This participant summary contains a correction for Specimen F-12 on pg 30.

COLLEGE of AMERICAN PATHOLOGISTS

Surveys and Anatomic Pathology Education Programs

Mycology
F-B 2020

Participant Summary/Final Critique

1.0 Credit of Continuing Education Available

This document is available online in e-LAB Solutions Suite.

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COLLEGE of AMERICAN PATHOLOGISTS

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The CAP designates this educational activity for a maximum of 1 credit of continuing education. Participants should only claim credit commensurate with the extent of their participation in the activity.

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This activity is approved for continuing education credit in the states of California and Florida.

Disclosure Statement

The following authors/planners have no financial relationships to disclose:
Kaede Ota-Sullivan, MD, FAAP; Angela Thiess MD; Robin Rolf MT(ASCP)

The following authors/planners have financial relationships to disclose:

Author	Commercial Interest	Your Role	What was received
Romney M. Humphries, PhD, D(ABMM), M(ASCP), MT(ASCP)	Accelerate Diagnostics	Chief Scientific Officer	Salary, stock
Allison McMullen, PhD, D(ABMM)	bioMerieux	Speakers Bureau	Honorarium
	Cepheid	Speakers Bureau	Honorarium
	Roche	Speakers Bureau	Honorarium
	Selux Diagnostics	Advisory Board	Consulting fee
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	Bio-Rad	Advisory Board Member	Consultant fee
	Quantamatrix	Advisory Board Member	Consultant fee

The following In-Kind Support has been received for this activity:
None

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None

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interest. All recommendations and/or planning criteria involving clinical medicine are based on evidence that is accepted within the profession of medicine as adequate justification for this indication and contraindication in the care of patients.

Learning Objectives

Upon completing the reading and answering the learning assessment questions, you should be able to:

1. Describe the typical colonial morphology, growth requirements, and clinical significance of isolated organisms.
2. State the key, distinguishing characteristics of the isolated organism.
3. Ensure the organism's identification is consistent with the source and clinical setting.
4. Identify appropriate reporting/interpretation of antimicrobial susceptibilities for an organism, considering the source of the specimen and any relevant clinical information.

2020 F-B
PARTICIPANT SUMMARY/FINAL CRITIQUE

Program Update

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With your participation in CAP's Surveys programs, *every member of your team* can take part in education activities: earn Continuing Education (CE) credits or receive Self-Reported Training* at no additional charge.

This Survey mailing includes an online education activity to earn **1.0** CE credit. To access the activity, see page 1.

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Evaluation Criteria

To provide a timely evaluation of your results, statistics presented in this Participant Summary reflect participant data received by the due date.

The CAP is required to submit PT results to the Centers for Medicare and Medicaid Services (CMS) for all labs that have provided a CLIA identification number. If you do not notify the CAP that your lab has discontinued testing of a regulated analyte, **a score of zero will be given**. Your reporting preferences are outlined on the CMS Analyte Reporting Selections document. If new products are ordered and/or canceled, this may affect your reporting selections, so it is recommended that you periodically check this report on e-LAB Solutions Suite, which will always reflect the most up-to-date information. This information can also be obtained by calling the Customer Contact Center at 800-323-4040, Option 1 (domestic) or 001-847-832-7000, Option 1 (international).

In the event a result is not graded, a numeric code will appear next to your result. A definition of the code will appear on the first page of your evaluation. Please see "Actions Laboratories Should Take when a PT Result is Not Graded" on page 31.

Analyte	Evaluation Criteria
Dermatophyte	**80% Participant or Referee Consensus**
Mold	**80% Participant or Referee Consensus**
Yeast	**80% Participant or Referee Consensus**
Antifungal susceptibility and testing	80% Participant Consensus and CLSI guidelines*
Cryptococcal antigen	80% Participant Consensus

* Only the qualitative interpretation (resistant, intermediate, susceptible, S-DD or No Interpretation) is formally evaluated. Grading is based on FDA and CLSI method interpretive tables.

The CAP wishes to thank Rosemary C. She, MD, FCAP; and Aida Mangahis, CLS, for providing these photographs. Unless permission is received from Dr. She and Ms. Mangahis, these photographs may not be used for any purpose except in connection with this Survey.

Specimen F-07

The F-07 challenge was a simulated blood culture specimen from a 66-year-old female after abdominal surgery. Participants were asked to determine the presence or absence of any yeast or aerobic Actinomycetes and identify any yeast or aerobic Actinomycetes present; and to perform antifungal susceptibility testing. The challenge contained *Candida albicans.* A response of *Candida albicans*, *Candida albicans/dubliniensis*, *Candida* sp., or Yeast, sent to reference lab for identification was considered satisfactory. Referee and participant responses are summarized below.

Table 1. Summary of Participant Responses

	Identification	Referees (71) No.	%	Participants (1006) No.	%
F-07	*Candida albicans*	66	93.0	898	89.3
	Candida albicans/dubliniensis	2	2.8	45	4.5
	Candida sp.	4	5.6	33	3.3
	Yeast, sent to reference lab for identification	-	-	27	2.7

Table 2. Results by Method.

		% of Laboratory Designation		
System	No. Labs	*Candida albicans*	*Candida albicans/ dubliniensis*	*Candida* sp.
API	58	82.8	8.6	8.6
BD Phoenix	17	100.0	-	-
Mass Spectrometry/Bruker MALDI	190	99.0	0.5	-
Mass Spectrometry/Vitek MS MALDI	168	100.0	-	-
MicroScan	24	37.5	50.0	8.3
Morphology and Bruker MALDI	79	98.7	1.3	-
Morphology and Vitek MS MALDI	71	100.0	-	-
Morphologic exam/biochemical	45	43.2	15.9	11.4
Remel RapID Yeast Plus	40	52.5	32.5	12.5
Vitek 2	268	94.4	0.8	3.7
Other[a]	41	51.2	9.8	12.2

[a] Includes other commercial kits and methods with <10 users.

Discussion

Taxonomy

The genus *Candida* contains approximately 200 species, leading some to refer to it as a “taxonomic pit”, into which yeasts without a known sexual stage or remarkable phenotypic traits have been historically placed.[1] In clinical laboratories, *Candida albicans* is the most commonly isolated species of the genus. The organism was first named *Oidium albicans* by Charles-Phillipe Robin in 1853. *O. albicans* was renamed *Synringospora robinii* in 1868 and reclassified as *Monilia albicans* in 1890. From these original names come the terms *oidiomycosis* and *moniliasis,* which are referred to as *candidiasis* today.[1]

In the late 19th century, various physicians independently cultivated *C. albicans* and named it *Mycoderma vini (*1877, Grawitz), *Oryctolagus cuniculus* (Rosentein 1867), *Saccharomyces albicans* (Reess, 1877), and *Endomyces albicans* (Vuillemin 1899).[2] The nearly-universally applied species name, albicans, means “to whiten”, referring to the whitish plaques found in the oropharynx, buccal mucosa or tongue that characterize thrush. The currently accepted genus name, *Candida*, was introduced in 1923 by Christine Berkhout when she created the genus *Candida* as part of her PhD thesis. Her description of the genus, translated from the original Dutch, was as follows: “Few hyphae, prostrate, breaking up into shorter or longer pieces. Conidia, arising by budding from the hyphae or on top of each other, are small and hyaline.”[1]

The name *Candida* refers to *toga candida*, a gleaming white robe worn by Roman Senators. As such, the name *Candida albicans* is somewhat redundant, referring to a white yeast that whitens.

Following the publication by Berkhout, a number of generic names were proposed for asexual filamentous yeasts, including *Blastodendrion*, *Myceloblastanon*, *Geotrichoides*, *Mycocandida*, *Mycotoruloides*, *Mycokluyveria*, leading to a long list of over 100 synonyms for *Candida albicans*. These were reconciled in 1952 by Lodder and van Kreger, with the publications of the first definitive taxonomic manual for yeasts.[1,3] Taxonomy of the genus continues to evolve, predominantly with the recognition of cryptic species or species complexes - i.e., genomically distinct organisms that are indistinguishable or closely related morphologically and biochemically.[4] Presently, the *Candida albicans* complex includes *C. albicans sensu stricto*, *C. dubliniensis* and *C. africana*.

Identification

Candida albicans is easily cultivated on routine mycology media, with colonies forming in as few as 24 hours, but usually 48 to 72 hours. Colonies are smooth and creamy. Many isolates of *C. albicans* produce colonies with ‘feet’ on blood agar, although this feature is not specific to the identification of *C. albicans*, as up to 25% *of Candida tropicalis* and *Candida krusei* may demonstrate this phenotype.[1]

A simple test to confirm the identification of *C. albicans* is demonstrating the formation of germ tubes, a technique first proposed by Claire Taschdjian in 1960.[2] *C. albicans*, *Candida dubliniensis* and *Candida africana* produce filamentous outgrowths when incubated in serum at 37°C. Of note, colonies with feet should not be used to perform germ tube tests, because the hyphal and pseudohyphal cells present within the inoculum will interfere with results. Chromogenic media are available for the presumptive identification of certain *Candida* species based on color, but between 2-10% of *C. albicans* strains produce white rather than colored colonies on chromogenic media.[5]

On cornmeal-Tween 80 agar, *C. albicans* produces pseudohyphae and some hyphae with clusters of round blastoconidia at the septa. Thick-walled, terminal chlamydospores are formed singly by *C. albicans* and often in clusters by *C. dubliniensis*. *C. africana* does not produce chlamydospores. Chlamydospores are thick-walled, asexual cells characteristic of *C. albicans* and *C. dubliniensis*. While the function of the chlamydospores is not definitively known, most researchers agree it likely aids *C. albicans* survival in unfavorable conditions.[2]

Automated biochemical-based ID systems identify *C. albicans* relatively well. A recent study comparing various conventional identification methods (morphology or biochemical kits) versus MALDI-TOF MS and ITS sequencing demonstrated superior accuracy of mass spectrometry over biochemical and morphologic methods for *Candida* species in general, including *C. albicans*.[6] *Candida* species identified as *C. famata* or *C. tropicalis* by conventional methods were identified as *C. albicans* by mass spectrometry and/or sequencing. Routine differentiation of *C. albicans*, *C. dubliniensis* and *C. africana* is not necessary as a routine practice in the clinical laboratory, but if not done, should be referred to collectively as *C. albicans* complex.

Clinical Significance

Candida albicans is a saprobe of humans and certain warm-blooded mammals. It colonizes the oral cavity of more than 25% of adults.[7] It also is part of the normal microbiota of the skin, vagina and gastrointestinal tract of

humans. *Candida* spp. are present throughout the gastrointestinal tract, and *C. albicans* is the most commonly isolated of these.[2] Environmental contamination of plants and foods by animals that carry *Candida* is also common.

The presence of *C. albicans* in a patient specimen in the laboratory may indicate disease, colonization, or environmental contamination from food or plant sources. However, isolation of *C. albicans* from the blood (candidemia) is indicative of invasive disease, and serious infection. Candidemia as a whole is associated with 47% attributable mortality, with prognosis closely linked to early administration of antifungals and source control (e.g., removal of colonized central venous catheters or drainage of infected material).[8]

The patient in this Survey developed candidemia following abdominal surgery. *Candida* species often contribute to polymicrobial intra-abdominal infections that occur following gut perforation, anastomotic leaks after bowel surgery or acute necrotizing pancreatitis.[9] *Candida albicans* is the predominant species isolated in these cases, although *Candida glabrata* is increasing. While the patient in this Survey had positive blood cultures, most patients with intra-abdominal candidiasis are blood-culture negative. In one multi-center study of 481 patients, only 14% had positive blood cultures.[10] A second study of 125 patients in the U.S. found only 6% were candidemic.[11] Diagnosis is best made in these cases by culture of aspirated peritoneal fluid or abscess (if present). Several studies demonstrate recovery of pathogens, including *Candida albicans*, is best if the fluid is cultured in blood culture bottles on an automated, continuous monitoring system.[12] Additionally, elevated serum beta-D-glucan assays may be of value to identify patients with intra-abdominal candidiasis,[13] as well as to rule out *Candida* spp. involvement in patients with intraabdominal infection.

Symptoms of *Candida* peritonitis are indistinguishable from those of bacterial peritonitis, including fever, chills and abdominal pain. Mortality associated with intra-abdominal candidiasis was 28%. Independent predictors of decreased mortality include presence of abscess, early source control and young age.[11]

Therapy Considerations

Intra-abdominal *Candida* infection requires early source control, such as surgical intervention, percutaneous drainage or transgastric drainage. Preferred initial treatment includes an echinocandin (e.g., micafungin, anidulafungin, or caspofungin). Fluconazole is an alternative therapy that can be used in patients who are not critically ill, have not received recently fluconazole therapy, or who are not considered to be likely to have fluconazole-resistant isolate. Lipid formulations of amphotericin B are also options, but used less frequently due to risk of nephrotoxicity. Once the species is known, therapy can be modified to fluconazole if the isolate is *C. albicans*.

Candida albicans is typically susceptible to agents used for treatment of candidiasis, including fluconazole and echinocandins. The Infectious Diseases Society of America recommends treatment with an echinocandin (caspofungin or anidulafungin) as initial therapy for candidemia, whereas fluconazole may be used for patients who are not critically ill or those for whom the isolate has been shown to be susceptible to fluconazole (ie, as de-escalation therapy). Because of the high mortality associated with candidemia, antifungal susceptibility testing, at minimum against fluconazole, is recommended for *Candida* isolates recovered from the blood.[8]

Key Points

- *Candida albicans* is a common commensal of the human body including the gastrointestinal tract, mouth, and other mucous membranes.
- *Candida albicans* species complex includes *C. albicans, C. dubliniensis and C. africana*. Routine differentiation of these by the clinical laboratory is not routinely required, provided the laboratory reports *Candida albicans* complex.

Table 3. Antifungal Susceptibility Testing

Antifungal Susceptibility Testing - MIC*

Antifungal Susceptibility Testing Intended	F/F1-07:	Antifungal agent	MIC Interpretation ♦
		Anidulafungin	S
		Amphotericin B	S,NI
		5-fluorocytosine	S,NI
		Fluconazole	R
		Itraconazole	R,NI
		Caspofungin	S
		Voriconazole	U
		Micafungin	S
		Isavuconazole	U

♦ S – Susceptible; I – Intermediate; R – Resistant; NC – Non-consensus; NI – No Interpretation; S-DD – Susceptible-Dose Dependent; NS – Non-Susceptible; U – Ungraded

Antifungal Susceptibility Testing - MIC*, cont'd

F/F1-07 *Candida albicans*

MIC testing	Interpretation	Participants No.	Participants %
Anidulafungin	Susceptible	101	98.1
	No Interpretation	2	1.9
Amphotericin B	Susceptible	73	36.7
	No Interpretation	126	63.3
5-fluorocytosine	Susceptible	64	44.8
	No Interpretation	79	55.2
Fluconazole	Susceptible	24	4.4
	Intermediate	59	10.9
	Resistant	441	81.7
	S-DD	12	2.2
	Non-Susceptible	1	0.2
	No Interpretation	3	0.6
Itraconazole	Resistant	23	21.9
	S-DD	1	0.9
	No Interpretation	81	77.1
Caspofungin	Susceptible	393	99.4
	Intermediate	1	0.2
	Resistant	1	0.2
	No Interpretation	1	0.2
Voriconazole**	Susceptible	99	24.4
	Intermediate	56	13.8
	Resistant	219	53.9
	S-DD	27	6.7
	Non-Susceptible	1	0.2
	No Interpretation	4	1.0
Micafungin	Susceptible	309	98.7
	Resistant	1	0.3
	No Interpretation	3	1.0
Isavuconazole***	No Interpretation	7	100.0

* The data for antifungal susceptibility has been combined with the F1 Survey to provide sufficient data to grade this challenge.

** Code 27, Non-consensus

*** Due to the limited number of participants (<10) reporting results, this drug/interpretation was not graded.

Antifungal Susceptibility Testing – Disk Agar*

Antifungal Susceptibility Testing Intended Interpretations	F/F1-07: Antifungal agent	Disk Agar Diffusion ♦
	Fluconazole**	U
	Capsofungin**	U
	Voriconazole**	U

♦ S – Susceptible; I – Intermediate; R – Resistant; S-DD – Susceptible-Dose Dependent; U - Ungraded

F/F1-07 *Candida albicans*

Disk Agar Diffusion	Interpretation	Participants No.	Participants %
Fluconazole**	Resistant	7	87.5
	S-DD	1	12.5
Capsofungin**	Susceptible	1	100.0
Voriconazole**	Susceptible	5	100.0

* The data for antifungal susceptibility has been combined with the F1 Survey.

** Due to the limited number of participants (<10) reporting results, this drug/interpretation was not graded.

Table 4. Supplemental questions for antifungal susceptibility testing of *Candida albicans* for F-07 2020

	Participant response:
1. Test methods:	
Broth microdilution	17
Disk Diffusion	7
YeastOne colorimetric microdilution	182
Gradient diffusion strips (eg, Etest, MTS)	35
Vitek 2	200
Other	11
2. Test performed according to:	
CLSIM27-S4/CLSI M60	372
CLSI M27-S3 (obsolete)	19
FDA	22
Other	19
3. Does your laboratory use or plan on using/reporting Epidemiologic cutoff values (ECVs)?	
Yes	65
No	346

Table 5. Distribution of antifungal MIC results by method for F/F1-07*

Occurrences at MIC (μg/mL)

5-fluorocytosine	<=	=	<=	=	<=	<=	>=	<=	=
Method	**0.050**	**0.060**	**0.120**	**0.250**	**0.500**	**1.000**	**1.000**	**2.000**	**4.000**
Broth microdilution	-	-	-	1	1	1	-	2	-
Vitek 2	-	-	-	-	-	41	2	1	1
YeastOne	1	1	4	11	32	21	-	4	-

Amphotericin B	=	<=	=	<=	=	<=	=	<=	<=	<	=
Method	**0.050**	**0.094**	**0.120**	**0.125**	**0.190**	**0.250**	**0.380**	**0.500**	**1.000**	**2.000**	**2.000**
Broth microdilution	-	1	1	2	-	1	1	4	2	-	1
Gradient diffusion strips	-	1	-	2	1	5	1	2	-	-	-
Vitek 2	-	-	-	-	-	10	-	26	18	1	-
YeastOne	1	-	-	-	-	10	-	67	5	-	-

Anidulafungin	=	=	=	<=	=	<=	<=	<=	<=	<=	<=	=	<=
Method	**0.002**	**0.003**	**0.006**	**0.008**	**0.012**	**0.015**	**0.016**	**0.030**	**0.060**	**0.080**	**0.120**	**0.125**	**0.250**
Broth microdilution	-	-	-	4	-	2	1	2	-	1	-	1	-
Gradient diffusion strips	1	1	1	-	1	-	1	-	-	-	-	-	-
YeastOne	-	-	-	-	-	4	1	10	22	-	32	1	1

Caspofugin	=	=	<=	=	<=	=	<=	<=	=	<=	>=
Method	**0.015**	**0.016**	**0.030**	**0.032**	**0.060**	**0.064**	**0.094**	**0.120**	**0.125**	**0.250**	**8.000**
Broth microdilution	-	1	3	1	3	-	-	-	1	3	-
Gradient diffusion strips	-	-	-	-	-	1	1	-	3	2	-
Vitek 2	-	-	-	-	-	-	-	106	-	102	1
YeastOne	1	-	16	-	63	1	-	21	-	3	-

Fluconazole	<=	>=	<=	>=	<=	>=	<=	>=	=	>=	>=	>=
Method	**8.000**	**8.000**	**16.000**	**16.000**	**32.000**	**32.000**	**64.000**	**64.000**	**96.000**	**128.000**	**256.000**	**512.000**
Broth microdilution	2	-	3	-	8	-	2	1	-	-	1	-
Gradient diffusion strips	-	-	2	-	7	8	7	-	1	2	4	-
Vitek 2	60	12	42	7	81	6	3	28	-	-	-	-
YeastOne	3	1	4	-	36	4	20	5	-	9	81	1

* Some MIC values may have been combined due to space limitations

Occurrences at MIC (μg/mL)

Isavuconazole	=	=	=
Method	**0.120**	**0.500**	**8.000**
Broth microdilution	1	2	1

Itraconazole	<=	=	<=	=	<=	>=	=	>=	>=
Method	**0.250**	**0.500**	**1.000**	**1.500**	**2.000**	**2.000**	**3.000**	**16.000**	**32.000**
Broth microdilution	3	3	2	-	-	-	-	2	-
Gradient diffusion strips	-	-	-	1	-	-	1	-	-
YeastOne	2	13	13	-	5	3	-	35	1

Micafungin	<=	=	<=	=	=	<=	=	<=	<=	<=	>=
Method	**0.008**	**0.012**	**0.015**	**0.016**	**0.023**	**0.030**	**0.047**	**0.060**	**0.150**	**0.250**	**8.000**
Broth microdilution	2	-	6	-	-	4	-	2	-	-	-
Gradient diffusion strips	1	2	-	3	4	-	-	1	-	-	-
Vitek 2	-	-	-	-	-	-	1	110	-	-	1
YeastOne	26	-	81	6	-	6	-	-	1	1	-

Posaconazole	=	<=	=	=	<=	=	=	>=	>=
Method	**0.125**	**0.250**	**0.500**	**0.750**	**1.000**	**2.000**	**4.000**	**8.000**	**16.000**
Broth microdilution	1	5	2	-	1	1	-	-	1
Gradient diffusion strips	-	-	-	1	1	-	-	-	-
YeastOne	-	-	10	-	16	-	1	30	1

Voriconazole	<=	<=	>=	<=	=	<=	>=	<=	=	>=	>=	>=	>
Method	**0.120**	**0.250**	**0.250**	**0.500**	**0.750**	**1.000**	**1.000**	**2.000**	**3.000**	**4.000**	**8.000**	**16.000**	**32.000**
Broth microdilution	-	5	-	5	-	3	-	1	-	-	1	1	-
Gradient diffusion strips	-	1	-	9	1	1	1	1	1	-	-	-	1
Vitek 2	17	17	1	7	-	99	6	10	-	12	1	-	-
YeastOne	-	4	-	29	-	25	2	3	-	8	56	9	-

* Some MIC values may have been combined due to space limitations

Table 6. Interpretation by Method

F/F1-07

Antimicrobial	Broth Microdilution					YeastOne Colorimetric					Gradient diffusion strips (eg, Etest, MTS)					Vitek 2					
	S	I	R	S-DD	NI	S	I	R	S-DD	NI	S	I	R	S-DD	NI	S	I	R	S-DD	NS	NI
Anidulafungin	10	-	-	-	1	70	-	-		-	4	-	-	-	1	-	-	-	-	-	-
Amphotericin B	1	-	-	-	12	9	-	-	-	73	6	-	-	-	6	48	-	-	-	-	9
Caspofungin	11	-	-	-	1	105	-	-	-	-	8	-	-	-	-	211	-	1	-	-	-
Fluconazole	1	-	17	-	1	-	-	164	1	-	-	-	31	-	-	20	47	163	9	1	1
Micafungin	13	-	-	-	1	121	-	-	-	-	10	-	-	-	1	114	-	1	-	-	-
Itraconazole	-	-	1	-	9	-	-	13	1	57	-	-	-	-	2	-	-	-	-	-	2
Voriconazole	-	8	6	1	1	3	20	101	10	1	2	4	6	4	-	80	14	67	8	1	1
5-Fluorocytosine	-	-	-	-	5	13	-	-	-	60	-	-	-	-	-	41	-	-	-	-	5
Isavuconazole	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Antimicrobial resistance and susceptibility testing

Candida albicans is generally susceptible to the azoles and echinocandins. In a 2014 SENTRY Antimicrobial Surveillance Program study, which monitors global susceptibility and resistance rates, 251 *C. albicans* isolates from around the world were tested. Resistance to fluconazole was 2.0% and voriconazole was 0.8%, based on CLSI breakpoints (Table 7). No echinocandin resistance was observed in this study, and all isolates had MICs below the echinocandin epidemiological cutoff value (ECV) (Table 8) ie, the cut-off that defines the wild-type population without acquired or mutational resistance mechanisms to echinocandins.[14]

Fluconazole acts by inhibiting the cytochrome P450 enzyme lanosterol demethylase, which is encoded by the *erg11* gene, in the ergosterol biosynthesis pathway. Ergosterol is an essential component of fungal cell membranes, including that of *Candida* spp., this inhibition is toxic leading to accumulation of methylated sterols in the cell membrane and arrest of cell growth. Because fluconazole is a fungistatic drug, treatment with fluconazole provides the opportunity for acquired resistance to develop. Almost all cases of fluconazole-resistant *C. albicans* are seen in patients with previous and extensive fluconazole therapy. Resistance can come about via several different pathways. In *C. albicans*, resistance has been attributed to overexpression of *erg11*, point mutations in *erg11* which lead to an altered protein structure and prevent azole binding, development of bypass pathways within sterol biosynthesis, and increased efflux of the azoles.[15]

Species-specific *Candida* interpretive criteria were published in 2012 by the Clinical and Laboratory Standards Institute (CLSI) in the M27-S4 document supplement.[16] The data from the M27-S4 is now compiled along with disk breakpoints in the M60 standard, the document laboratories should use when evaluating *Candida* susceptibility testing.[17] A comparison between the current and old (CLSI M27-S3) fungal breakpoints for *C. albicans* is listed in Table 7. Notably, not all *Candida* spp. are represented in the M27-S4/M60 document, nor are there breakpoints for all antifungal agents that may warrant testing. In October 2018, FDA recognized many of the M60 *Candida* spp. breakpoints on the Susceptibility Test Interpretive Criteria website (Table 7), including those for fluconazole, voriconazole, anidulafungin, caspofungin and micafungin. The only exception to the CLSI breakpoints is the FDA does not currently recognize the “susceptible-dose-dependent” (SDD) interpretive category, which CLSI applies to fluconazole breakpoints (Table 7). Rather, FDA applies the “intermediate” category to isolates with MICs that fall within the SDD range (Table 7). Recognition of the CLSI breakpoints by FDA now allows commercial antifungal susceptibility testing manufacturers, such as Vitek 2 (bioMerieux) or Sensititre (Thermofisher), to obtain clearance for *Candida* MICs with the current CLSI interpretations. Laboratories should confirm with their manufacturers the breakpoints applied by the test system in use, as well as the timelines by which the manufacturer will update to current CLSI/FDA breakpoints. Of note, the M27-S3 breakpoints are now obsolete, and laboratories should endeavor to update to current breakpoints as soon as possible. CLSI and FDA breakpoints for fluconazole are <=2 mcg/mL (susceptible), 4 mcg/mL (intermediate) and

>=8 mcg/mL (resistant). Laboratories performing testing for fluconazole where the lowest measurable fluconazole concentration is 8 mcg/mL should be aware that a result of <= 8 mcg/mL could indicate susceptible, intermediate or resistant and presents a patient safety issue. Similarly, laboratories that report a result of <=8 mcg/mL as susceptible should be aware that this practice may result in very major errors (i.e., false susceptibility).

In addition to clinical breakpoints, the CLSI recently published epidemiological cut-off values (ECVs) for several *Candida* species, in the M59 document.[18] These are listed in Table 8 for *C. albicans*. The meaning of ECVs, in particular when a clinical breakpoint exists such as for micafungin and anidulafungin, is confusing to many. Laboratories must keep in mind that the most reliable tool for predicting whether an antifungal agent will be active in a clinical infection is the clinical breakpoint. Breakpoints are developed using pharmacokinetic/ pharmacodynamics analysis (ie, analysis of how the drug is dosed, distributed in the body, eliminated and how the drug affects the organism at the site of infection), clinical trial outcome data and MIC distributions. In contrast, MIC distribution data alone are used to establish ECVs. ECVs define the upper limit of the wild-type (WT) MIC distribution – ie, the highest "normal" MIC for a given species. Thus, ECVs can be useful to distinguish between WT isolates (those with no acquired or mutational resistance mechanisms) and non-wild-type (NWT) isolates. ECVs are primarily used by public health and reference laboratories to herald and track the emergence of resistance mechanisms in a given species to a given antifungal agent, by routine MIC susceptibility testing. The CLSI has published M57,[19] which is a companion document to M59. M57 explains the rationale for ECVs as well as provides some suggestions on how to discuss and report ECVs to clinicians. For example, if a laboratory is requested to test amphotericin B against *C. albicans*, the ECV could be used in the absence of an amphotericin B *C. albicans* breakpoint. A report comment could indicate:[19]

C. albicans amphotericin B MIC	Example Comment Adapted from CLSI M59
2 µg/mL	There are currently no breakpoints or interpretive criteria for C. *albicans* and amphotericin B. The Amphotericin B MIC is below the WT cutoff, which suggests the isolate does not have any acquired mechanism of resistance. The clinical implication of an isolate with an MIC below the WT cutoff is currently unknown. Infectious diseases consultation strongly advised.
4 µg/mL	There are currently no breakpoints or interpretive criteria for *C. albicans* and amphotericin B. The Amphotericin B MIC is above the WT cutoff, which suggests the isolate may have an acquired mechanism of resistance. The clinical implication of an isolate with an MIC above the WT cutoff is currently unknown. Infectious diseases consultation strongly advised.

Table 7. Comparison of FDA and Current versus Old CLSI Interpretive Guidelines for *Candida albicans*

Antifungal Agents	Current M60 CLSI Interpretive Guidelines (MIC µg/mL)				Old M27-S3 CLSI Interpretive Guidelines (MIC µg/mL) – **Now Outdated**					Current FDA Breakpoints (MIC µg/mL)					EUCAST MIC (mg/L)	
	S	**SDD**	**I**	**R**	**S**	**SDD**	**I**	**R**	**NS**	**S**	**SDD**	**I**	**R**	**NS**	**S**	**R**
Fluconazole	≤2	4	---	≥8	≤8	16-32	---	≥64	---	≤2	---	4	≥8	--	≤2	>4
Itraconazole*	No breakpoint recognized				≤0.12	0.25-0.5	---	≥1		≤0.12	---	0.25-0.5	≥1	--	0.06	>0.06
Posaconazole	No breakpoint recognized				No breakpoint recognized					No breakpoint recognized					≤0.06	>0.06
Voriconazole	≤0.12	---	0.25-0.5	≥1	≤1	2	---	≥4	---	Recognizes CLSI M60					≤0.06	>0.06
Anidulafungin	≤0.25	---	0.5	≥1	≤2	---	---	---	≥4	Recognizes CLSI M60					≤0.03	>0.03
Caspofungin	≤0.25	---	0.5	≥1	≤2	---	---	---	≥4	Recognizes CLSI M60, except disk breakpoints					No breakpoint recognized**	
Micafungin	≤0.25	---	0.5	≥1	≤2	---	---	---	≥4	Recognizes CLSI M60					≤0.016	>0.016
Flucytosine	No breakpoint recognized				≤4	---	8-16	≥32	---	Recognizes CLSI M27-S3					No breakpoint recognized	
Amphotericin B	No breakpoint recognized				No breakpoint recognized					No breakpoint recognized					≤1	>1

S= susceptible; SDD = susceptible-dose dependent; I = intermediate; R = resistant; NS = non-susceptible.
*FDA itraconazole breakpoint is for the oral solution, not oral capsules
** EUCAST has not recognized caspofungin breakpoints due to inter-laboratory variation in MIC ranges. Laboratories instructed to test anidulafungin and micafungin.

Table 8. Epidemiological Cutoff Values for In Vitro Susceptibility Testing of *Candida albicans*

Antifungal Agent	M59-ED1 Epidemiological Cutoff Values (MIC µg/mL)	
	WT	NWT
Amphotericin B	≤2	≥4
Anidulafungin	≤0.12	≥0.25
Micafungin	≤0.03	≥0.06

WT= wild-type; NWT= not wild-type

Listed below are some of the changes to CLSI breakpoints and ECVs for *Candida* spp.:

1. Not all *Candida* species share the same breakpoints for a drug, as they did historically. For example, in the current M60, the S/SDD/R interpretive guidelines for *C. albicans* for voriconazole are ≤0.12/0.25-0.5/≥1. However, the S/SDD/R interpretive guidelines for *C. krusei* for voriconazole are ≤0.5/1/≥2.
2. Not all antifungal agents are included in the current guidelines. For instance, flucytosine and itraconazole are not included in M60.
3. Not all *Candida* species are included in the current guidelines. For instance, there are no guidelines for interpretation of MICs for *C. guillermondii*.
4. ECVs may be found in the M59 document, for some *Candida* spp. and antifungal agents not listed in M60. However, if a breakpoint is found in M60 for the *Candida* spp. under investigation, it should be used to interpret MICs, and not the ECV.

If a laboratory is following the current M60 document, the laboratorians need to determine with their Infectious Disease and other healthcare providers how to report susceptibilities for organisms and antifungal agents for

which there are no interpretive breakpoints. Options for reporting in such instances may include: a) reporting a drug MIC without an interpretation, b) suggesting a clinical consult with the Infectious Disease team or a Clinical Microbiologist if treatment for this organism is warranted; c) reporting using an ECV, if available. Also, given the decrease in the azole MIC breakpoints, more isolates will test SDD or resistant if the new breakpoints are used, so clinicians need to be made aware of this change. From an epidemiological perspective, it is important to be aware of these new breakpoints as they will undoubtedly have an impact on antibiograms generated for the *Candida* spp.

Key points

- Azole resistance in *Candida albicans* is rare.
- The CLSI M60 breakpoints are species specific, but not all *Candida* species are covered in the document.
- FDA has now recognized most M60 breakpoints, meaning the historical M27-S3 breakpoints are no longer acceptable for testing.
- Laboratorians need to determine with their Infectious Disease and other healthcare providers how to report susceptibilities for organisms and antifungal agents for which there are no interpretive breakpoints.

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Specimen F-08

The F-08 challenge was a simulated sinus specimen from a 36-year-old female with a history of chronic sinusitis. Participants were asked to determine the presence or absence of any fungus or aerobic Actinomycetes and identify any fungus or aerobic Actinomycetes present. The challenge contained *Curvularia* sp. A response of *Curvularia* sp, Dematiaceous mold, or Mold recognized, sent to reference lab for identification was considered satisfactory.

Table 1. Summary of Participant Responses

F-08

Identification	Referees (71) No.	%	Participants (1008) No.	%
Curvularia sp.	54	77.1	793	78.7
Dematiaceous mold	2	2.9	16	1.6
Mold recognized, sent to reference lab for identification	13	18.6	182	18.1
Bipolaris sp.	2	2.9	14	1.4

Table 2. Results by Method

System	No. Labs	% of Laboratory Designation *Curvularia* sp.
Mass Spectrometry/Bruker MALDI	10	100.0
Morphology and Bruker MALDI	27	96.3
Morphology and Vitek MS MALDI	26	96.2
Morphology and sequencing	18	100.0
Morphologic exam/biochemical	831	80.3
Other[a]	50	64.0

[a] Includes other commercial kits and methods with <10 users.

Discussion

Taxonomy

Curvularia spp. are dematiaceous (brown pigmented) fungi commonly found as an environmental mold in tropical and temperate climates. Over 80 species of *Curvularia* have been characterized.[1] In general, *Curvularia* spp. may be identified based on typical growth and microscopic characteristics. Based on morphology, *C. lunata* has been the most commonly reported species associated with human disease. Not surprisingly, multiple studies indicate disagreement between morphologic and molecular methods of identification of *Curvularia* to species level. Definitive identification of *Curvularia* may require additional techniques such as PCR targeting ribosomal DNA (rDNA) of the 18S or 23S regions, or internal transcribed spacer regions (ITS).[2] Based on sequence analysis of a large collection of clinical isolates of *Curvularia* spp. collected over the last 5 years, specimens morphologically identified as *C. lunata* were actually re-classified as two separate species; *C. lunata* and *C. aeria,*[1] with *C. lunata* most likely a species complex. A recent study which combined phylogeny data from four individual genetic loci (ITS, large subunit rDNA, II, *gpd*) revealed that *Curvularia* sp. could be additionally categorized into six clades, each with specific morphologic features; *C. lunata, americana, eragrostidis, spicifera, trifolii* and *hominis*.[3]

Identification

The challenge mold grew rapidly (4-8 days) on Sabouraud dextrose agar (SDA), BHI agar, or Inhibitory Mold agar at 30° C. No growth was detected on Mycosel agar. Wooly pink, orange-to-brown colonies were noted, with a dark reverse (Figure 1A-1B). Upon microscopic examination, transversely multiseptate, clavate, smooth-walled conidia containing four cells were noted. Uniquely curved conidia are the result of an asymmetrically swollen intermediate cell within the conidia. Hyphae are septate with a brown pigment, distinctive for dematiaceous molds (Figure 2). Germ tubes are not formed.[4] Production of conidia from the hyphae in a sympodial fashion (geniculate conidiophores) is often observed, but not specific to *Curvularia* spp.[1,4] The characterization of *Curvularia* sp. as euseptate vs. distoseptate is a matter of some controversy. Euseptate refers to a single cell wall, with septations formed as inward expansions of the wall. Distoseptate is used to describe a common outer wall containing multiple cells, each surrounded by an individual inner cell wall. Historically, *Curvularia* spp. have often been described as euseptate, and this characteristic has often been used to differentiate *Curvularia* sp. from *Bipolaris* sp. However, recent studies indicate the presence of distoseptate conidia in multiple species of *Curvularia*. Two cell wall layers may be discernable in young conidia, but become more difficult to visualize in older cultures (thus appearing euseptate). Morphologic features such as conidial curvature, a protruding single walled hilum delimited by a septum and conidial size are now considered to be of more use in the differentiation of *Curvularia* spp. from *Bipolaris* sp. than the type of conidial septation.[3]

Conidia of *Curvularia* spp. may also be confused with *Drechslera* or *Exserohilum*. However, these other genera may be differentiated from *Curvularia* sp. based on careful review of macroscopic colony morphology and pigment, number and type of septations within conidia, and formation of germ tubes.

Clinical Significance

Curvularia lunata has been implicated as a causative agent in a wide spectrum of human infections, ranging from skin and soft tissue mycetoma,[5] non-invasive allergic fungal rhinosinusitis[6], to cerebral phaeohyphomycosis.[7] Darkly pigmented, septate fungal hyphae may be noted in biopsy specimens and aspirates upon staining with GMS, PAS, Giemsa and Fontana-Masson, but negative by hematoxylin and eosin stains. Tissue necrosis and granulomatous inflammation may be significant in tissue specimens.[7-9] Although immunosuppression appears to play a role in systemic dissemination of *C. lunata*,[8,9] localized CNS, skin, sinus, and ocular infections have been reported in immunocompetent hosts.[2,5,7] Invasive pulmonary infections and fungal pneumonia with consolidation have been reported.[10] Due to the abundance of *Curvularia* sp. in the environment, recovery of this mold in respiratory specimens is not uncommon, and differentiation of contamination vs true pathogenicity may be a challenge.

Antimicrobial Resistance and Therapy Considerations

Clinical cure of localized infections have been achieved in cases where itraconazole, or voriconazole have been used. Surgical debridement may also play an important role in clearance of infection.[2,5,6] Amphotericin B has been used with variable success in cases of systemic dissemination of *C. lunata*.[9] Although CLSI breakpoints do not specifically address antifungal susceptibility of *C. lunata*, *in vitro* testing of 10 unique clinical isolates using a broth microdilution method (CLSI M-38-A2)[11] demonstrated a 90% MIC of ≤ 1 µg/mL to amphotericin B, caspofungin, micafungin, itraconazole, voriconazole, and posaconazole. A 90% MIC of ≥ 8 µg/mL was noted with anidulafungin, and fluconazole; 5-flucytosine was ineffective.[1]

Key Points[4,5]

- *Curvularia lunata* has been the most commonly reported species of *Curvularia* associated with human disease, but may actually be composed of 2 species; *C. lunata* species complex and *C. aeria.*
- *Curvularia* spp. are rapidly growing (4-8 days) dematiaceous molds demonstrating smooth, clavate, and characteristically curved conidia.

- *Curvularia lunata* species complex has been reported in a wide variety of human infections, both localized and systemic. Immunosuppression may play a role in systemic infections, but not necessarily localized infections.
- Successful treatment of infection due to *C. lunata* species complex has been reported with use of itraconazole and voriconazole. Reports of clinical cure using amphotericin B in cases of systemic disseminated of *Curvularia* spp. have been variable.

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Specimen F-09

The F-09 challenge was a simulated corneal scraping specimen from a 46-year-old female horticulturist. Participants were asked to determine the presence or absence of any fungus or aerobic Actinomycetes and identify any fungus or aerobic Actinomycetes present. The challenge contained *Fusarium* sp. and *Staphylococcus epidermidis* as a contaminant. A response of *Fusarium* sp., or Mold recognized, sent to reference lab for identification was considered satisfactory.

Table 1. Summary of Participant Responses

	Identification	Referees (71) No.	%	Participants (1008) No.	%
F-09	*Fusarium* sp.	53	75.7	812	80.6
	Mold recognized, sent to reference lab for identification	17	24.3	182	18.1
	Acremonium sp.	1	1.4	9	0.9

Table 2. Results by Method

System	No. Labs	% of Laboratory Designation *Fusarium sp.*
Mass Spectrometry/Vitek MS MALDI	14	100.0
Morphology and Bruker MALDI	33	93.9
Morphology and Vitek MS MALDI	36	97.2
Morphology and sequencing	18	100.0
Morphologic exam/biochemical	811	81.8
Other[a]	50	68.0

[a] Includes other commercial kits and methods with <10 users.

Discussion

Taxonomy

Fusarium spp. are common soil saprobes and plant pathogens. *Fusarium* is a large genus with over 70 well described species. Clinical species typically demonstrate only the anamorph on common fungal media. The members of the *Fusarium solani* complex (*F. solani, F. falciforme*, *F. lichenicola*, and *Neocosmospora vasinfecta*) are most commonly associated with human disease, although *F. oxysporum*, *F. verticillionides,* and *F. dimerum* groups are also frequent.[1] *Fusarium* have teleomorphs in *Gibberella*, *Neocosmospora*, *Nectria* and other genera. Their taxonomy is complex, with species recently reassigned from *Acremonium*, *Cylindrocarpon* and others.[2] O'Donnell *et al.* have provided a validated online molecular taxonomic resource for the genus *Fusarium*.[3]

Identification

Fusarium spp. grow rapidly, typically within 3-5 days on standard fungal media with the exception of media containing cycloheximide. Colonies are white and fluffy, developing pink-lavender to salmon shades when mature (Figure 3). The colony reverse can be pale to darkly colored. Morphology is media dependent. Hyphae are hyaline and septate, and conidiophores are monophialides, i.e., phialides have a single pore from which conidia are released. *Fusarium* usually demonstrates two types of conidia: microconidia (Figure 4A), usually 2-4 µm x 4-8 µm oval with 1-2 septa, and cells born singly or in small clusters; and macroconidia, usually 3-6 µm wide x 30-60 µm long with 3 to 5 septa. *Fusarium* have annelloconidiation, and thus, a hilar scar is typically visible on macroconidia, although the scar can be seen on microconidia and conidiophores as well. This hilar scar, best visualized under oil,

helps differentiate *Fusarium* from *Acremonium* and *Cylindrocarpon* species. Macroconidia may become more sickle-shaped with age, and characteristically have an acutely bent foot cell or “bent-knee morphology” and a “beaked” terminal cell (Figure 4B). The different species complexes of *Fusarium* can be described by their morphologic features. However, species and complex level identification based on morphology is difficult, and usually requires highly specialized media such as carnation leaf agar or high phosphate water agar.[1]

Use of ribosomal ITS region and D1 and D2 domains of the 28S ribosomal large subunit for sequence analysis is insufficient for identification to species level, thus alternative gene targets such as EF-1α are recommended for this purpose.[4] MALDI-TOF analysis has been able to identify some *Fusarium* species to at least the complex level.[5] At this time, certain filamentous fungi including *Fusarium* are included in the Vitek MS (v3.2) but not the Bruker Biotyper FDA-cleared database.

Table 3. Abbreviated summary of representative species from the major *Fusarium* complexes

	Colony	**Conidiophore**	**Macroconidia**	**Microconidia**	**Chlamydospores**
F. solani	Rapid Cream, lavender-to-blue green	Long Monophialides	Abundant Stubby thick-walled Curved	Abundant 0-1 Septa Kidney shaped	Present Single or pairs
F. oxysporum	Rapid Lavender, orange-salmon	Short Monophialides	Moderately abundant Delicate walls Curved	Abundant 0 septa	Abundant
F. verticillioides	Rapid Lavender	Medium length Monophialides	Low abundance Straight-walled	Abundant 0-1 Septa	Absent
F. dimerum	Slow = 8 days Yeast-like Orange-red Slimy	Short peg-like Monophialides	Abundant 0-1 Septa Curved	Low abundance Ellipsoidal-to-curved Usually one celled	Modified macroconidia Rare hyphal chlamydospores

Clinical Significance

Fusarium infections have a spectrum of clinical presentations. In immunocompetent hosts, usual infections include sinusitis, keratitis following trauma or contamination of contact lens solutions, onychomycosis of subungual tissue and nail beds, tinea pedis, and intertrigo. More severe infections are seen in burns, decubitus ulcers, and septic arthritis associated with steroid injections. Invasive fungal infection occurs in immunocompromised patients, particularly in acute myeloid leukemia and hematopoietic stem cell transplant patients with prolonged neutropenia. Invasive fusariosis is somewhat less common than invasive aspergillosis or mucormycosis, but mortality exceeds 75%. Sinusitis and onychomycosis are frequent precursor lesions in patients with hematological malignancies; however, the majority of infections present *de novo* as a nodular pneumonia.[6,7] Skin lesions are common in disseminated fusariosis. Interestingly, the fusaria are one of the few hyaline molds that produce a yeast form (blastospore) in addition to hyphae in tissue. They are also angioinvasive. Thus, *Fusarium* infections can rapidly disseminate and are frequently first identified in blood cultures (either fungal or routine aerobic blood culture bottles), in which the organism can initially appear as a yeast. There are reports of some clinical samples cross-reacting in the BioRad *Aspergillus* galactomannan assay.[8]

Fusarium spp. are also associated with a wide range of toxin related syndromes; the most well-known is Alimentary Toxic Aleukia due to trichothecene mycotoxin T2, which killed over 1 million Russians following consumption of wheat that had lain under winter snows, in the 1940s. More than 200 toxins have been identified in *Fusarium* species.[9] The relationship of toxin production to clinical infectivity and pathogenicity is unknown.[10]

Fusarium can be frequently found on fresh fruits and vegetables.[11] Outdoor and indoor air appears to be the dominant source of the mold spores; however, hospital water supplies and the presence of live plants with soil may also pose a hazard for immunosuppressed patients.[12]

Resistance and Therapy

Fusarium is one of the most resistant fungi to modern antifungal agents. *Fusarium solani* is the most resistant species complex within the genus, as it is intrinsically resistant to echinocandins, is frequently resistant to the azoles, and has variable MICs for amphotericin B.[13] There is no current standardized therapeutic regimen. Liposomal amphotericin B remains a mainstay of therapy and has been shown to reduce fungal burden in mice while amphotericin deoxycholate has not. Posaconazole also has activity and has been effective clinically in approximately 50% of cases in the highly challenging salvage setting.[14]

Key points

- The hilar scar helps differentiate *Fusarium* spp. from morphologically similar genera but species level identification is difficult with currently available methods. Species level identification and susceptibility testing should be considered in invasive infections.
- *Fusarium* spp. can produce a yeast form in tissue. Blood cultures are frequently positive in disseminated infection.
- *Fusarium* is resistant to multiple antifungal agents and disseminated infections are highly fatal.

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Specimen F-10

The F-10 challenge was a simulated skin biopsy from a 30-year-old male with fever and necrotic skin lesions after bone marrow transplant. Participants were asked to determine the presence or absence of any fungus or aerobic Actinomycetes and identify any fungus or aerobic Actinomycetes present. The challenge contained *Lomentospora prolificans* and *Staphylococcus epidermidis* as a contaminant. A response of *Lomentospora prolificans, Lomentospora* sp., *Scedosporium* sp., Dematiaceous mold, or Mold recognized, sent to reference lab for identification was considered satisfactory.

Table 1. Summary of Participant Responses

F-10

Identification	Referees (70) No.	%	Participants (1005) No.	%
Lomentospora prolificans	29	41.4	314	31.2
Lomentospora sp.	1	1.4	26	2.6
Scedosporium sp.	15	21.4	308	30.6
Dematiaceous mold	5	7.1	43	4.3
Mold recognized, sent to reference lab for identification	20	28.6	262	26.1
Scedosporium apiospermum complex	1	1.4	27	2.7

Table 2. Results by Method

		% of Laboratory Designation		
System	No. Labs	*Lomentospora prolificans*	*Lomentospora* sp.	*Scedosporium* sp.
Mass Spectrometry/Vitek MS MALDI	19	73.7	-	26.3
Morphology and Bruker MALDI	35	82.9	5.7	5.7
Morphology and Vitek MS MALDI	36	69.4	-	30.6
Morphologic exam/biochemical	800	27.1	2.8	33.4
Morphology and sequencing	21	61.9	-	33.3
Other[a]	48	22.9	2.1	22.9

[a] Includes other commercial kits and methods with <10 users.

Discussion

Taxonomy

The taxonomy of the genus *Scedosporium* has undergone revision in the last decade due to emerging genetic sequencing data. The genus *Scedosporium* was first described in 1911 in a patient with mycetoma. This isolate only developed in the asexual state (anamorph) and was eventually named *Scedosporium apiospermum*. The fungus in the sexual phase (teleomorph) was reported in 1922 as *Allescheria boydii*. In 1944, the name was changed to *Petriellidium boydii* and in 1970 to *Pseudallescheria boydii*.[1] Today, the genus *Scedosporium* contains 10 species: *S. aurantiacum*, *S. minutisporum*, *S. desertorum*, *S. cereisporum*, *S. dehoogii*, and the *S. apiospermum* complex (*S. boydii*, *S. ellipsoideum*, *S. apiospermum*, S. angustum*, S. fusoideum*).[2]

In 1984, *Scedosporium inflatum* was described in a bone biopsy from an immunocompetent child with osteomyelitis. In its anamorphic state, *Scedosporium inflatum* had distinctive swollen, flask-shaped conidiophores.

In 1994, sequencing of the ribosomal DNA internal transcribed spacers (ITS) of *S. inflatum* and *Lomentospora prolificans* resulted in a merging of the two organisms under the name *Scedosporium prolificans*. Subsequently, it was shown that *Lomentospora prolificans* is genetically unrelated to the genus *Scedosporium* and the genus *Lomentospora* was reinstated for this species.[2,3]

Identification

Colonies of *L. prolificans,* a dematiaceous mold, are initially flat, white, and suede-like and become brown, olive-grey to black over time with a dark reverse (Figure 5). *Lomentospora prolificans* grows well on standard mycology media (SAB, BHI) but is inhibited by cyclohexamide. This mold typically grows within 5 days at 25° C.[4] Unlike many other molds *L. prolificans* is readily recovered from automated blood culture systems in patients with disseminated fungemia.[5] Microscopically, hyphae are septate and conidiophores have a characteristic flask-shaped appearance with a swollen base and a thin, elongated neck (Figure 6). Conidia are arranged in small clusters at the apex and are smooth, olive-brown colored, and one-celled.[4]

Clinical Significance

Lomentospora prolificans and members of the genus *Scedosporium* are ubiquitous in the environment. They are readily found in soil and water.[5] Direct exposure to the fungus through penetrating injuries may result in skin and soft tissue infections, ocular infections which may progress to invasive disease.[6] *Lomentospora prolificans* has been associated with disseminated infections in immunocompromised patients. In a large meta-analysis, hematological malignancies and neutropenia were identified as risk factors for disseminated *L. prolificans* disease. In a recent ten-year case review at a single transplant center, 6-month mortality was reported to be 55%.[7]

Therapy

There are no published interpretive breakpoints for *Lomentospora prolificans* or *Scedosporium* species, but *L. prolificans* is largely considered pan-antifungal resistant.[8] In invasive infections, European guidelines recommend the use of voriconazole with surgical debridement when possible. Combination antifungal therapy has also been described although this strategy remains investigational.[2,8]

Key Points

- The taxonomic classification of *Scedosporium prolificans* has undergone change. This organism is now under a new genus and is called *Lomentospora prolificans*.
- *Lomentospora prolificans* is readily recovered from automated blood culture systems in patients with disseminated fungemia.
- Patients with neutropenia and hematological malignancies are at highest risk of disseminated *Lomentospora prolificans*.
- When grown in culture, microscopic features include septate hyphae and conidiophores with a characteristic flask-shaped appearance with a swollen base and a thin, elongated neck.

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Specimen F-11

The F-11 challenge was a simulated lung biopsy from a 50-year-old male with acute myeloid leukemia and cavitating lung disease. Participants were asked to determine the presence or absence of any fungus or aerobic Actinomycetes and identify any fungus or aerobic Actinomycetes present. The challenge contained *Mucor* sp. and *Pseudomonas aeruginosa* as a contaminant. A response of *Mucor* sp., Mucorales (Zygomycete), presumptive I.D., or Mold recognized, sent to reference lab for identification was considered satisfactory.

Table 1. Summary of Participant Responses

F-11

Identification	Referees (70) No.	%	Participants (1007) No.	%
Mucor sp.	29	41.4	470	46.7
Mucorales (Zygomycete), presumptive I.D.	14	20.0	227	22.5
Mold recognized, sent to reference lab for identification	25	35.7	253	25.1
Rhizomucor sp.	1	1.4	10	1.0
Rhizopus sp.	2	2.9	12	1.2

Table 2. Results by Method

System	No. Labs	% of Laboratory Designation *Mucor* sp.
Morphology and Bruker MALDI	38	89.5
Morphology and Vitek MS MALDI	30	86.7
Morphologic exam/biochemical	812	43.7
Morphology and sequencing	20	95.0
Other[a]	61	42.6

[a] Includes other commercial kits and methods with <10 users.

Discussion

Mucor spp. are ubiquitous fungi found in soil and on decaying plant material, and can cause opportunistic disease in immunocompromised individuals including diabetic patients, patients with hemochromatosis, trauma patients, as well as patients with malignancy and neutropenia.[1]

Taxonomy

Fungi in the class Zygomycetes, order Mucorales, family Mucoraceae contain several genera with pathogenic potential; *Mucor*, *Rhizomucor*, *Rhizopus*, *Apophysomyces*, and *Lichtheimia (Absidia).*[1] The genus *Mucor*, currently encompass over 250 species with multiple varieties and subspecies. The number and classification of species are in constant flux, based on results of molecular sequencing studies of rRNA and internal transcribed spacer (ITS) sequences.[2] There are five species of *Mucor,* which are currently considered to be human pathogens, and have been reported in clinical infections: *M. ramosissimus, M. racemosus, M. hiemalis, M. indicus, and M. circinelloides*. Of these, *M. circinelloides* was reported to be most frequently associated with human disease.[3]

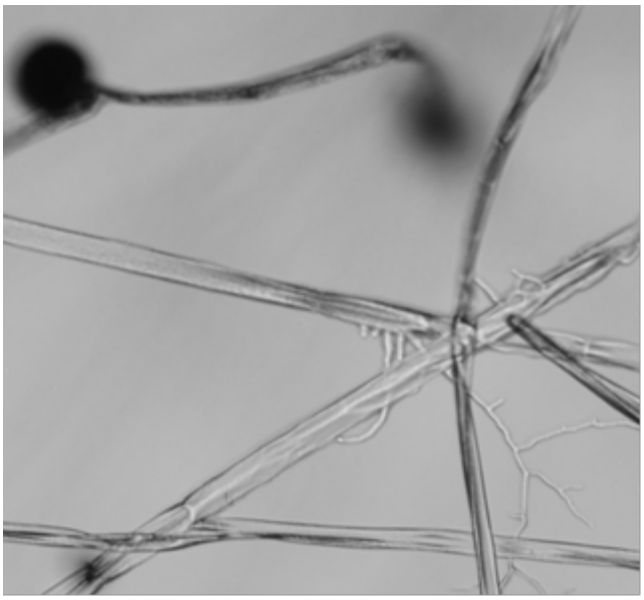
Identification

Mucor spp. grow rapidly and usually mature by four days. These hyaline molds are initially white-to-brown, becoming tan, brown, gray, or gray/brown with sporulation (Figure 7). They have a cottony or woolly texture and may be two or more centimeters high by day five. Due to the rapid growth of these molds, it is strongly recommended that plate cultures be taped shut or sealed during incubation. *Mucor circinelloides* grows poorly at temperatures >35˚C, which may be a useful method of differentiating this species from other morphologically similar heat-tolerant zygomycetes such as *Rhizomucor* spp.[1]

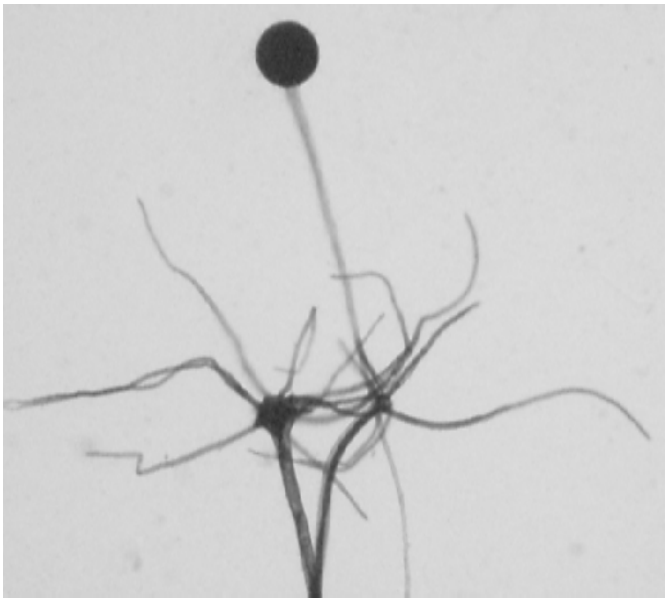
On a macroscopic level, it is not possible to differentiate *Mucor* spp. from *Rhizopus* or *Rhizomucor* spp. as all three genera produce similar floccose mycelia with overlapping color on standard fungal culture media. *Mucor* spp. are generally not identified to the species level in most clinical laboratories due to the difficulties in microscopic differentiation between species. However, various molecular methods, including MALDI-TOF, are becoming more readily available in the clinical laboratory which will allow species-level identification of *Mucor*.[4]

Microscopically, *Mucor* spp. may be generally differentiated from *Rhizopus* or *Rhizomucor* species based on lack of rhizoids and complete absence of an apophysis on the sporangiophore (Figure 8A).[1] *Mucor* spp. appear similar to other Mucoraceae in that they have broad hyphae, and have no or rare septations (pauciseptate) which can be noted in both culture specimens, and histopathologic sections of infected tissues. Fungal hyphae may also be coenocytic, in that multiple nuclei within cells are noted.[1] On fungal culture media, individual sporangia are large (50 to 300 µm), and usually demonstrate columellae which may be globose, obovoid, or ellipsoidal (Figure 8B). Most *Mucor* spp. do not develop rhizoids; however, *M. circinelloides* can occasionally show rare thread-like structures or rootlets. They are thin and very infrequent in any one culture but this rare phenomenon can lead to misidentification. (See Image 1a and 1b) Note, the *M. circinelloides* photo 1a is taken at a higher magnification. In contrast *Rhizopus* spp. should have frequent rhizoids, *Rhizomucor* consistently has rhizoids, although a thorough search may be necessary. Indeed, *Rhizomucor* spp. are frequently misidentified as *Mucor*, emphasizing the need to check other morphologic features such as the apophysis prior to identification. Tall sporangiophores of *M. circinelloides*, may branch sympodially with both long and short branches. Short sporangiophores may form circular branching patterns (circinate), which is a unique and characteristic feature of this species. Sporangiospores for *M. circinelloides* are round to ellipsoidal. Thick-walled chlamydospores may be noted in older cultures.[5]

Image Comparison of hair-like structure to true rhizoids.



1a. The rare thread-like structures of *M. circinelloides*



1b. True rhizoids seen in *Rhizopus* spp.

Clinical Significance

Mucor spp. have been implicated in a wide variety of clinical manifestations such as invasive rhino-orbital, rhinocerebral, pulmonary, cutaneous, gastrointestinal, and hematogenous infections with systemic dissemination. Disease is generally associated with hosts with underlying diabetic ketoacidosis, have penetrating trauma, or are immunocompromised.[3] Mortality rates in disseminated infections are significant, and may exceed >80% in rhinocerebral disease, even with treatment.[4] Interestingly, a 2013 outbreak of more than 300 cases of gastrointestinal illness consisting of nausea, vomiting and diarrhea was associated with ingestion of yogurt contaminated with *M. circinelloides.* Infections were self-limiting, with no reports of disseminated disease or deaths reported in association with this incident.[6]

Therapy

A high level of clinical suspicion in susceptible hosts, with rapid diagnosis and identification of Mucoraceae, are key in management of mucormycosis. Treatment of infections due to *Mucor* spp. remains challenging, with surgical debridement, and administration of lipid formulations of Amphotericin B being the primary approaches to therapy. Azoles such as fluconazole, voriconazole and itraconazole demonstrate limited to no therapeutic effect and are not recommended. Echinocandins demonstrate only modest activity, and only limited evidence for their use in combination with a lipid formulation of Amphotericin B exists. Posaconazole is a triazole agent with reported activity against Mucoraceae, which has been available for prophylaxis of neutropenic patients, and as a component of salvage therapy in refractory infections.[7,8]

Although species-specific MIC values or clinical breakpoints have not been developed for *Mucor* spp., epidemiologic cutoff values (ECV's), based on MIC distributions have recently been proposed for filamentous fungi with the most commonly used antifungal agents. Espinel-Ingroff *et al* reported calculated *M. circinelloides* ECV's (≥95%) of 1.0 µg/mL for Amphotericin B, and 4.0 µg/mL for posaconazole.[9] However, a recent case report of proven *M. circinelloides* with reduced susceptibility to posaconazole (>32 µg/mL) has been reported.[10]

Key Points[4,5]

- Of the five species of *Mucor* sp. considered to be human pathogens, *M. circinelloides* was reported to be most frequently associated with human disease.

- *Mucor* sp. grow rapidly and usually mature by four days. They are initially white-to-yellow, becoming tan, brown, gray or gray/brown with sporulation.
- *Mucor* sp. have broad hyphae, and may be pauciseptate. Short sporangiophores forming circular branching patterns is a characteristic finding in *M. circinelloides.*
- Rhino-orbital, rhinocerebral, pulmonary, cutaneous and hematogenous infections with dissemination are associated with *Mucor* sp. A food-borne outbreak of gastrointestinal disease has been reported due to *M. circinelloides.*
- Recent reports of *M. circinelloides* resistant to posaconazole is of concern, and may limit use of this agent in treatment of disease.

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CORRECTED

Specimen F-12

The F-12 challenge was a simulated cerebrospinal fluid specimen from an HIV patient with worsening headaches. Participants were asked to determine the presence, or absence, of cryptococcal antigen using the method(s) commonly employed by their laboratory. The challenge contained cryptococcal antigen. A response of “Positive” for cryptococcal antigen was considered satisfactory.

Table 1. Summary of Participant Responses

F-12 Identification	Participants (578) No.	%
Positive	575	99.5
Negative	3	0.5

Table 2. Results by Method

Method	No. Labs	Positive	Negative
Immy Latex	23	100.0	-
Immy LFA	346	100.0	-
Meridian Bioscience	114	99.1	0.9
Remel	74	97.3	2.7
Other[a]	13	100.0	-

[a] Includes other commercial kits and methods with <10 users.

Table 3. Titers

Method	No. Labs	Undiluted	1:2	1:4	1:5	1:8	1:10	1:16	1:20	1:40	≥1:32	≥1:80
Immy Latex	21	-	-	-	-	-	-	-	-	-	17	4
Immy LFA	342	13	-	-	-	-	-	-	-	-	18	311
Meridian Bioscience	113	3	-	-	-	-	-	-	-	-	97	13
Remel	67	2	-	-	-	-	-	-	-	-	60	5
Other[a]	11	1	-	-	-	-	-	-	-	-	5	5

[a] Includes other commercial kits and methods with <10 users.

Discussion

As in previous cryptococcal antigen challenges, the participant performance in this challenge was excellent, with 99.5% of participants reporting the intended response. Should a participant’s result differ from the intended response, it is recommended that laboratories review their current procedures, especially related to the known causes of false-positive or false-negative results.

Actions Laboratories Should Take when a PT Result is Not Graded

The CAP uses exception reason codes that signify the proficiency testing (PT) for an analyte has not been graded. The exception reason code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all analytes with an exception reason code, review, and document the acceptability of performance as outlined below and retain documentation of review for at least 2 years. The actions laboratories should take include, but are not limited to:

Code	***Exception Reason Code Description***	***Action Required***
11	Unable to analyze	Document why the specimens were not analyzed (eg, instrument not functioning or reagents not available). Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
20	Response was not formally graded due to insufficient peer group data. Please see the participant summary for additional information.	Applies to a response that is not formally evaluated when a peer group is not established due to fewer than 10 laboratories reporting. Document that the laboratory performed a self-evaluation using the data presented in the participant summary and compared its results to a similar method, all method, all participant statistics, or data tables for groups of 3-9 laboratories, if provided. Perform and document the corrective action of any unacceptable results. If self-evaluation is not possible, it is up to the laboratory director/designee to determine an alternative performance assessment.
21	Specimen problem	Document that the laboratory has reviewed the proper statistics supplied in the participant summary. Perform and document alternative assessment for the period that commercial PT was not tested to the same level and extent that would have been tested. Credit is not awarded in these cases.
22	Result is outside the method/ instrument reportable range	Document the comparison of results to the proper statistics supplied in the participant summary. Verify detection limits. Perform and document the corrective action of any unacceptable results.
24	Incorrect response due to failure to provide a valid response code	Document the laboratory's self-evaluation against the proper statistics and evaluation criteria supplied in the participant summary. Perform and document the corrective action of any unacceptable results. Document corrective action to prevent future failures.
25	Inappropriate use of antimicrobial	Document the investigation of the results as if they were unacceptable and review the proper reference documents to gain knowledge of the reason your response is not appropriate.
26	Educational challenge	Review participant summary for comparative results and document performance accordingly. Evaluation criteria are not established for educational challenges. Laboratories should determine their own evaluation criteria approved by their laboratory director for self-evaluation. Response to the CAP is not required.
27,31	Lack of participant or referee consensus	Document that the laboratory performed a self-evaluation and compared its results to the intended response when provided in the participant summary. If comparison is not available, perform and document alternative assessment (ie, split samples) for the period that commercial PT reached non-consensus to the same level and extent that would have been tested.
28	Response qualified with a greater than or less than sign; unable to quantitate	Applies to a response that is not formally evaluated when a less than or greater than sign is reported. Document that the laboratory performed a self-evaluation and compared its results to the proper statistics supplied in the participant summary. Verify detection limits. Perform and document the corrective action of any unacceptable results.
30	Scientific committee decision	Applies to a response that is not penalized based on scientific committee decision. Document that the laboratory has reviewed the proper statistics supplied in the participant summary.

Actions Laboratories Should Take when a PT Result is Not Graded

The CAP uses exception reason codes that signify the proficiency testing (PT) for an analyte has not been graded. The exception reason code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all analytes with an exception reason code, review and document the acceptability of performance as outlined below and retain documentation of review for at least 2 years. The actions laboratories should take include but are not limited to:

Code	***Exception Reason Code Description***	***Action Required***
33	Specimen determined to be unsatisfactory after contacting the CAP	Document that the laboratory has contacted the CAP and no replacements specimens were available. Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
40 41	Results for this kit were not received. Results for this kit were received past the evaluation cut-off date.	Document why results were not received, corrective action to prevent recurrence and the laboratory's self-evaluation of the results by comparing results to the proper statistics and evaluation criteria supplied in the participant summary. If PT specimens were not analyzed, perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
42	No credit assigned due to absence of response	The participant summary indicates which tests are graded (see evaluation criteria) and which tests are not evaluated/educational. Updates to grading will also be noted. If a test is educational, the laboratory is not penalized for leaving a result(s) blank. If a test is graded (regulated and non-regulated analytes) and your laboratory performs that test, results cannot be left blank. The laboratory is required to submit results for **all** challenges within that test or use an appropriate exception code or indicate test not performed/not applicable/not indicated. Exceptions may be noted in the kit instructions and/or the result form. Document corrective actions to prevent future failures.
44	This drug is not included in our test menu. Use of this code counts as a correct response.	Verify that the drug is not tested on patient samples and document to ensure proper future reporting.
45	Antimicrobial agent is likely ineffective for this organism or site of infection	Document that the laboratory performed a self-evaluation of written protocols and practices for routine reporting of antimicrobial susceptibility reports to patient medical records. Document that routine reporting of this result to clinicians for patient care is compliant with specific recommendations of relevant medical staff and committees (eg, infectious diseases, pharmacy and therapeutics, infection control). Response to the CAP is not required.
77	Improper use of the exception code for this mailing	Document the identification of the correct code to use for future mailings.
91	There was an insufficient number of contributing challenges to establish a composite grade.	Document the investigation of the result as if it were an unacceptable result. Perform and document the corrective action if required.
35, 43, 46, 88, 92	Various codes	No action required.

NOTES

This concludes the report.

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The CAP wishes to thank Rosemary C. She, MD, FCAP; and Aida Mangahis, CLS for providing these photographs. Unless permission is received from Dr. She and Aida Mangahis, these photographs may not be used for any purpose except in connection with this Survey.

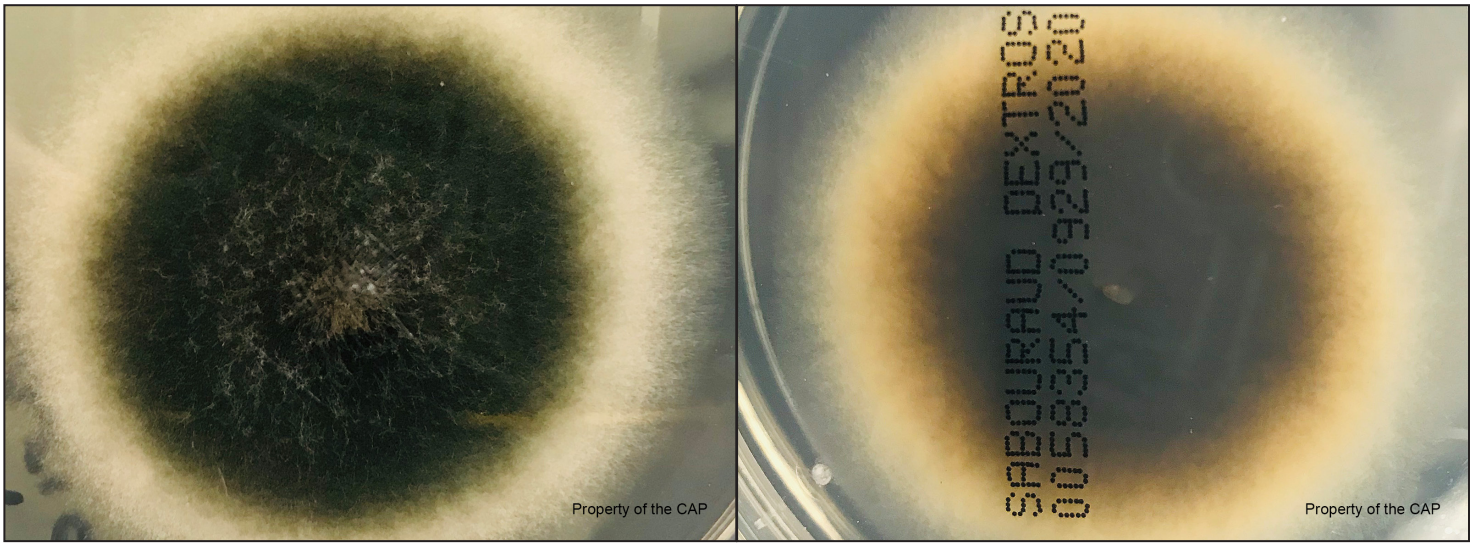

Figure 1A - 1B

Curvularia spp. demonstrates a wooly dark colony with a dark reverse on Sabouraud Dextrose agar.

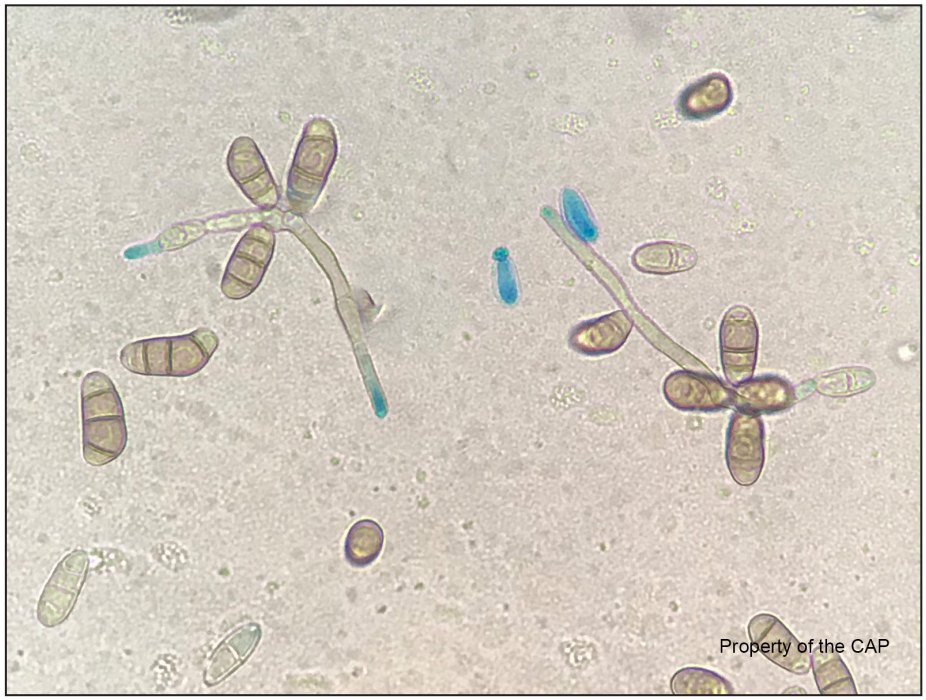

Figure 2

Transversely multiseptated, clavate, smooth-walled conidia with four cells are noted. The curvature of mature macroconidia results from an asymmetrically swollen intermediate cell.

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Figure 3

Fusarium sp. as white fluffy colonies turning pink-to-salmon on Sabouraud Dextrose agar at 6 days.

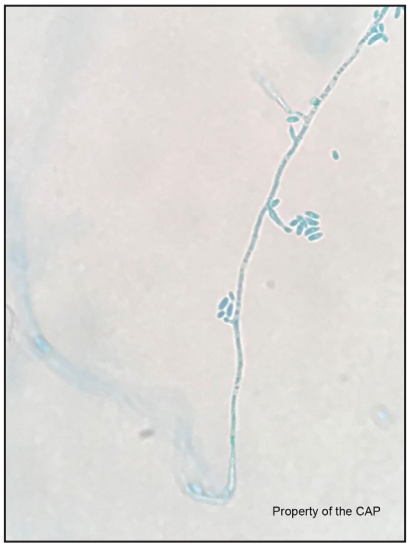

Figure 4A

Microscopic field demonstrating a phialide bearing a cluster of oval microcondia, typical of *Fusarium* sp.

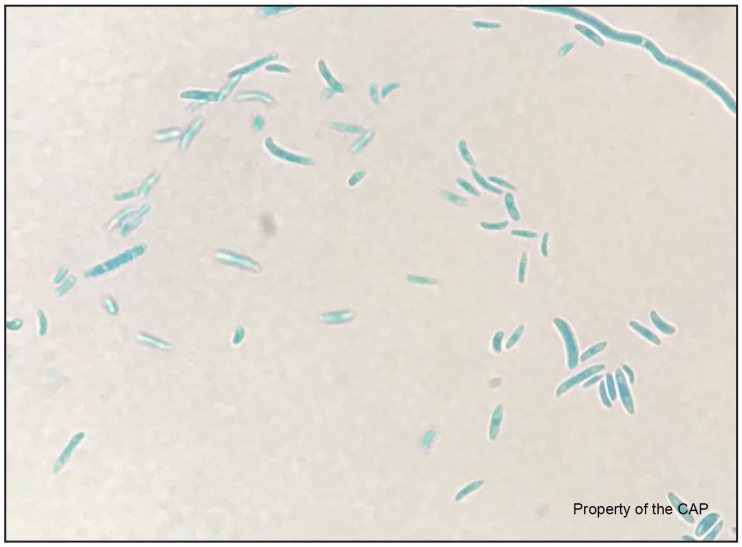

Figure 4B

Developing macroconidia elongate and become sickle-shaped with pointed ends over time.

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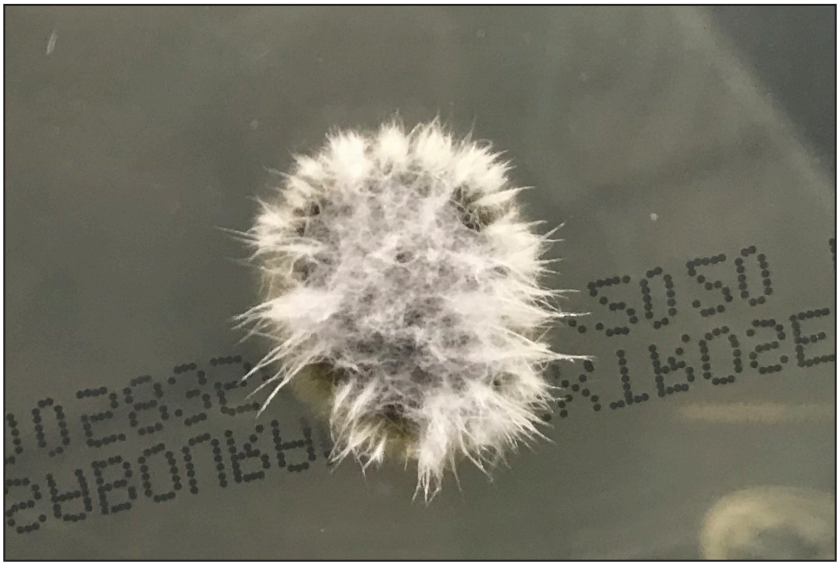

Figure 5

Lomentospora prolificans as a dematiaceous mold is initially white, turning brown-to-olive green with a dark reverse.

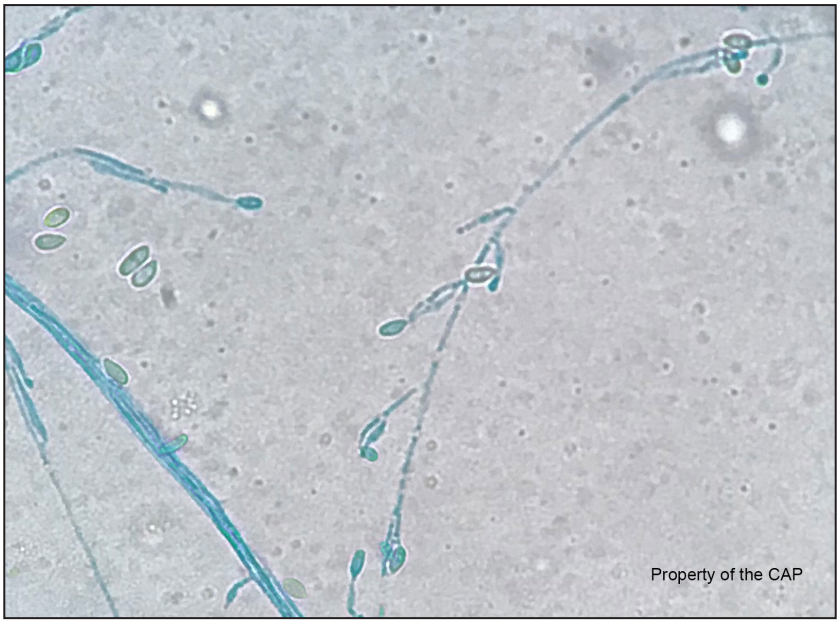

Figure 6

Conidiophores of *Lomentospora prolificans* with a characteristic flask shaped appearance. In this field several of the conidia are noticeably pigmented.

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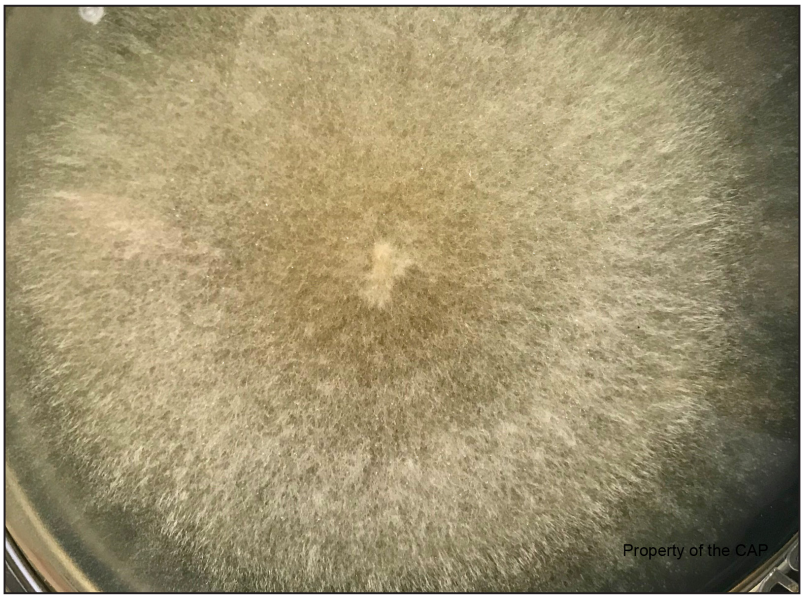

Figure 7

In this challenge, *Mucor* colonies grew rapidly and were white-to-tan-brown with a woolly texture on Sabouraud Dextrose agar.

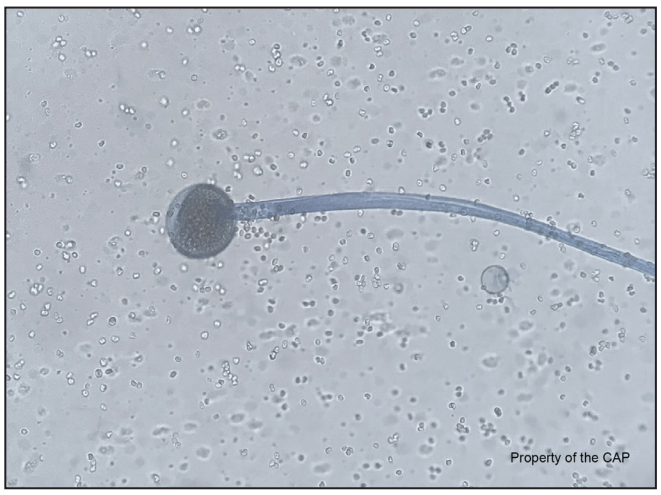

Figure 8A

Note the lack of rhizoids and complete absence of an apophysis on the sporangiophore of *Mucor* spp.

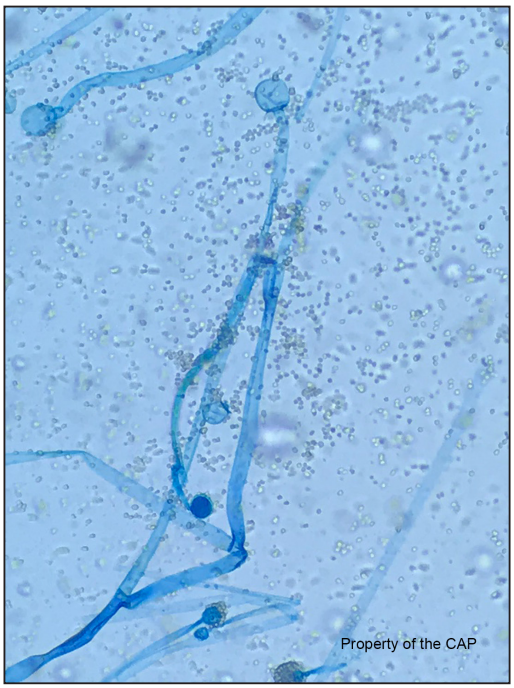

Figure 8B

Mucor spp. with large broad hyphae and large sporangia.