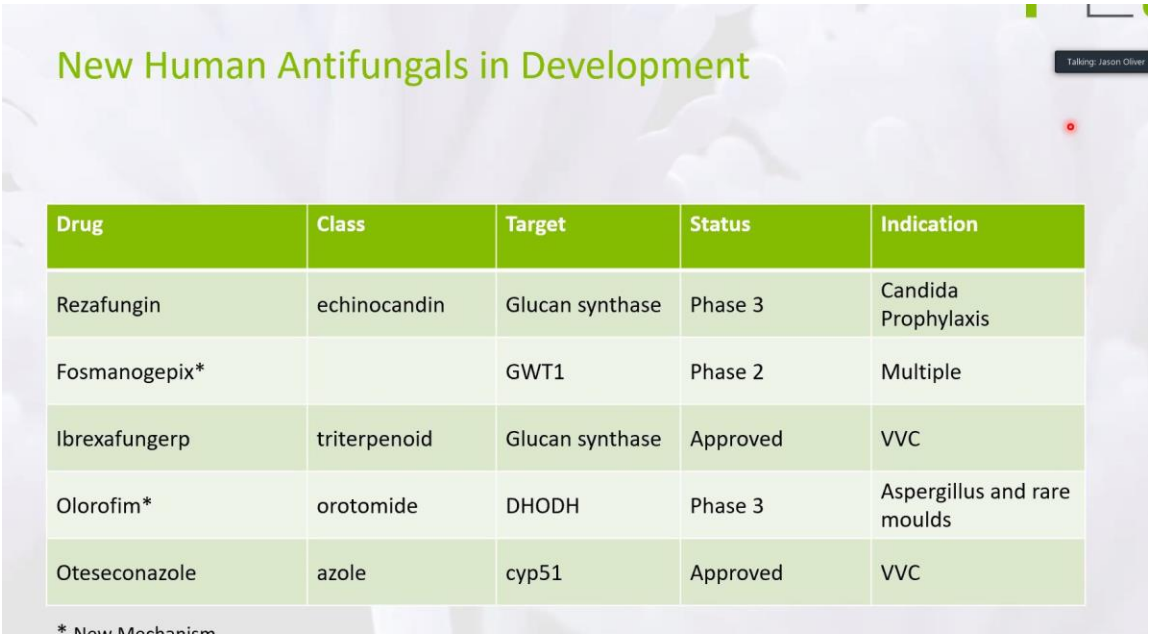
	
	Ongoing Work and Future IR Assessments
	• *L. prolificans* and posaconazole
	• *L. prolificans* and voriconazole
	• *L. prolificans* and echinocandins (had been awaiting more data but will complete assessment with or without additional data)
	• *Fusarium* and echinocandins (had been awaiting more data but will complete assessment with or without additional data)
	• Journal of Clinical Microbiology supports 2 mini reviews covering updates from CLSI Antifungal Tests Subcommittee re: intrinsic resistance and body site reporting restrictions
	  • 2 manuscripts – one for yeasts and one for molds
	  • Yeast manuscript is in progress; goal for submission is Q2 2023

16.	**Afternoon Break**

SUMMARY MINUTES Saturday, 21 January 2023	
#	Description
17.	**DHODH Inhibitor Fungicide/Herbicide and Potential for Resistance Development to Olorofim (Dr. Oliver)**

- Invasive aspergillosis
- Azole resistance mutations in cyp51 target gene, also upregulation of drug efflux (less common)
- Azoles are used as fungicides in agriculture. Include tebuconazole, propiconazole. Millions of tons sprayed onto fields each year, relation between generation of azole resistance in environment and patients. Often common genotypes are seen in clinical isolates.
- Literature back to 2009 questioning if azole resistance is a side effect of environmental fungicide use. Genetic similarity 2022 US and UK studies. Strong evidence for agricultural origin of azole resistance.

- New human antifungals in development:



Drug	Class	Target	Status	Indication
Rezafungin	echinocandin	Glucan synthase	Phase 3	Candida Prophylaxis
Fosmanogepix*		GWT1	Phase 2	Multiple
Ibrexafungerp	triterpenoid	Glucan synthase	Approved	VVC
Olorofim*	orotomide	DHODH	Phase 3	Aspergillus and rare moulds
Oteseconazole	azole	cyp51	Approved	VVC

* New Mechanism

#	Description

Agribusiness increasingly using human antifungal targets

Talking: Jason Oliver

Drug	Target	Source
Aminopyrifen	GWT1	AGRO-KANESHO Co., Ltd
Ipflufenoquin	DHODH	Japanese Soda Co Ltd
Tetflupyrolimet	DHODH	FMC Corp

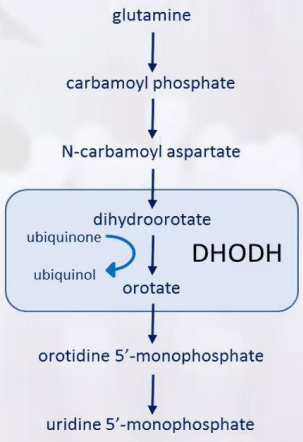
- Using validated human targets reduces discovery time
- Development and approval timelines inherently shorter
- Fungicides can be in use before human approval

- Olorofim
  - New in clinical development. Spectrum includes *Aspergillus* spp., *Coccidioides*, difficult to treat *Scedosporium*, *Lomentospora*, *Scopulariopsis*
  - Novel mechanism. Inhibits DHODH enzyme in protein synthesis.

Orotomide Mechanism of Action

Talking: Jaso

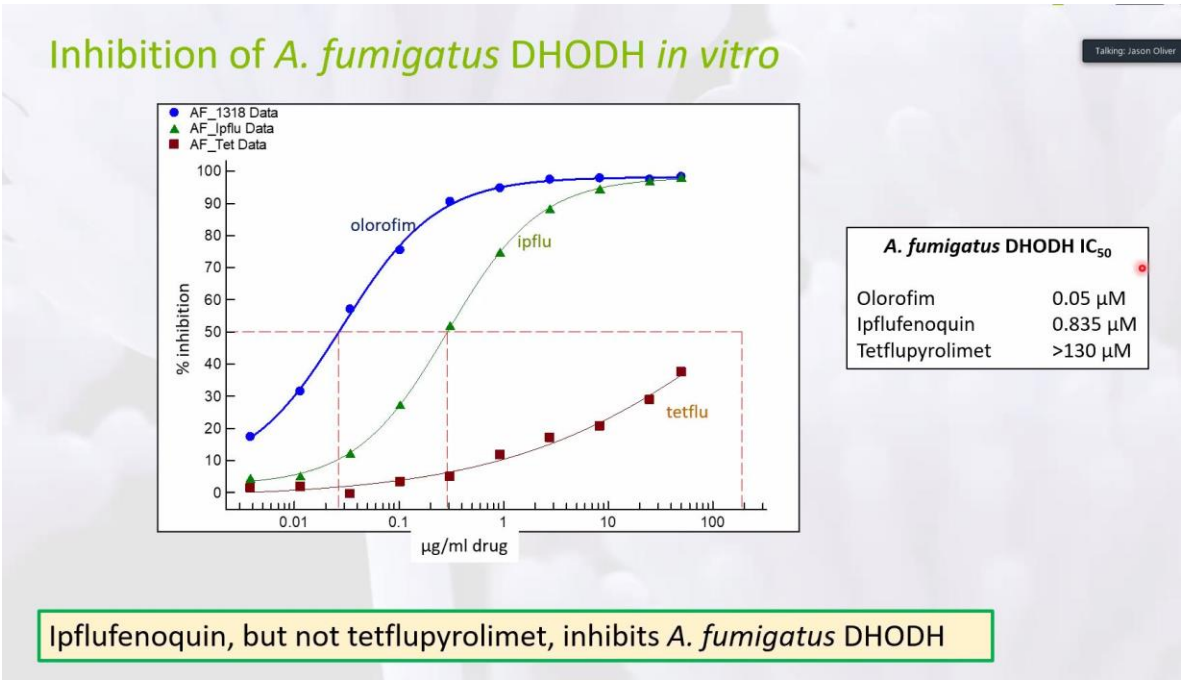
- Olorofim is a potent inhibitor of *A. fumigatus* DHODH
  - DHODH (Dihydroorotate dehydrogenase) is a key enzyme involved in pyrimidine biosynthesis
- Humans also have this enzyme
  - But, > 2000-fold difference in IC_{50} between human and fungal enzymes
- Pyrimidine inhibition has profound effects as it interferes with
  - DNA synthesis and cell cycle regulation
  - RNA synthesis and protein production
  - Cell wall synthesis
  - Phospholipid synthesis



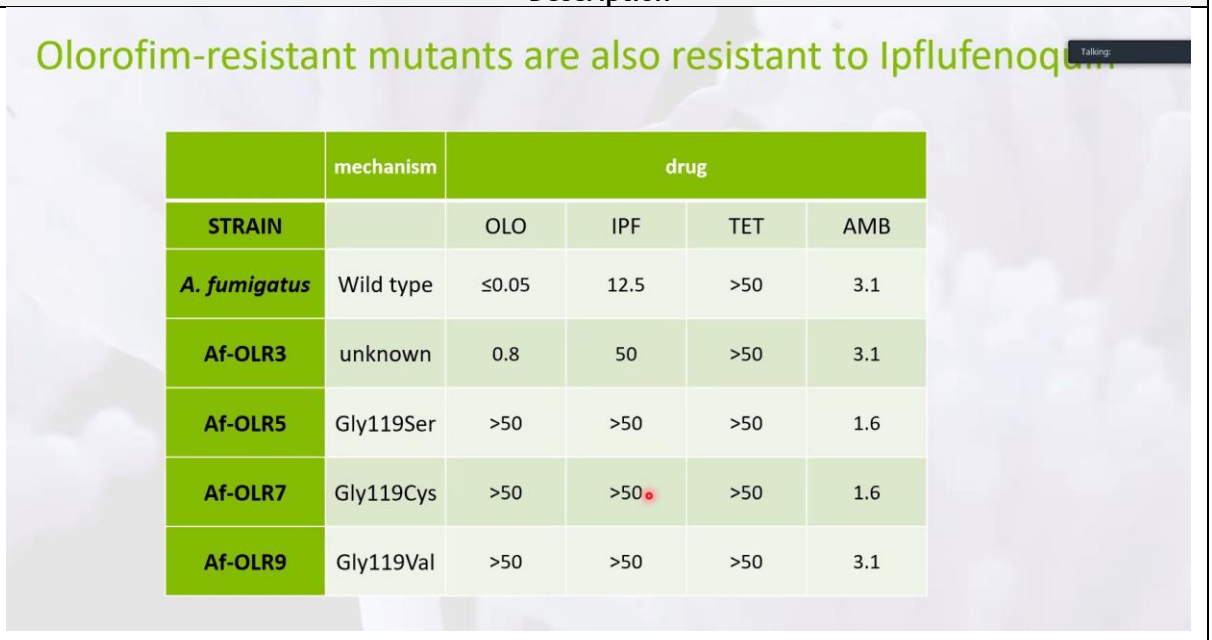
#	Description

- Agricultural DHODH inhibitors have been developed and approved. Concerns are do the agricultural drugs have activity against any fungi we target with Olorofim. Will this give rise to cross-resistance?

- Ipflufenoquin and Tetflupyrolimet against *Aspergillus* spp., Ipflufenoquin has MIC activity against some species *A. terreus*, Tetflupyrolimet does not. Ipflufenoquin quite potent. Introducing exogenous pyrimidines can reverse the effect of Ipflufenoquin similar to Olorofim.



- Similarly, Ipflufenoquin is also a potent inhibitor of *Coccidioides immitis* DHODH. Line on this graph for the Ipflufenoquin almost lays completely on top of Olorofim.
- Inhibition of human DHODH in vitro shows Olorofim is a poor inhibitor of human DHODH. Tetflupyrolimet is a potent inhibitor of human DHODH. Ipflufenoquin is weak. Teriflunomide is licensed for treatment of human MS and is also a DHODH inhibitor.
- Buil et al Emerg Microbes Infect 2022 11 :703. Hotspot of resistance identified as Gly119 in *A. fumigatus*. Mutation of Gly 119 affects IPF and TET inhibition. Side chain either prevents Olorofim binding or prevents Olorfim from entering binding site (stearic hindrance).
- Tetflupyrolimet IC50 DECREASES with Gly119 mutation (!!!) indicating it binds better.

#	Description
	

	mechanism	drug			
STRAIN		OLO	IPF	TET	AMB
A. fumigatus	Wild type	≤0.05	12.5	>50	3.1
Af-OLR3	unknown	0.8	50	>50	3.1
Af-OLR5	Gly119Ser	>50	>50	>50	1.6
Af-OLR7	Gly119Cys	>50	>50	>50	1.6
Af-OLR9	Gly119Val	>50	>50	>50	3.1

- Concerns that proposed Ipflufenoquin use against Almond leaf spots overlaps with *Coccidioides immitis* endemic area in California. Side effect concerns with tetflupyr.
- F2G has not looked at *Scedosporium* or *Lomentospora*, has not done the experiments. They do have *L. prolificans* enzyme assay going. F2G thinks the results would be similar.

18. **Other Business (Dr. Dufresne)**
- None

19. **Plans for Next Virtual Meeting**
Summer virtual meeting to be planned. Ms. Lam will send out a doodle poll. Normally June but everyone prefers August so there is more time, however we may need to align with CLSI days. TBD.

20. **Adjournment**
Dr. Dufresne thanked the participants for their time. The meeting was adjourned at 3:30 PM Eastern (US) time.

ACTION ITEMS			
#	**Description**	**Responsible**	**Status**
1.	Isavuconazole breakpoints will have accompanying comments and subcommittee will craft a statement at summer meeting about how we need to be cautious for the intermediate category and comment to refer to voriconazole. Also need a comment about how to report when isavuconazole/voriconazole don't agree.	Antifungal Subcommittee	In progress
2	Posaconazole CLSI BP determination: analyze FTL azole, MIC data, constitute and send CYP51 mutant panel to a few high and low mode PSC CLSI labs, animal studies with isolates with MICs in 0.25 to 2 µg/mL range.	Antifungal subcommittee	In progress
3	Create a WG for antifungal reading and interpretation with audiovisual support from CLSI leadership about mold susceptibility reading.	Antifungal Subcommittee	In progress
4	Yeast susceptibility according to genetic group/clade: Decisions to be made: a) Which species to include? Probably exclude those that are rare. b) Which output format table or tree? c) Also decide on a definition of reduced susceptibility designation. d) Reviewed by ECV and IR WG then submit to subcommittee for approval.	Antifungal Subcommittee	In progress

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

SC Reviewers and Guest Attendees

Rebecca Abelman	Sharon Erdman
Supriya Aher	Gina Ewald-Saldana
Sarah Alsamarai	Michelle Fang
Karl Anthony Ramos	Halyna Filonenko
Stella Antonara	Andrew Fratoni
Sophie Arbefeville	Zoe Freeman Weiss
Mari Ariyasu	Marcelo Galas
Tomefa Asempa	Barb Gancarz
Shukal Bala	Guillermo Garcia-Effron
Faiza Benahmed	Akela Ghazawi
Jill Bennett	Darcy Gill
Timothy Bensman	Melissa Gitman
Amira Bhalodi	Beth Goldstein
Amelia S. Bhatnagar	Eriyanto Ginting
Bhaskar Bhattacharya	Heather Glasgow
Sujata Bhavnani	Avery Goodwin
Tanaya Bhowmick	Christopher Haddock
Paul Bien	Diane Halimi
James Birch	Lauren Hamilton
Michael Birch	Itzel Harriott
Melissa Boddicker	Stephen Hawser
Maryann Brandt	Sarah Hepler
Derrek Brown	Evann Hilt
Alexandra Bryson	Maren Hnaya
Shelley Campeau	Rita Hoffard
Jerry Capraro	Stephanie Horiuchi
Cecilia Carvalhaes	Michael Huband
Darcie Carpenter	Dmitri Iarikov
Nydia A. Castillo-Martinez	Muhammad Irfan
Sukantha Chandrasekaran	Edwin Kamau
Sudha Chaturvedi	Shivaramu Keelara Veerappa
Jennifer Chau	Haziq Khalid
Melvili Cintron	Abdullah Kilic
Kia Cox	Scott Killian
Arryn Craney	Anna Klavins
Kausik Datta	Cynthia Knapp
Animesh Dhara	Jennifer Krauss
Alhagie Dibbasey	Sarah Leppanen
Cau Dinh Pham	Xian-Zhi Li
Rebekah Dumm	Luiz Lisboa
Mervat Elanany	Jeff Locke
Divyaa Elangovan	Zabrina Lockett

SC Reviewers and Guest Attendees (continued)

Jordan Mah	Josh Shirley
Allie Malmberg	Simone Shurland
Matt Mason	Jennifer Slaughter
Ron Master	Jennifer Smart
Sandra McCurdy	Dallas Smith
CT Meenachi	Paula Snippes Vagnone
Anali Milagros Salad Fitzcarrald	Zhanna Sobkova
Crystal Minchew	Chalwe Sokoni
Susan Mindel	Dylan Staats
Anisha Misra	Muriel Starck
Nicholas Moore	Judith Steenbergen
Yesenia Morales	Mohammed Suaudi Hassen
Madhavi Motati	Dillon Thai
Mary Motyl	Susan Thomson
Besarta Mullalli	Daouda Touré
Chie Ohno	Allison Tsan
Jason Oliver	John Turnidge
Luis Ostrosky-Zeichner	Valentine Usongo
Olayide Oyelaja	Bahar Vafadar
David Paisey	Tam Van
Elizabeth Palavecino	Benjamin von Bredow
Robin Patel	Wayne Wang
Jeffrey Pearson	Eric Wenzler
Chris Pillar	Christine Yang
Eric Ransom	Cheung Yee
Hallo Rashid	Ingrid Yu Ying Cheung
Mark Redell	Jean-Yves Ressot
Will Rotunno	Priyanka Uprety
Madiha Shah	Stephen Vella
Ribhi Shawar	Hadjer Zemmouri
Amanda Sheets	Yanan (Nancy) Zhao