COLLEGE of AMERICAN PATHOLOGISTS

Surveys and Anatomic Pathology Education Programs

Mycology
F-C 2021

Participant Summary/Final Critique

Self-Reported Training Available

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2021 F-C
PARTICIPANT SUMMARY/FINAL CRITIQUE

Program Update

Don't Miss Out on this Educational Opportunity!

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 **0.5** CE credit. To access the activity, see page 28.

**CAP Self-Reported Training activities do not offer CE credit but can be used towards fulfilling requirements for maintenance of certification (MOC) by agencies such as the American Society of Clinical Pathology (ASCP). Please verify with your certifying agency to determine your education requirements.*

Evaluation Criteria

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 contacting the Customer Contact Center at 800-323-4040, Option 1, or 001-847-832-7000, Option 1 (international).

As published in the January 24, 2003 Federal Register, (42 CFR Part 493, Medicare, Medicaid, and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; Final Rule) effective April 24, 2003, proficiency-testing (PT) providers are required to grade all analytes regulated for PT at 80% participant or referee consensus, with the exception of Transfusion Medicine. For information on criteria for grading analytes not regulated for PT, please review your participant summary.

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

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 26. Laboratories should perform a self-evaluation. For more information, go to cap.org.

1. Hover over Laboratory Improvement and click **Proficiency Testing.**
2. Under Proficiency Testing (PT) Programs, Surveys, click **PT Resources.**
3. Under Existing Customers, click **Performing a Self-Evaluation When PT is Not Graded.**

Analytes regulated for proficiency testing appear in **bold** type.

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*

* 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-13

The F-13 challenge was a simulated abdominal abscess culture from a 75-year-old male with a history of colectomy. 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 glabrata.* A response of *Candida glabrata* complex (*C. glabrata, C. bracarensis, C. nivariensis*); *Candida* sp., not *albicans*; *Candida* sp.; Yeast, sent to reference lab for identification; or Yeast was considered satisfactory.

Table 1. Summary of Participant Responses

Total Respondents	Referees (71)		Participants (984)	
Identification	**LABS**	**%**	**LABS**	**%**
Candida glabrata complex (*C. glabrata, C. bracarensis, C. nivariensis*)	66	93.0	914	92.9
Candida sp., not *albicans*	3	4.2	13	1.3
Candida sp.	-	-	19	1.9
Yeast, sent to reference lab for identification	-	-	30	3.0
Yeast	2	2.8	7	0.7

Table 2. Results by Method

		% of Laboratory Designation		
System	**No. Labs**	***Candida glabrata* complex**	***Candida* sp., not *albicans***	***Candida* sp.**
API	53	92.4	3.8	3.8
BD Phoenix	15	100.0	-	-
Mass spectrometry/Bruker MALDI	194	100.0	-	-
Mass spectrometry/Vitek MS MALDI	187	99.5	-	-
MicroScan	24	91.7	4.2	-
Morphology and Bruker MALDI	96	100.0	-	-
Morphology and Vitek MS MALDI	71	98.6	-	1.4
Morphologic exam/biochemical	40	27.5	7.5	10.0
Remel RapID Yeast Plus	19	94.7	-	5.3
Vitek 2	247	93.5	2.0	3.2
Other[a]	27	55.6	7.4	11.1

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

Discussion

Taxonomy

The *Candida glabrata* species complex includes *C. glabrata* as well as the related but phenotypically indistinguishable *C. bracarensis* and *C. nivariensis*.[1,2]

Identification

Candida glabrata is typically slow-growing. On Sabouraud dextrose agar (SDA), *C. glabrata* colonies are shining, smooth, and cream-colored and are somewhat indistinguishable from those of other commonly-isolated *Candida* species, except for their relatively smaller colony size, due to slower growth. *Candida glabrata* cells are small (3-4 µm) and oval with unipolar budding. They do not form chlamydospores, true hyphae, or pseudohyphae. The rapid

trehalose test can provide a presumptive identification of *C. glabrata* within three hours which is helpful in guiding antimicrobial therapy.[3] Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) performs well in identification of *Candida* isolates, including *C. glabrata*.[4] In a multicenter study by Westblade et al 62/62 clinical *C. glabrata* isolates were identified to the species level (100% identification) using direct, on-target extraction with formic acid overlay via the Vitek MS system.[5] The Bruker system also performs well in identifying *C. glabrata* by mass spectrometry.[6]

In recent years a variety of assays that rapidly identify yeast in positive blood cultures have become commercially available. *Candida* PNA FISH (OpGen, Gaithersburg, MD), FilmArray BCID (bioMerieux, Durham, NC), Accelerate PhenoTest BC (Accelerate Diagnostics, Tucson, AZ), and ePlex BCID-FP (GenMark, Carlsbad, CA) detect various *Candida* species from positive blood cultures within 1-2 hours. Also, use of MALDI-TOF MS on aliquots of blood culture broth from positive blood culture bottles for identification of yeast has also been reported in literature.[7] All of these assays require incubation of inoculated blood cultures bottles to amplify organism(s) prior to testing. By contrast, the T2Candida assay (T2Biosystems, Lexington, MA) uses PCR and magnetic resonance technology to detect various *Candida* species directly from whole blood without incubation. None of the systems provide rapid antifungal susceptibility testing results.

Clinical Significance

Candida spp. are normal inhabitants of the oral cavity and the genitourinary tract, gastrointestinal tract, and respiratory tract. *Candida* colonization of the bronchial tree is common in patients receiving mechanical ventilation.[8,9] *Candida glabrata* is a common cause of vulvovaginits, second to *C. albicans.*[4] Urinary tract infection due to *C. glabrata* is encountered in the nosocomial setting, associated with indwelling urinary catheters, broad-spectrum antibiotic use, and previous exposure to antifungal agents.[10] Invasive candidiasis is typically healthcare related, with risk factors that include exposure to broad-spectrum antibiotics, intensive care unit (ICU) admission, abdominal surgery, dialysis, parenteral nutrition, and *Candida* colonization.[8] Bloodstream infection with *C. glabrata* reportedly accounts for up to a quarter of all candidemia cases and is second most common agent of candidemia behind *C. albicans*.[11]

Treatment

In cases of candidemia and invasive candidiasis, the Infectious Diseases Society of America (IDSA) Clinical Practice Guidelines for the Management of Candidiasis recommend treatment with an echinocandin (eg, caspofungin, micafungin) due to better patient outcomes as compared to fluconazole therapy. High dose fluconazole is an acceptable alternative only for confirmed fluconazole-susceptible (susceptible-dose dependent) *C. glabrata.* Lipid formulation amphotericin B is another alternative but not considered first-line due its nephrotoxic potential. In the case of intra-abdominal abscess, as in this challenge, source control (eg, abscess drainage, surgical repair, etc.) must additionally be achieved for successful therapy.[8]

Resistance

Candida glabrata has a special significance in that it shows higher resistance to fluconazole in comparison to most other *Candida* spp. Resistance is easily acquired during prolonged azole therapy. Other factors that are believed to be associated with antifungal resistance in *C. glabrata* include tolerance of the yeast to physiologic stresses such as temperature, oxidative stress, presence of an extracellular matrix which protects the cells from hostile factors, and “persister cells” which are dormant variants of regular cells persisting in chronic infections.[12] Recently, *C. glabrata* strains resistant to both fluconazole and echinocandins have been demonstrated to have acquired glucan synthase *FKS1* or *FKS2* mutations. Acquisition of these strains were linked to therapeutic failures.[13]

Table 3. Antifungal Susceptibility Testing – MIC*

F/F1-13 Antifungal Agent	Intended MIC Interpretation ♦
Anidulafungin	S
Amphotericin B	S,NI
5-fluorocytosine	NI
Fluconazole	R
Itraconazole	NI
Caspofungin	NC
Voriconazole	NI
Micafungin	S
Isavuconazole	U
Posaconazole	U

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

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

Fungal Susceptibility Testing – MIC*

F/F1-13 *Candida glabrata* complex MIC Testing	Interpretation	Participants LABS	%
Anidulafungin	Susceptible	110	98.2
	Intermediate	1	0.9
	Resistant	1	0.9
Amphotericin B	Susceptible	80	37.6
	No Interpretation	133	62.4
5-fluorocytosine	Susceptible †	60	37.5
	No Interpretation	100	62.5
Fluconazole	Susceptible	4	1.4
	Resistant	272	93.5
	S-DD	9	3.1
	No Interpretation	6	2.1
Itraconazole	Resistant †	12	11.0
	No Interpretation	97	89.0
Caspofungin	Susceptible ††	150	54.5
	Intermediate ††	43	15.6
	Resistant ††	79	28.7
	Non-Susceptible ††	1	0.4
	No Interpretation ††	2	0.7
Voriconazole	Susceptible †	1	0.4
	Resistant †	94	36.3
	Non-Susceptible †	1	0.4
	No Interpretation	163	62.9
Micafungin	Susceptible	415	99.0
	Intermediate	2	0.5
	Resistant	1	0.2
	S-DD	1	0.2
Isavuconazole**	No Interpretation	9	100.0
Posaconazole**	No Interpretation	1	100.0

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

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

See Susceptibility Testing in discussion for details

† Scientific committee decision – Code 30

†† Not graded due to lack of consensus – Code 27

Table 4. Antifungal Susceptibility Testing – Disk Agar*

F/F1-13 Antifungal Agent	Intended Disk Agar Interpretation ♦
Fluconazole**	U
Capsofungin**	U

Disk Agar Diffusion	Interpretation	Participants LABS	%
Fluconazole**	Resistant	7	77.8
	S-DD	2	22.2
Capsofungin**	Susceptible	1	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 5. Supplemental questions for antifungal susceptibility testing of *Candida glabrata* complex for F-13 2021

	Participant response:
1. Test methods:	
Broth macrodilution	-
Broth microdilution	20
Disk Diffusion	8
YeastOne colorimetric microdilution	198
Gradient diffusion strips (eg, Etest, MTS)	36
Vitek 2	170
Other	6
2. Test performed according to:	
CLSIM27-S4/CLSI M60	381
CLSI M27-S3 (obsolete)	10
FDA	15
Other	10
3. Does your laboratory use or plan on using/reporting Epidemiologic cutoff values (ECVs)?	
Yes	66
No	330

Table 6. Distribution of Antifungal MIC Rresults by Method for F/F1-13*

Occurrences at MIC (μg/mL)

5 - FLUOROCYTOSINE									
	0.006	**0.030**	**0.060**	**0.060**	**0.120**	**0.125**	**1.000**	**1.000**	**2.000**
Method	<=	<=	<	<=	<=	<	<=	=	<=
BROTH MICRODILUTION	-	1	-	-	1	1	-	-	1
VITEK 2	-	-	1	-	-	-	55	4	-
YEAST ONE COLORIMETRIC	2	10	3	52	3	-	-	-	-

AMPHOTERICIN B								
	0.125	**0.190**	**0.250**	**0.380**	**0.500**	**1.000**	**1.000**	**2.000**
Method	=	=	<=	=	<=	<=	>=	=
BROTH MICRODILUTION	2	1	2	-	7	-	3	1
GRADIENT DIFFUSN STRPS	1	-	-	1	2	1	-	-
VITEK 2	-	-	10	-	13	8	32	-
YEAST ONE COLORIMETRIC	-	-	3	1	32	3	47	2

ANIDULAFUNGIN									
	0.003	**0.012**	**0.015**	**0.016**	**0.030**	**0.031**	**0.060**	**0.120**	**2.000**
Method	=	<=	<=	=	<=	=	<=	<=	=
BROTH MICRODILUTION	-	-	-	-	2	1	8	2	-
GRADIENT DIFFUSN STRPS	1	1	-	1	-	-	-	1	-
VITEK 2	-	1	-	1	-	-	-	-	-
YEAST ONE COLORIMETRIC	1	-	3	-	9	-	38	20	1

CASPOFUNGIN											
	0.032	**0.047**	**0.060**	**0.120**	**0.120**	**0.125**	**0.128**	**0.250**	**0.250**	**0.500**	**0.500**
Method	<=	=	=	<=	>=	=	=	<=	>=	<=	>=
BROTH MICRODILUTION	-	-	6	-	3	3	-	-	1	1	-
GRADIENT DIFFUSN STRPS	-	-	-	-	-	2	-	-	2	-	-
VITEK 2	1	1	-	9	-	-	-	5	8	70	10
YEAST ONE COLORIMETRIC	1	-	10	5	47	1	1	5	25	2	-

FLUCONAZOLE											
	0.500	**1.000**	**2.000**	**8.000**	**32.000**	**64.000**	**128.000**	**128.000**	**256.000**	**256.000**	**512.000**
Method	=	<=	<=	=	=	>=	<=	>=	<=	>=	=
BROTH MICRODILUTION	-	-	-	-	2	10	5	1	2	-	-
GRADIENT DIFFUSN STRPS	-	-	-	-	-	1	-	1	1	22	1
VITEK 2	-	3	1	1	2	1	-	-	-	7	-
YEAST ONE COLORIMETRIC	1	-	-	-	1	10	38	17	65	33	-

ISAVUCONAZOLE		
	4.000	**8.000**
Method	=	=
BROTH MICRODILUTION	4	1

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

Table 6. Distribution of Antifungal MIC Results by Method for F/F1-13*, cont'd

Occurrences at MIC (μg/mL)

ITRACONAZOLE								
	0.120	**0.500**	**2.000**	**4.000**	**8.000**	**16.000**	**16.000**	**32.000**
Method	=	=	>=	>=	>	<=	>=	>=
BROTH MICRODILUTION	-	1	1	-	-	3	5	2
GRADIENT DIFFUSN STRPS	-	-	-	-	-	-	-	1
YEAST ONE COLORIMETRIC	1	-	6	1	1	14	49	1

MICAFUNGIN											
	0.003	**0.008**	**0.012**	**0.015**	**0.016**	**0.030**	**0.030**	**0.032**	**0.060**	**0.060**	**2.000**
Method	=	<=	<=	<=	=	<=	>	=	<=	>=	=
BROTH MICRODILUTION	-	2	-	5	-	4	-	-	1	1	-
GRADIENT DIFFUSN STRPS	-	-	2	-	2	-	-	-	-	1	-
VITEK 2	-	-	-	-	-	1	-	-	172	16	-
YEAST ONE COLORIMETRIC	1	4	-	44	2	72	1	1	2	3	1

POSACONAZOLE								
	0.060	**1.000**	**2.000**	**4.000**	**8.000**	**8.000**	**16.000**	**32.000**
Method	=	=	>=	=	<=	>=	>=	>
BROTH MICRODILUTION	-	2	1	1	1	-	4	-
GRADIENT DIFFUSN STRPS	-	-	-	-	-	-	-	1
YEAST ONE COLORIMETRIC	1	-	2	-	2	48	1	-

VORICONAZOLE										
	0.015	**0.500**	**1.000**	**2.000**	**4.000**	**4.000**	**8.000**	**8.000**	**12.000**	**16.000**
Method	=	<=	=	=	<=	>	<=	>=	=	=
BROTH MICRODILUTION	-	1	2	3	7	-	1	-	-	1
GRADIENT DIFFUSN STRPS	-	1	2	1	-	-	-	-	1	-
VITEK 2	-	-	-	-	5	-	-	88	-	-
YEAST ONE COLORIMETRIC	1	-	3	15	56	1	1	18	-	-

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

Table 7. Interpretation by Method, F/F1-07

	Broth Microdilution				**YeastOne Colorimetric**						**Gradient diffusion strips (eg, Etest, MTS)**				**Vitek 2**					
Antimicrobial	**S**	**R**	**S-DD**	**NI**	**S**	**I**	**R**	**S-DD**	**NI**	**NS**	**S**	**I**	**R**	**NI**	**S**	**I**	**R**	**S-DD**	**NI**	**NS**
Anidulafungin	13	-	-	-	70	-	1	-	-	-	4	-	-	-	2	-	-	-	-	-
Amphotericin B	1	-	-	15	12	-	-	-	75	-	2	-	-	4	54	-	-	-	14	-
Caspofungin	13	-	1	-	71	26	2	-	-	-	5	1	-	-	35	9	66	-	2	1
Fluconazole	1	-	20	-	-	-	163	2	-	-	-	-	26	1	3	-	10	3	5	-
Micafungin	13	-	-	-	131	-	1	-	-	-	5	-	-	-	194	-	-	1	-	-
Itraconazole	-	-	-	12	-	-	10	-	63	-	-	-	-	1	-	-	-	-	-	-
Voriconazole	-	-	-	15	-	-	7	-	86	1	1	-	-	4	-	-	68	-	29	-
5-Fluorocytosine	-	-	-	4	11	-	-	-	58	-	-	-	-	-	42	-	-	-	22	-
Isavuconazole	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

S= susceptible; SDD = susceptible-dose dependent; I = intermediate; R = resistant; NI = no interpretation

Table 8. Summary of results reported by laboratories for this PT challenge according to antifungal agent, result interpretation, and breakpoint guideline used, F/F1-13

	Susceptible	Intermediate	S-DD	Resistant	No Interpretation	Non-susceptible
ANIDULAFUNGIN						
OTHER	2	-	-	-	-	-
CLSI M27-S4/M60	104	1	-	1	-	-
AMPHOTERICIN B						
OTHER	26	-	-	-	7	-
CLSI M27-S3	3	-	-	-	-	-
CLSI M27-S4/M60	43*	-	-	-	111	-
5 - FLUOROCYTOSINE						
OTHER	11	-	-	-	-	-
CLSI M27-S3	3	-	-	-	-	-
CLSI M27-S4/M60	39*	-	-	-	95	-
FLUCONAZOLE						
OTHER	1	-	-	4	1	-
CLSI M27-S3	-	-	-	3	-	-
CLSI M27-S4/M60	2**	-	8	254	5	-
ITRACONAZOLE						
OTHER	-	-	-	2	3	-
CLSI M27-S3	-	-	-	1	-	-
CLSI M27-S4/M60	-	-	-	9*	89	-
CASPOFUNGIN						
OTHER	8	-	-	4	-	1
CLSI M27-S3	3	-	-	2	-	-
CLSI M27-S4/M60	132	41	-	66	2	-
VORICONAZOLE						
OTHER	-	-	-	25	6	1
CLSI M27-S3	-	-	-	5	-	-
CLSI M27-S4/M60	1*	-	-	53*	147	-
MICAFUNGIN						
OTHER	30	-	-	-	-	-
CLSI M27-S3	10	-	-	-	-	-
CLSI M27-S4/M60	353	2	-	1	-	-
ISAVUCONAZOLE						
OTHER	-	-	-	-	1	-
CLSI M27-S4/M60	-	-	-	-	8	-
POSACONAZOLE						
CLSI M27-S4/M60	-	-	-	-	1	-

* Although reported by the number of laboratories shown, interpretations for these drugs against *C. glabrata* are not provided in the document indicated (CLSI M27-S3 or CLSI M27-S4/M60). Laboratories should verify the source of their breakpoint interpretations and ensure usage of current guidelines.

** Interpretive categories for fluconazole against *C. glabrata* in CLSI M27-S4 and M60 include only S-DD and resistant and not susceptible.

Susceptibility Testing

CLSI Subcommittee on Antifungal Susceptibility Testing recommendations for susceptibility testing of yeasts have evolved over recent years. Major changes include publication of species-specific MIC interpretive criteria (breakpoints) in 2012, in the M27-S4 document.[14] In 2018, CLSI combined broth and disk susceptibility testing recommendations and breakpoints for yeast, including *C. glabrata*, into a single document, M60, the second edition of which was published in 2020.[15] The M27-S4 document will continue to be available, as it describes the specifics for performing broth dilution testing, as will M44-S3, which describes the disk diffusion method for yeast.

Candida glabrata breakpoints published by CLSI in M60 include anidulafungin, caspofungin, micafungin, and

fluconazole.[15] For fluconazole, no susceptible category exists, but rather isolates with MICs ≤32ug/mL are considered “susceptible dose-dependent” which reflects the fact that susceptibility depends on clinicians using maximally tolerated doses of fluconazole when treating infections caused by *C. glabrata*.

There was consensus among respondents for anidulafungin and micafungin but lack of consensus for caspofungin results. In instances where both caspofungin and micafungin susceptibility testing are performed, discrepancies should be resolved. Some feel that micafungin may be the more reliable drug for testing and may serve as an acceptable surrogate marker for the prediction of susceptibility and resistance of *Candida* species to caspofungin.[16] Further, breakpoints for caspofungin in current CLSI M60 guidelines may not be covered in the interpretive criteria used by older commercial assays. Laboratories should consider their ability to accommodate current CLSI breakpoint interpretations as past breakpoints were based on minimal clinical data, may be incorrect, and should not be used.

There are no CLSI or EUCAST clinical breakpoints for *C. glabrata* and voriconazole or itraconazole as current data are insufficient to demonstrate a correlation between *in vitro* susceptibility testing results to these drugs and clinical outcomes for *C. glabrata*. Participants who responded with NI received a “good” grading. Others received a code 30 but should note that old voriconazole or itraconazole breakpoints are likely incorrect and should not be used.[12] The CLSI M59 document, “Epidemiologic Cut-off Values”, provides an ECV of ≤0.25 µg/mL for voriconazole and an ECV of ≤4 µg/mL for itraconazole with *C. glabrata*.[17] An ECV is the minimum inhibitory concentration/minimum effective concentration value that separates fungal populations into those with and without acquired and/or mutational resistance based on their MIC. In other words, the ECV defines the wild-type (WT) distribution limit and distinguishes WT from non-WT. ECVs are not meant to predict clinical outcome to therapy.[17]

While CLSI’s M60 does not provide breakpoints for *C. glabrata* and amphotericin B, EUCAST specifies *C. glabrata* isolates with MICs ≤1 µg/mL as susceptible and >1 µg/mL as resistant.[18] The M59 document also provides an ECV ≤2 µg/mL for *C. glabrata* and amphotericin B.[17]

Finally, many respondents reported flucytosine results. There are no CLSI or EUCAST breakpoints for *C. glabrata* against flucytosine and the M60 recommends against use of previously published breakpoints due to insufficient data. NI was graded as the correct response. As the FDA still uses the breakpoints from CLSI M27-S3, a code 30 was applied to respondents who reported a result of susceptible.

In a recent publication of the SENTRY Antimicrobial Surveillance Program group which monitors global susceptibility and resistance rates, 12% of 251 clinical *C. glabrata* isolates collected worldwide during 2013 were resistant to fluconazole using the CLSI M27-S4 antifungal susceptibility guidelines (MIC ≥64 µg/mL).[19] Isolates were tested by the CLSI-recommended broth microdilution method in this study. The prevalence of echinocandin resistance of *C. glabrata* from the 2013 SENTRY isolates ranged from 0.8% resistance to micafungin, 2.0% to caspofungin and 2.4% to anidulafungin. Some *C. glabrata* with elevated minimum inhibitory concentrations (MICs) to echinocandins were shown to have mutations in *FKS*. The authors of the SENTRY publication conclude that *C. glabrata* should remain a focus of antifungal surveillance given its ability to express resistance to both azoles and the echinocandins.

Table 9. Current Clinical Breakpoints and ECVs for *Candida glabrata*

	Current M60 CLSI Interpretive Guidelines (MIC µg/mL)				M59-ED3 Epidemiologic Cutoff Values (ECVs) (µg/mL)
Antifungal Agents	**S**	**S-DD**	**I**	**R**	**WT**
Fluconazole	-	≤32	-	≥64	≤8
Posaconazole	-	-	-	-	≤1.0
Voriconazole*	-	-	-	-	≤0.25
Anidulafungin	≤0.12	-	0.25	≥0.5	≤0.25
Caspofungin	≤0.12	-	0.25	≥0.5	-
Micafungin	≤0.06	-	0.12	≥0.25	≤0.03
Amphotericin B	-	-	-	-	≤2.0
Itraconazole	-	-	-	-	≤4.0

*According to CLSI M60, current data are insufficient to demonstrate a correlation between *in vitro* susceptibility testing and clinical outcome for *C. glabrata* and voriconazole.

I – Intermediate; R – Resistant; NS – Non-Susceptible; S – Susceptible; S-DD – Susceptible-Dose-Dependent.

Key points

- *Candida* spp. are normal inhabitants of the oral cavity and the genitourinary tract, gastrointestinal tract, and respiratory tract.
- *Candida glabrata* are relatively slow-growing, small oval yeast that do not form chlamydospores, true hyphae, or pseudohyphae.
- *Candida glabrata* exhibits higher MICs to fluconazole as compared to most other commonly isolated *Candida* species.

Table 10. Comparison of FDA and Current Versus Old CLSI Interpretive Guidelines for *Candida glabrata*

Antifungal Agents	Current M60 CLSI Interpretive Guidelines (MIC µg/mL)				Old M27-S3 CLSI Interpretive Guidelines (MIC µg/mL) – **Now Obsolete**					Current FDA Breakpoints (MIC µg/mL)					EUCAST Breakpoints (MIC µg/mL)	
	S	**SDD**	**I**	**R**	**S**	**SDD**	**I**	**R**	**NS**	**S**	**SDD**	**I**	**R**	**NS**	**S**	**R**
Fluconazole	---	≤32	---	≥64	≤8	16-32	---	≥64	---	Recognizes CLSI M60					≤0.001	>16
Itraconazole	No breakpoint recognized				≤0.12	0.25-0.5	---	≥1		No breakpoint recognized					No breakpoint recognized	
Voriconazole	No breakpoint recognized				≤1	2	---	≥4	---	No breakpoint recognized					No breakpoint recognized	
Anidulafungin	≤0.12	---	0.25	≥0.5	≤2	---	---	---	>2	Recognizes CLSI M60					≤0.064	>0.064
Caspofungin	≤0.12	---	0.25	≥0.5	≤2	---	---	---	>2	Recognizes CLSI M60					No breakpoint Recognized**	
Micafungin	≤0.06	---	0.12	≥0.25	≤2	---	---	---	>2	Recognizes CLSI M60					≤0.03	>0.03
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.

* CLSI M27-S3 is an obselete document that has been replaced by CLSI M60 in which there are no flucytosine breakpoints for *C. glabrata*.

** EUCAST states that “Isolates that are susceptible to anidulafungin as well as micafungin should be considered susceptible to caspofungin, until caspofungin breakpoints have been established.”

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Specimen F-14

The F-14 challenge was a simulated sputum specimen from a 56-year-old HIV-positive patient with pneumonia. 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 *Nocardia cryiacigeorgica*. A response of *Nocardia cryiacigeorgica*; *Nocardia* sp.; Aerobic actinomycete isolated, sent to reference lab for identification; or Specimen negative for dermatophytes was considered satisfactory.

Table 1. Summary of Participant Responses

Total Respondents	Referees (70)		Participants (974)	
Identification	**LABS**	**%**	**LABS**	**%**
Nocardia cryiacigeorgica	22	31.4	232	23.8
Nocardia sp.	12	17.1	287	29.5
Aerobic actinomycete isolated, sent to reference lab for identification	33	47.1	360	37.0
Specimen negative for dermatophytes	-	-	2	0.2
Incorrect responses				
Nocardia farcinica complex	1	1.4	1	0.1
Specimen negative for fungi	2	2.9	64	6.6

Table 2. Results by Method

		% of Laboratory Designation	
System	**No. Labs**	***Nocardia cryiacigeorgica***	***Nocardia* sp.**
Mass spectrometry/Bruker MALDI	123	59.4	37.4
Mass spectrometry/Vitek MS MALDI	79	72.2	25.3
Morphology and Bruker MALDI	94	42.5	52.1
Morphology and sequencing	17	70.6	23.5
Morphology and Vitek MS MALDI	67	55.2	32.8
Morphologic exam/biochemical	491	0.8	26.5
Other[a]	56	14.3	21.4

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

Discussion

Taxonomy

Aerobic actinomycetes include the genus *Nocardia* and seven other genera, as well as genera *Corynebacterium* and *Mycobacterium*, which have mycolic acids in their cell walls. In some classification schemes, these 9 genera are grouped together in the suborder Corynebacterineae. There are approximately 100 *Nocardia* species described to date; more than 50 of which are considered clinically significant.[1,2,3]

The taxonomy of the genus *Nocardia* has been challenging and the genus has undergone multiple rounds of reclassification over the past 50 years.[2] Historically, *N. asteroides* complex was accepted as the type species (ATCC 19247) of the genus from its original description in 1890 until 1962, however, with molecular sequencing methods that are now able to discriminate between species, the term *N. asteroides* complex is no longer valid.[1,2]

The identification of *Nocardia* sp. was traditionally based on organism growth and reactivity to a variety of biochemical substrates. Unfortunately, due to the slow growth rate of aerobic actinomycetes, limited biochemical activity and significant overlap of biochemical reactions between species, this means slow turnaround times and indeterminate results are common and biochemical testing is not considered sufficient for accurate and reliable species-level identification of *Nocardia* sp.[2]

A 1988 study by Wallace et al proposed an alternative classification system using six antibiotic susceptibility groups.[4] The "Group VI" susceptibility pattern (susceptibility to sulfonamides, broad-spectrum cephalosporins, amikacin, imipenem, and linezolid and resistance to penicillins, clarithromycin, and variable resistance to ciprofloxacin) was determined to include isolates that were, for the most part, "*N. asteroides*" but that have since been shown to belong to the species *N. cyriacigeorgica*, suggesting that *N.cyriacigeorgica* was responsible for a considerable share of human *Nocardia* infections reported before the availability of molecular sequencing tests.[2]

Sequencing analysis of the *hsp65* and the 16S rRNA genes of isolates belonging to *N. asteroides* complex showed that gene sequences were sufficiently unique to warrant retirement of the term *N. asteroides* complex and the creation of six new taxa including *N. abscessus, Nocardia brevicatena/N. paucivorans, Nocardia nova* complex, *N. transvalensis* complex, *N. farcinica*, and *N. cyriacigerogica*.[2,5] *Nocardia cyriacigeorgica* is now considered the species most frequently implicated in disease in the U.S.[2] Other species reported as human pathogens include *N. abscessus*, *N. brasiliensis*, *N. farcinica*, *N. nova*, *N. otitidiscaviarum*, *N. paucivorans*, *N. pseudobrasilensis*, *N. transvalensis*, *N. veteran*, and *N. wallacei*.[2] Molecular sequencing is now considered the gold standard method for identification of *Nocardia* to the species level.

Identification

Microscopic examination of clinical specimens is a fundamental first step if aerobic actinomycetes are suspected. *Nocardia* sp. exhibit characteristics that can inform the laboratory to culture on the appropriate media and to incubate at the appropriate temperature and for an extended time to have the best chance of isolate recovery. Gram stain and modified acid-fast stain are both important and can be performed directly from sputum, drainage, aspirates, and other body fluids. *Nocardia* sp. are gram-positive, thin, filamentous branching rods, 0.5-1.0mm in diameter, and beading may be apparent. They are weakly or partially acid fast. If smear is performed directly on the specimen, *Nocardia* sp. are commonly seen in association with polymorphonuclear leukocytes.[2,3]

Aerobic actinomycetes including *Nocardia* sp. grow readily on standard bacteriological media (eg, blood agar, chocolate agar, brain heart infusion agar); fungal media (eg, Sabouraud dextrose agar, inhibitory mold agar) and mycobacterial media (eg, Lowenstein-Jensen). Other media, such as buffered charcoal yeast extract agar may also be useful in the recovery or *Nocardia* sp., as well as selective media if the sample is from a non-sterile site. Although it should be noted that growth of some strains may be suppressed in the presence of gentamicin and chloramphenicol. Cultures should be held for a minimum of 2-3 weeks as *Nocardia* sp. tend to grow slowly. The colonial morphology of *Nocardia* sp. is highly variable. *Nocardia cyriacigeorgica* are characteristically dry white colonies and can have a powdery appearance with aerial hyphae covering the surface of colonies. Microscopic examination of the isolate reveals delicately beaded, branching gram-positive filaments that are weakly acid-fast when stained with a modified acid-fast stain.[3]

To accurately identify *Nocardia* sp. to species or group/complex level, an algorithm has been proposed that integrates matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) with sequencing methodologies.[2] Matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is being used increasingly to identify *Nocardia* sp.[6] Recent reports have shown that the manufacturers of MALDI-TOF MS instruments have improved their spectral IVD databases to allow more accurate and reliable identification of *Nocardia* sp.[7,8] However, less common species may be harder to identify and are dependent on the

coverage provided by the database. Laboratories may want to supplement the IVD database to expand their ability to accurately identify species by MALDI-TOF MS, or laboratories may perform gene sequencing of *hsp65* and 16S rRNA.

Laboratories that do not have access to newer technologies may only be able to identify *Nocardia* sp. to the genus level. These isolates should be referred out for accurate identification and antimicrobial susceptibility testing, if clinically appropriate.

Clinical Significance

Nocardia sp. are commonly found in soil and water and the vast majority of infections due to *Nocardia* sp. stem from environmental sources through trauma-related introduction or inhalation. Found throughout the United States and across the globe, certain species have been reported to exhibit certain geographic tendencies. For example, *N. nova* complex appears to be less commonly reported in the Southwestern regions of the U.S. compared to other parts of the country, although geographic distribution may change over time with more accurate species identification and continued changes in classification for this genus.[2,9]

Nocardia sp. have been implicated in pulmonary infections as well as disseminated infections through hematogenous spread, often from a pulmonary focus. Serious infection due to *Nocardia* sp. is frequently associated with immunosuppression with the brain being a common site of secondary infection, as reported for *N. cyriacigeorgica*.[10,11] These infections can be difficult to treat and have an increased risk of poor outcomses.[12]

Therapy

Infections involving *Nocardia* sp. are typically treated with trimethoprim-sulfamethoxazole. Severe, disseminated, infections, and those involving the central nervous system may require the addition of amikacin or a β-lactam to ensure antimicrobial susceptibility to at least two agents.[13,14] A 2010 survey reported 69% resistance to sulfamethoxazole and 53% resistance to trimethoprim-sulfamethoxazole, raising concerns about trimethoprim-sulfamethoxazole monotherapy.[15] However, it should be noted that the challenges related to performing antimicrobial susceptibility testing of *Nocardia* sp. may account for the observed *in vitro* resistance.[16]

Key Points

- The taxonomy of the genus *Nocardia* is evolving and the term "*Nocardia asteroides* complex" is no longer valid. Although relatively recently acquiring a valid name, *Nocardia cyriacigeorgica* has been determined to be the same species as those strains in the *N. asteroides* drug pattern VI. Historically, these species were the most commonly identified in human infection and can now likely be attributed to *N. cyriacigeorgica*.
- Accurate and reliable identification of *Nocardia* sp. requires advanced techniques, including MALDI-TOF MS and/or gene sequencing.
- Trimethoprim-sulfamethoxazole is the empiric antibiotic treatment of choice for treatment of infections involving *Nocardia* sp., but resistance may be emerging. This highlights the importance of performing antimicrobial susceptibility testing for clinically relevant isolates.

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Specimen F-15

The F-15 challenge was a simulated corneal scraping from a 63-year-old female with keratitis. 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 *Alternaria alternata* and *Staphylococcus epidermidis* as a contaminant. A response of *Alternaria* sp.; Dematiaceous mold, sent to reference lab for identification; Specimen negative for dermatophytes; or Mold recognized, sent to reference lab for identification was considered satisfactory.

Table 1. Summary of Participant Responses

Total Respondents	Referees (70)		Participants (981)	
Identification	**LABS**	**%**	**LABS**	**%**
Alternaria sp.	55	78.6	761	77.6
Dematiaceous mold, sent to reference lab for identification	4	5.7	28	2.9
Specimen negative for dermatophytes	-	-	1	0.1
Mold recognized, sent to reference lab for identification	11	15.7	180	18.4

Table 2. Results by Method

		% of Laboratory Designation
System	**No. Labs**	***Alternaria* sp.**
Mass spectrometry/Bruker MALDI	14	92.9
Mass spectrometry/Vitek MS MALDI	13	100.0
Morphology and Bruker MALDI	30	100.0
Morphology and sequencing	20	95.0
Morphology and Vitek MS MALDI	33	97.0
Morphologic exam/biochemical	792	77.7
Other[a]	43	60.5

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

Discussion

Taxonomy

The genus *Alternaria* currently contains well over 250 species, only a few of which are pathogenic: *Alternaria alternata* and *Alternaria infectoria* are the most frequently reported species complexes associated with human disease.[1,2] Identification of *Alternaria* to the species level by morphology alone is technically difficult and not commonly done in the clinical microbiology laboratory; genus-level identification is generally considered sufficient for clinical use. In addition, as with many fungi, the taxonomy of *Alternaria* is actively being revised as molecular investigations challenge long-standing classifications.[3] While a recent study suggested a comprehensive framework for correct molecular identification of a significant portion of *Alternaria*, it is clear that this will remain a work in progress for the next several years.[4]

Identification

Alternaria species demonstrate moderately rapid growth at 30ºC; most grow poorly (if at all) at 37ºC. *Alternaria* typically produce wooly and pigmented colonies - dark olive green to brown (Figures 1A and 1B) - with a dark reverse coloration whenmature (4-5 days on standard fungal media) reflecting the extensive melanization.[2]

Alternaria spp. also typically produce medium-to-dark brown pigmented hyphae, best appreciated at the points of septation (Figure 2). Isolates typically have multi-celled macroconidia with both cross and longitudinal septations in the "drumstick" morphology often seen in *Alternaria*; branching is common and may suggest species identification but is not trivial to quantify accurately. Occasional oval spores are also seen. Importantly, some species sporulate poorly on routine clinical media - *A. infectoria* in particular - further complicating identification. As noted above, most clinical laboratories do not identify *Alternaria* to the species level due to the complex and contradictory morphological findings.

Alternaria can be confused with *Ulocladium* species in culture. Both fungi are dematiaceous, with filamentous growth not extending high above agar surface. Both genera have multicellular macroconidia with cross and longitudinal septations, and each may demonstrate overlap in macroconidia shape when the oval morphology is favored instead of the "drumstick" morphology in some species of *Alternaria*. Classically, morphological identification to the species level was primarily based on the pattern of the tertiary conidium (the final conidium in a chain of conidia): *Alternaria* spores arise sequentially in a chain-like pattern (although fragmentation can lead to free conidia and chaining may not be easy to demonstrate), while *Ulocladium* spores proceed directly from the conidiophore by geniculate ("bent knee") conidiation producing "tree" or "bouquet" morphologies.[2] Finally, *Alternaria* species lack the rough-walled conidia morphology often seen in *Ulocladium* species.

Clinical Significance

Alternaria are ubiquitous soil-dwelling saprophytes associated with decaying plant material and spores are widely distributed in both the indoor and outdoor environment.[1] Allergies and allergic responses to the dematiaceous fungi - including *Alternaria* spp. - represent the most common clinical manifestations of these fungi in immunocompetent patients: asthma, hypersensitivity pneumonitis, and allergic sinusitis and rhinitis are frequently associated with exposure to these environmental organisms. Patients with Cushing's syndrome (hypercortisolism) appear to be at significantly increased risk for cutaneous/subcutaneous disease.[1] Ocular disease in immunocompetent individuals with gross environmental exposures (eg, soil, refuse) or trauma are occasionally reported.[5]

Alternaria species have been found as part of the normal skin flora of human and other animals and are rare opportunistic pathogens in human disease: roughly half of reported cases have been associated with immunocompromised states.[1,5] Most *Alternaria* cases in immunosuppressed patients involve cutaneous and subcutaneous infections (including onychomycoses: fungal infections of the nails), though ocular and invasive rhinocerebral infections have also been reported in the literature.[1]

Antifungal Resistance and Therapy Considerations

In general, *Alternaria* infections have been successfully treated with most of the available antifungals (azoles, amphotericin B, echinocandins), though data is limited: isolate susceptibility testing is recommended for clinically significant infections.[6] At the species level, *A. infectoria* resistance to echinocandins (≥4 mg/L) has been reported; other *Alternaria* spp. are more frequently susceptible.[6] Ocular infections may be treated with topical, oral, or intraocular antifungal therapy; intravitreal amphotericin B is the drug of choice for endophthalmitis.[1] Amphotericin B is the drug most commonly used for invasive rhinocerebral disease, while systemic itraconazole is most frequently used to treat nail involvement and cutaneous and subcutaneous infections.[1] Posaconazole appears to have good efficacy against *Alternaria* - particularly in the post-transplant population - though the published literature is somewhat sparse.[6]

Key Points

- *Alternaria* spp. are ubiquitous in the environment but rarely directly pathogenic in immunocompetent patients.
- Genus-level identification of *Alternaria* is generally considered sufficient for clinical purposes.
- Systemic itraconazole, posaconazole, and amphotericin B are used to treat significant *Alternaria* infections, particularly in immunosuppressed patients. Topical or oral antifungals are appropriate for most superficial ocular infections.

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Specimen F-16

The F-16 challenge was a simulated blood culture specimen from a 70-year-old female in ICU with fever. 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 *Beauveria* and *Streptococcus* viridans group as a contaminant. A response of *Beauveria* sp.; or Mold recognized, sent to reference lab for identification was considered satisfactory.

Table 1. Summary of Participant Responses

Total Respondents	Referees (71)		Participants (974)	
Identification	**LABS**	**%**	**LABS**	**%**
Beauveria sp.	35	49.3	476	48.9
Mold recognized, sent to reference lab for identification	31	43.7	441	45.3
Incorrect responses				
Specimen negative for fungi	2	2.8	26	2.7
Aspergillus sp.	1	1.4	1	0.1
Fusarium sp.	1	1.4	2	0.2
Trichosporon sp.	1	1.4	1	0.1

Table 2. Results by Method

System	No. Labs	% of Laboratory Designation *Beauveria* sp.
Morphology and Bruker MALDI	29	86.2
Morphology and sequencing	29	96.5
Morphology and Vitek MS MALDI	12	25.0
Morphologic exam/biochemical	802	48.1
Other[a]	61	41.0

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

Discussion

Taxonomy

The genus *Beauveria* comes from the Family of Cordycipitaceae, the Order of Clavicipitales, the Sub-division of Pezizomycotina, and the Order of *Ascomycota*. *Beauveria bassiana* is the most common species encountered in the clinical laboratories.

Identification

This *Beauveria* isolate grew well on routine mycology media including media containing cycloheximide. The colony demonstrated a moderately fast growth rate at 30°C, producing a 4 cm colony at 7 days. The colony was white to cream color, velvety at the border, and showed cottony aerial tufts toward the center (Figure 3A). The colony reverse was variably pigmented. Microscopic preparations revealed delicate hyaline septate hyphae with patchy sporulation from areas of dense hyphae (Figure 4B). With close observation, amphora-shaped conidiogenous cells (3-6 x 2.5 to 3.5 µm) are seen which end with long filamentous extensions (rachi) connecting small, smooth, conidia (2 to 4 µm), singly at each bend of the filament to form a zig-zag geniculate arrangement (Figure 4A). The

presence of conidia along, or at the end of the zig-zag filament will differentiate *Beauveria* from other fungal genera. For example, oblong clusters of conidia of *Acremonium* sp. form directly on the tapering phialide, with no apparent structure connecting the conidia to the phialide. Care must be taken to examine the colony early and before heavy sporulation obscures the delicate structures required for identification. *Trichoderma* may look somewhat similar with hyaline hyphae and flask-shaped phialides, but conidia of *Trichoderma* cluster tightly at the end of each phialide and the colonies are green at maturation (Image1). These tight conidial clusters of *Trichoderma* differ from the delicate rachis bearing conidia seen in *Beauveria*. In a similar manner, the conidia of *Verticillium* appear singly or in small clusters at the ends of phialides. The bushy conidial masses of a mature colony of *Beauveria* would not be expected with either *Trichoderma* or *Verticillium*.

Clinical Significance

Beauveria species grow poorly or not at all at 35°C, which may account for their rarity as human pathogens. There have been several examples of keratitis.[2,3] *Beauveria* pneumonia has been reported in an acute myelogenous leukemia (AML) patients,[4] and a case of disseminated disease was historically documented in an acute lymphoblastic leukemia (ALL) patient with possible exposure to *Beauveria* through “organic” agricultural practices.[5] *Beauveria* are commonly found in association with insects and habitats contaminated with insect remains. *Beauveria* was first described by Agostino Bassi in 1835 as the cause of muscardine disease in silkworms.[6] *Beauveria bassiana* is one of the most common members of this genus and is now commonly used as an “organic” biological insecticide.

Image 1 - *Trichoderma* produces tight clusters of conidia at the end of flask-shaped phialides (lactophenol aniline blue, original magnification x1000). These clusters are easily disrupted. Undisturbed conidia need to be located to help distinguish *Trichoderma* from *Beauveria*.

Antifungal therapy and resistance

There are very limited *in vitro* data on antifungal susceptibility testing of *Beauveria*, mostly from case reports in which showed the organism was mostly sensitive to itraconazole, voriconazole, micafungin, moderately sensitive to amphotericine B, and less susceptible to fluconazole and flucytosine.[7,8]

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Specimen F-17

The F-17 challenge was a simulated lung tissue specimen from a 44-year-old male with history of bone marrow transplant now with pulmonary infiltrate. 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 *Aspergillus fumigatus* and *Streptococcus* viridans group as a contaminant. A response of *Aspergillus fumigatus; Aspergillus fumigatus* complex; *Aspergillus* sp.; *Aspergillus* sp., presumptive I.D.; or Mold recognized, sent to reference lab for identification was considered satisfactory.

Table 1. Summary of Participant Responses

Total Respondents	Referees (70)		Participants (980)	
Identification	**LABS**	**%**	**LABS**	**%**
Aspergillus fumigatus	21	30.0	379	38.7
Aspergillus fumigatus complex	16	22.9	163	16.6
Aspergillus sp.	22	31.4	285	29.1
Aspergillus sp., presumptive I.D.	-	-	2	0.2
Mold recognized, sent to reference lab for identification	10	14.3	141	14.4
Incorrect responses				
Aspergillus sp. not *fumigatus*	1	1.4	8	0.8

Table 2. Results by Method

System	No. Labs	% of Laboratory Designation *Aspergillus fumigatus*	*Aspergillus fumigatus* complex	*Aspergillus* sp.	*Aspergillus* sp., presumtive I.D.
Mass spectrometry/Bruker MALDI	13	92.3	-	-	-
Mass spectrometry/Vitek MS MALDI	15	86.7	13.3	-	-
Morphology and Bruker MALDI	35	77.1	22.9	2.9	-
Morphology and sequencing	20	65.0	30.0	-	-
Morphology and Vitek MS MALDI	44	84.1	9.1	6.8	-
Morphologic exam/biochemical	774	34.4	18.1	33.0	0.3
Other[a]	43	14.0	2.3	41.9	-

Discussion

Taxonomy

Aspergillus fumigatus is the clinically common anamorph form of the teleomorph, *Neosartorya fumigate,* and belongs to the *A. fumigatus* complex (or section Fumigati).

Identification

Aspergillus fumigatus grows rapidly and is typically mature within three days. On Sabouraud dextrose agar, colonies have a suede-like surface with blue-green pigmentation that becomes grayer with age (Figure 5A). The reverse of the agar surface is white-to-light yellow (Figure 5B). Microscopically, the hyphae of *A. fumigatus* are septate and hyaline. Conidiophores are non-pigmented and relatively short in comparison to several other *Aspergillus* species. Conidiophores give rise to club-like vesicles which lend this fungus its genus name, after the

liturgical implement aspergillum which is used to sprinkle holy water. The phialides are compact and uniseriate and form on the upper two-thirds of the vesicle. Conidia are generally smooth and round (Figures 6A and 6B). *Aspergillus fumigatus* grows well at 45ºC, which differentiates it from several other *Aspergillus* species.

Publications within the last decade have demonstrated that there are less recognized *Aspergillus* species which can appear phenotypically similar to *A. fumigatus* but can only be distinguished by DNA sequence analysis of the beta-tubulin or other genes.[1] Examples of such species include *Aspergillus lentulus*, *Aspergillus thermomutatus*, *Aspergillus novofumigatus*, *Aspergillus fumigatiaffinis*, and the teleomorphic species *Neosartorya udagawae*.[2,3] Some differences among these species include rapidity of sporulation, ability to grow at 48ºC, and colony color, but sequencing must be performed to differentiate among them. Different patterns of susceptibilities also exist (ie, *A. lentulus* demonstrates higher minimal inhibitory concentrations [MICs] to certain antifungals than *A. fumigatus*).[2] As such, if laboratories are not performing sequencing, reporting *Aspergillus* species that morphologically resemble *A. fumigatus* should be reported as *A. fumigatus* species complex.

Clinical Significance

Aspergillus fumigatus is the major cause of invasive aspergillosis (IA). It is a ubiquitous saprophytic soil organism to which humans are exposed continuously and is the dominant fungus in garden and greenhouse soil.[4] Self-heating compost heaps are significant environmental reservoirs for *A. fumigatus* due to the mold's ability to withstand higher temperatures. Many features of this mold make it a significant human pathogen, including: its survival and growth in a wide range of environmental conditions (conidia can even survive in liquid nitrogen for up to 18 years); its ability to disperse widely in the air due to hydrophobic conidia; the ability of conidia to easily reach the lower airways; and adaptation to the immunocompromised host environment.[4]

Infection is due to inhalation of conidia which can be cleared quickly in the immunocompetent host, but may cause disease in the immunocompromised host. Intensive aspergillosis occurs in 4-23% of lung transplant patients.[5] Due to the routine employment of antifungal prophylaxis in the early post-transplant period, the time to onset of IA in the current era in lung transplant patients is usually greater than one year post-transplant.[6] Factors which may lead to development and earlier onset of IA include lack of antifungal prophylaxis, poor absorption of antifungal medications, inhibitory interactions of the antifungals with other medications, and the possibility of azole-resistant *A. fumigatus*.[7] While *Aspergillus* spp. can be detected in the airway of approximately 25-30% of lung transplant recipients, positive cultures do not always infer infection but may portend a higher risk for subsequent invasive disease.[8]

Antimicrobial Resistance and Therapy Considerations

Azole-resistant *A. fumigatus* isolates are a significant clinical concern. Resistance can emerge with prolonged clinical exposure and can occur in patients with chronic pulmonary aspergillosis. Such resistance occurs via acquisition of mutations in the CYP51A gene, which encodes the enzyme that converts lanosterol to ergosterol in *Aspergillus*. Mutations at different sites in this gene may differentially affect azole susceptibility, affecting susceptibility to posaconazole and itraconazole, or to voriconazole and isavuconazole, or to all azoles.[9] Such mutations have also been documented in azole-naïve patients; and are linked to the mold's exposure to azoles in the environment, which are used in some agricultural practices. Efflux mechanisms are also important factors leading to resistance. The global prevalence of azole resistance in *Aspergillus* appears to be 3-6%.[10]

Antimicrobial Susceptibility Testing

Most routine laboratories do not perform antifungal susceptibility testing on *Aspergillus* isolates. However, susceptibility testing may be considered in cases of invasive and cutaneous infections in immunocompromised hosts when the patient is not responding to azoles, and such testing is best performed at a reference laboratory that specializes in antifungal susceptibility testing. CLSI has established MIC breakpoints for voriconazole and *A. fumigatus,*[11] and species-specific epidemiological cutoff values (ECVs) for a variety of antifungal agents for

A. fumigatus, *A. flavus*, *A. niger, A. terreus,* and *A. versicolor*, which illustrate the inherent differences in azole MICs between these species.[12] For example, the voriconazole ECV for *A. fumigatus* is 1 µg/mL whereas the ECV for *A. flavus*, *A. niger*, and *A. versicolor* is a dilution higher, at 2 µg/mL. Similarly, the ECV for isavuconazole is 1 µg/mL for *A. fumigatus*, *A. flavus*, and *A. terreus*, whereas that of *A. niger* is 4 µg/mL.[12] While many laboratories find species identification of aspergilli difficult, species identification of *Aspergillus* is suggested due to such differences in intrinsic and acquired antifungal resistance.

It is critical to recognize the ECV is not a clinical breakpoint, but rather an MIC cut-off that differentiates the wild-type population of *Aspergillus* from those with acquired or mutational resistance mechanisms. Factors outside of resistance mechanisms, including clinical data on patient outcomes and pharmacokinetic/pharmacodynamic data are lacking to establish a clinical breakpoint that predicts treatment outcomes.

Key Points

- *Aspergillus fumigatus* is a ubiquitous saprophyte found in garden and greenhouse soil.
- There are several less-recognized *Aspergillus* species which appear phenotypically similar to *A. fumigatus* but are more resistant to certain antifungal agents.
- The time to onset of invasive aspergillosis in lung transplant patients is usually greater than one year post-transplant.
- Resistance of *A. fumigatus* to azoles is rare, but may occur in both patients with prolonged exposure and naïve to azole therapy. Susceptibility testing is best performed at a reference laboratory that specializes in antifungal susceptibility testing.

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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.
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).
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.

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We the participants below have completed the review of the F-C, 2021 CAP Program
Product Mailing, Year

Participant Summary/Final Critique report and can self-report this activity towards fulfilling education and maintenance of certification (MOC) requirements. Time spent on activity* _____.

Participant	Date	Participant	Date
_____	_____	_____	_____
_____	_____	_____	_____
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_____	_____	_____	_____

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Director (or Designee) Signature - I have verified that the individuals listed above have successfully participated in this activity. Date

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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.

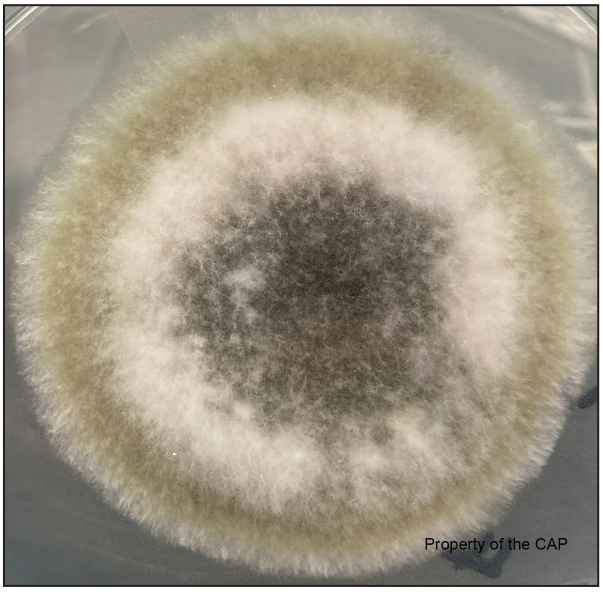

Figure 1A

Figure 1B

Growth on Sabouraud Dextrose agar showing a surface image of wooly dark green mold (1A) and a dark reverse (1B) characteristic of *Alternaria*.

Figure 2

Alternaria present as large club-shaped conidia, typically with both longitudinal and transverse septations, although in this field longitudinal septations are not evident. Conidia are seen both alone and in chains. The tapered ends of the conidia face the conidiophores. Dark hyphae are septate.

The CAP wishes to thank Rosemary C. She, MD, FCAP; and Aida Mangahis, CLS, for providing these photographs.

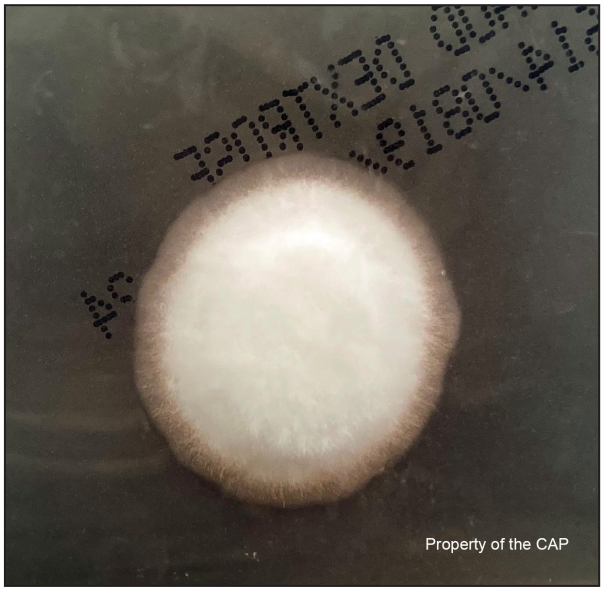

Figure 3A

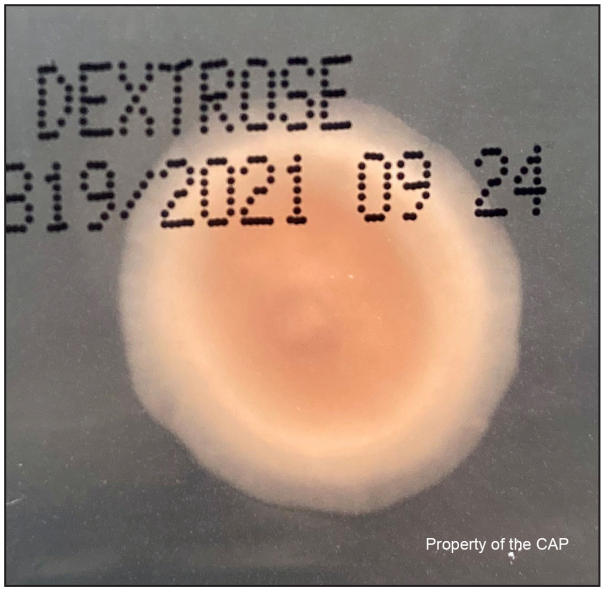

Figure 3B

Growth of *Beauveria* on Sabouraud Dextrose agar demonstrating a cottony texture; Surface (3A): white; Reverse (3B): pale yellow to pink.

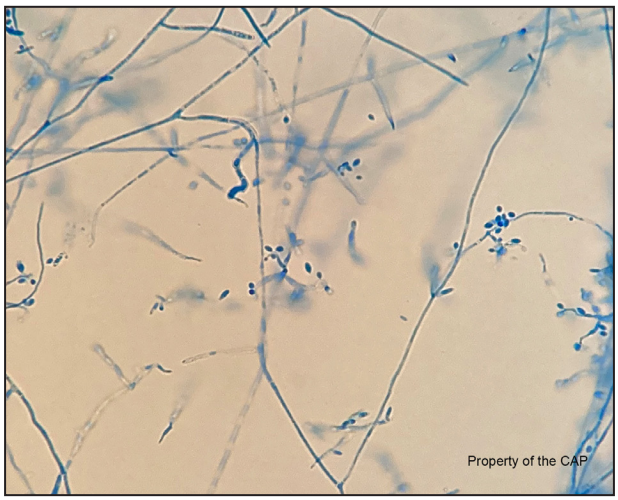

Figure 4A

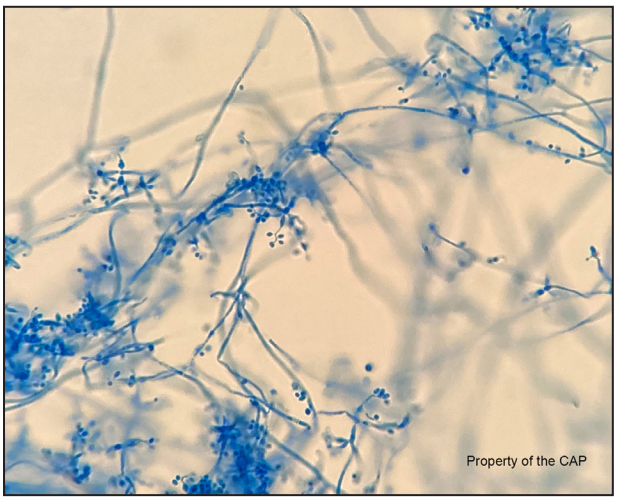

Figure 4B

Beauveria hyphae are septate and hyaline. Conidia-bearing cells are wide at the base or flask-shaped. Geniculate (zig-zag) extensions of the conidiophore bear round to ovoid conidia on each side.

The CAP wishes to thank Rosemary C. She, MD, FCAP; and Aida Mangahis, CLS, for providing these photographs.

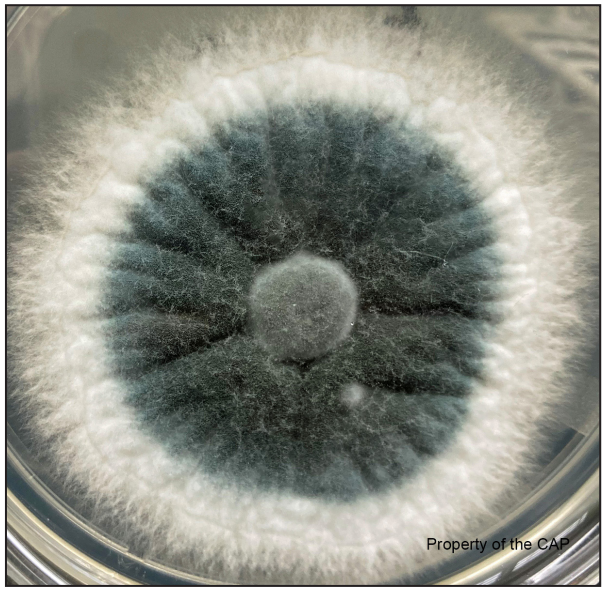

Figure 5A

Figure 5B

Surface and reverse macroscopic features of *Aspergillus fumigatus* on Sabouraud Dextrose Agar. *Aspergillus fumigatus* grows rapidly to produce a blue-to-gray green colony.

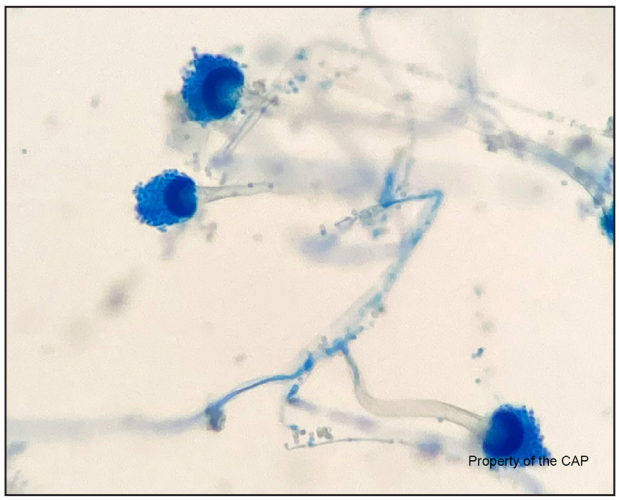

Figure 6A

Figure 6B

Aspergillus fumigatus hyphae are septate and hyaline. Short, smooth conidiophores support flask-shaped vesicles with uniserate phialides on upper surfaces. Conidia are round and form in chains.