Mycology Proficiency Testing Program

Test Event Critique
February 2012

Wadsworth Center
NEW YORK STATE DEPARTMENT OF HEALTH
Mycology Laboratory

Table of Contents

Mycology Laboratory

Mycology Laboratory at the Wadsworth Center, New York State Department of Health (NYSDOH) is a reference diagnostic laboratory for fungal diseases. The services include testing for dimorphic pathogenic fungi, unusual molds and yeasts pathogens, antifungal susceptibility testing including tests with research protocols, molecular tests including rapid identification and strain typing, outbreak and pseudo-outbreak investigations, laboratory contamination and accident investigations and environmental samples related to fungal diseases. The laboratory maintains proficiency and certification for handling Select Agents and to assist clinical laboratories in compliance with the latest regulations. Fungal Culture Collection of mycology laboratory is an important resource for high quality cultures used for proficiency testing program and for in house development of new diagnostic tests.

Mycology Proficiency Testing Program provides technical expertise to NYSDOH Clinical Laboratory Evaluation Program (CLEP). The program is responsible for conducting the CLIA-compliant proficiency testing (mycology) for clinical laboratories in New York. All analytes for these test events are prepared and standardized internally. The program also provides continuing educational activities in form of detailed critiques of test events, workshops and occasional one-on-one training of laboratory professionals.

Mycology Laboratory Staff and Contact Details

Name	Responsibility	Phone	Email
Dr. Vishnu Chaturvedi	Director	518-474-4177	vishnu@wadsworth.org
Dr. Sudha Chaturvedi	Deputy Director (In-Charge, Diagnostics)	518-474-4177	schaturv@wadsworth.org
Dr. Ping Ren	PT Program Coordinator	518-474-4177	mycologypt@wadsworth.org renp@wadsworth.org
Ms. Xianjiang Li	Research Scientist (Diagnostic Section)	518-486-3820	mycologydianostics@wadsworth.org
Ms. Tanya Victor	Research Scientist (Molecular Section)	518-474-4177	mycologydiagnostics@wadsworth.org

Mycology Proficiency Testing Program (PTP)

CATEGORY DESCRIPTION

COMPREHENSIVE: This category is for laboratories that examine clinical specimens for pathogenic molds and yeasts routinely encountered in a clinical microbiology laboratory. These laboratories are expected to identify fungi to the genus and species level as appropriate. Laboratories holding this category may also perform antifungal susceptibility testing, antigen detection, molecular identification or other tests described under any of the categories listed below.

RESTRICTED: This category is for laboratories that restrict their testing to one or more of the following:

IDENTIFICATION YEAST ONLY: This category is for laboratories that isolate and identify to genus and species, as appropriate, yeast-like fungi routinely encountered in a clinical microbiology laboratory. Laboratories holding this category may also perform susceptibility testing on yeast. These laboratories are expected to refer mold specimens to another laboratory holding Mycology – Comprehensive permit.

ANTIGEN DETECTION: This category is for laboratories that perform direct antigen detection methods.

MOLECULAR METHODS: This category is for laboratories that use FDA-approved or lab-developed molecular methods for detecting, identifying, typing, characterizing or determining drug resistance of infectious agents. Laboratories using molecular methods under another Restricted permit category (e.g. Restricted: Antigen detection) or those holding a Comprehensive category permit, do not need to request this molecular method category.

OTHER: This category is for laboratories that perform only specialized tests such as KOH mounts, wet mounts, PNA-FISH or any other mycology test not covered in the categories above or when no New York State proficiency test is available.

PROFICIENCY TESTING ANALYTES OFFERED

(CMS regulated analytes or tests are indicated with an asterisk)

COMPREHENSIVE

- Culture and Identification*
- Susceptibility testing
- *Cryptococcus neoformans* Antigen Detection

RESTRICTED

Identification Yeast Only

- Culture and Identification of yeast*
- Susceptibility testing of yeasts and molds

Antigen Detection

- Antigen detection of Cryptococcus neoformans*

Molecular Methods

- No proficiency testing is offered at this time.

TEST SPECIMENS & GRADING POLICY

Test Specimens

At least two strains of each mold or yeast specimens are examined for inclusion in the proficiency test event. The colony morphology of molds is studied on Sabouraud dextrose agar. The microscopic morphologic features are examined by potato dextrose agar slide cultures. The physiological characteristics such as cycloheximide sensitivity and growth at higher temperatures are investigated with appropriate test media. The strain that best demonstrates the morphologic and physiologic characteristics typical of the species is included as a test analyte. Similarly, two or more strains of yeast species are examined for inclusion in the proficiency test. The colony morphology of all yeast strains is studied on corn meal agar with Tween 80 plates inoculated by Dalmau or streak-cut method. Carbohydrate assimilation is studied with the API 20C AUX identification kit (The use of brand and/or trade names in this report does not constitute an endorsement of the products on the part of the Wadsworth Center or the New York State Department of Health). The fermentations of carbohydrates, i.e., glucose, maltose, sucrose, lactose, trehalose, and cellobiose, are also documented using classical approaches. Additional physiologic characteristics such as nitrate assimilation, urease activity, and cycloheximide sensitivity are investigated with the appropriate test media. The strain that best demonstrates the morphologic and physiologic characteristics of the proposed test analyte, is included as test analyte. The morphologic features are matched with molecular identification using PCR and nucleotide sequencing of ribosomal ITS1 – ITS2 regions.

Grading Policy

A laboratory's response for each sample is compared with the responses that reflect 80% agreement of 10 referee laboratories and/or 80% of all participating laboratories. The referee laboratories are selected at random from among hospital laboratories participating in the program. They represent all geographical areas of New York State and must have a record of excellent performance during the preceding three years. The score in each event is established by total number of correct responses submitted by the laboratory divided by the number of organisms present plus the number of incorrect organisms reported by the laboratory multiplied by 100 as per the formula shown on the next page.

$$\frac{\text{\# of acceptable responses} \times 100}{\text{\# of fungi present + \# incorrect responses}}$$

For molds and yeast specimens, a facility can elect to process only those analytes that match the type of clinical materials included within the scope of the facility's standard operating procedures (SOP). Similarly, the participating laboratory can elect to provide only genus level identification if it reflects the SOP for patient testing in the concerned facility. In all such instances, a maximum score of 100 will be equally distributed among the number of test analytes selected by the laboratory. The rest of the score algorithm will be similar to the aforementioned formula.

Acceptable results for antifungal susceptibility testing are based on consensus/references laboratories MIC values within +/- 2 dilutions and interpretation per CLSI (NCCLS) guidelines or related, peer-reviewed publications. One yeast and/or mold is to be tested against following drugs: amphotericin B, anidulafungin, caspofungin, flucytosine (not for molds), fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. The participating laboratories are free to select any number of antifungal drugs from the test panel based upon test practices in their facilities. A maximum score of 100 is equally distributed to account for the drugs selected by an individual laboratory. If the result for any drug is incorrect then laboratory gets a score of zero for that particular test component or set.

For *Cryptococcus neoformans* antigen test, laboratories are evaluated on the basis of their responses and on overall performance for all the analytes tested in the Direct Detection category. The maximum score for this event is 100. Appropriate responses are determined by 80% agreement among participant responses. Target values and acceptable ranges are mean value +/- 2 dilutions; positive or negative answers will be acceptable from laboratories that do not report antigen titers. When both qualitative and quantitative results are reported for an analyte, ten points are deducted for each incorrect result. When only qualitative OR quantitative results are reported, twenty points are deducted from each incorrect result.

A failure to attain an overall score of 80% is considered unsatisfactory performance. Laboratories receiving unsatisfactory scores in two out of three consecutive proficiency test events may be subject to 'cease testing'.

TEST ANALYTE MASTER LISTS

Mold Master List

The mold master list is intended to provide guidance to the participating laboratories about the scope of the Mycology (Comprehensive) Proficiency Testing Program. The list includes most common pathogenic and non-pathogenic fungi likely to be encountered in the laboratory. The list is compiled from published peer-reviewed reports as well as current practices in other proficiency testing programs. This list is meant to illustrate acceptable identification used in grading of responses received after each test event. However, the laboratory can elect to provide only genus level identification if it reflects the standard operating procedures (SOP) for patient testing. This list does not include all molds that might be encountered in a clinical laboratory nor is it intended to be used for competency assessment of laboratory personnel in diagnostic mycology.

The nomenclature used in the mold master list is based upon currently recognized species in published literature, monographs and in catalogues of recognized culture collections. No attempt has been made to include teleomorphic states of fungi if they are not routinely encountered in the clinical specimens. Where appropriate, current nomenclature has been included under parentheses to indicate that commonly accepted genus and/or species name is no longer valid, e.g. *Phaeoannellomyces werneckii* (*Hortea werneckii*). These guidelines supersede any previous instructions for identification of molds. The list is subject to change in response to significant changes in nomenclature, human disease incidence or other factors.

Absidia corymbifera
Absidia species
Acremonium species
Alternaria species
Arthrographis species
Aspergillus clavatus
Aspergillus flavus
Aspergillus fumigatus species complex
Aspergillus glaucus
Aspergillus glaucus group
Aspergillus nidulans
Aspergillus niger
Aspergillus species
Aspergillus terreus
Aspergillus versicolor
Aureobasidium pullulans
Aureobasidium species
Basidiobolus ranarum
Beauveria species
Bipolaris species
Blastomyces dermatitidis
Chaetomium globosum
Chaetomium species
Chrysosporium species
Cladophialophora bantiana
Cladophialophora boppii
Cladophialophora carrionii species complex
Cladophialophora species
Cladosporium species
Coccidioides immitis
Coccidioides species
Cokeromyces recurvatus
Conidiobolus coronatus
Cunninghamella bertholletiae
Cunninghamella species
Curvularia species
Drechslera species
Emmonsia parva
Epicoccum species
Epidermophyton floccosum
Exophiala (Wangiella) dermatitidis
Exophiala jeanselmei species complex
Exophiala species
Exserohilum species
Fonsecaea species
Fusarium oxysporum species complex
Fusarium solani species complex
Fusarium species
Gliocladium species
Helminthosporium species
Histoplasma capsulatum
Hormonema dematioides
Malbranchea species
Microsporum audouinii
Microsporum canis
Microsporum cookei
Microsporum gypseum species complex
Microsporum nanum
Microsporum persicolor

Microsporum species
Mucor circinelloides
Mucor plumbeus
Mucor racemosus
Mucor species
Nigrospora species
Paecilomyces lilacinus
Paecilomyces species
Paecilomyces variotii
Penicillium marneffei
Penicillium species
Phaeoannellomyces werneckii (Hortaea werneckii)
Phialophora richardsiae
Phialophora species
Phialophora verrucosa species complex
Phoma species
Pithomyces species
Pseudallescheria boydii species complex
Pseudallescheria species
Rhizomucor pusillus
Rhizomucor species
Rhizopus oryzae
Rhizopus species
Scedosporium apiospermum (Pseudallescheria apiospermum)
Scedosporium prolificans (inflatum)
Scedosporium species
Scopulariopsis brevicaulis
Scopulariopsis brumptii
Scopulariopsis species
Scytalidium hyalinum
Scytalidium species
Sepedonium species
Sporothrix schenckii species complex
Stachybotrys atra (chartarum / alternans)
Stachybotrys species
Syncephalastrum racemosum
Syncephalastrum species
Trichoderma species
Trichophyton ajelloi
Trichophyton interdigitale
Trichophyton mentagrophytes species complex
Trichophyton rubrum
Trichophyton schoenleinii
Trichophyton species
Trichophyton terrestre
Trichophyton tonsurans
Trichophyton verrucosum
Trichophyton violaceum
Trichothecium species
Ulocladium species
Ustilago species
Verticillium species

Yeast Master List

The yeast master list is intended to provide guidance to the participating laboratories about the scope of the Mycology - Restricted to Yeasts Only Proficiency Testing Program. This list includes most common pathogenic and non-pathogenic yeasts likely to be encountered in the clinical laboratory. The list is compiled from published peer-reviewed reports as well as current practices in other proficiency testing programs. The list is meant to illustrate acceptable identifications used in grading of responses received after each test event. However, the laboratory can elect to provide only genus level identification if it reflects the standard operating procedures (SOP) for patient testing. This list does not include all yeasts that might be encountered in a clinical laboratory nor is it intended to be used for competency assessment of the laboratory personnel in diagnostic mycology.

The nomenclature used in this list is based upon currently recognized species in published literature, monographs, and catalogues of recognized culture collections. No attempt has been made to include teleomorphic states of fungi if they are not routinely encountered in the clinical specimens. Where appropriate, current nomenclature has been included under parentheses to indicate that commonly accepted genus and/or species name is no longer valid, e.g. *Blastoschizomyces capitatus* (*Geotrichum capitatum*). These guidelines supersede any previous instructions for identification of yeasts. The list is subject to change in response to significant changes in nomenclature, human disease incidence or other factors.

Blastoschizomyces capitatus (Geotrichum capitatum)
Blastoschizomyces species
Candida *albicans*
Candida dubliniensis
Candida famata
Candida glabrata
Candida guilliermondii species complex
Candida kefyr
Candida krusei
Candida lipolytica (Yarrowia lipolytica)
Candida lusitaniae
Candida norvegensis
Candida parapsilosis species complex
Candida rugosa
Candida species
Candida tropicalis
Candida viswanathii
Candida zeylanoides
Cryptococcus albidus
Cryptococcus gattii
Cryptococcus laurentii
Cryptococcus neoformans
Cryptococcus neoformans-Cryptococcus gattii species complex
Cryptococcus species
Cryptococcus terreus
Cryptococcus uniguttulatus
Geotrichum candidum
Geotrichum species
Hansenula anomala (Candida pelliculosa)
Malassezia furfur
Malassezia pachydermatis
Malassezia species
Pichia ohmeri (Kodamaea ohmeri)
Prototheca species
Prototheca wickerhamii
Prototheca zopfii
Rhodotorula glutinis
Rhodotorula minuta
Rhodotorula mucilaginosa (rubra)
Rhodotorula species
Saccharomyces cerevisiae
Saccharomyces species
Sporobolomyces salmonicolor
Trichosporon asahii
Trichosporon inkin
Trichosporon mucoides
Trichosporon species

Summary of Laboratory Performance:

Mycology – Mold

	Specimen key	Validated specimen	Acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
M-1	*Acremonium* species	*Acremonium* species		63/66 (95%)
M-2	*Alternaria* species	*Alternaria* species		65/66 (98%)
M-3	*Cladophialophora boppii*	*Cladophialophora boppii*	*Cladophialophora* species *Cladophialophora carrionii* species complex	61/66 (92%)
M-4	*Mucor* species	*Mucor* species	*Mucor circinelloides* *Mucor racemosus*	62/66 (93%)
M-5	*Curvularia* species	*Curvularia* species		66/66 (100%)

Mycology – Yeast Only

	Specimen key	Validated specimen	Acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
Y-1	*Candida guilliermondii*	*Candida guilliermondii*		52/58 (90%)
Y-2	*Candida lusitaniae*	*Candida lusitaniae*		57/58 (98%)
Y-3	*Saccharomyces cerevisiae*	*Sacharomyces cerevisiae*		58/58 (100%)
Y-4	*Candida pelliculosa*	*Candida pelliculosa*		57/58 (98%)
Y-5	*Prototheca wickerhamii*	*Prototheca wickerhamii*	*Prototheca* species	57/58 (98%)

Mycology – Direct detection (*Cryptococcus* Antigen Test)

	Specimen key (Titer)	Validated specimen	Correct responses / Total laboratories (% correct responses)	
			Qualitative	Quantitative
Cn-Ag-1	Positive (1:128)	Positive (1:128)	69/69 (100%)	63/64 (98%)
Cn-Ag-2	Negative	Negative	68/69 (99%)	NA
Cn-Ag-3	Positive (1:16)	Positive (1:16)	69/69 (100%)	NA
Cn-Ag-4	Negative	Negative	69/69 (100%)	69/69 (100%)
Cn-Ag-5	Negative	Negative	69/69 (100%)	NA

Antifungal Susceptibility Testing for Yeast (S-1: *Candida glabrata* M956)

Drugs	Acceptable MIC (μg/ml) Range	Acceptable interpretation	Laboratories with acceptable responses/ Total laboratories (% correct responses)
Amphotericin B	0.12 – 1	Susceptible / No interpretation	23/23 (100%)
Anidulafungin	0.015 – 0.03	Susceptible	17/17 (100%)
Caspofungin	0.06 – 1	Susceptible	22/22 (100%)
Flucytosine (5-FC)	0.03 – 0.125	Susceptible	26/26 (100%)
Fluconazole	≥ 64	Resistant	29/32 (91%)
Itraconazole	≥ 1	Resistant	28/30 (93%)
Ketoconzole	0.5 – 8	No interpretation	5/5 (100%)
Micafungin	0.008 – 0.03	Susceptible	17/17 (100%)
Posaconazole	2 – 32	Susceptible-dose dependent / Resistant / No interpretation	17/18 (94%)
Voriconazole*	2 – 8	Susceptible-dose dependent / Resistant	18/24 (75%)

*This analyte is not validated as less than 80% participants reported acceptable results.

Antifungal Susceptibility Testing for Mold (MS-1: *Aspergillus fumigatus* M2039)

Drugs	Acceptable MIC (μg/ml) Range	Laboratories within MIC range / Total laboratories (%)
Amphotericin B	0.06 – 1.0	6/6 (100%)
Anidulafungin*	0.008 – 0.12	3/4 (75%)
Caspofungin	0.008 – 1.0	4/5 (80%)
Fluconazole	≥ 64	5/5 (100)
Itraconazole	2.0 – 16	6/6 (100)
Ketoconzole	4.0 – 64	2/2 (100%)
Micafungin*	0.008 – 0.12	3/4 (75%)
Posaconazole	0.06 – 1.0	5/5 (100)
Voriconazole	0.25 – 2.0	5/5 (100)

*This analyte is not validated as less than 80% participant reported acceptable results.

Commercial Device Usage Statistics:

(Commercial devices/ systems/ methods used for fungal identification, susceptibility testing or antigen detection)

Device	No. laboratories
Yeast Identification*	
API 20C AUX	46
AMS Vitek	4
Vitek2	23
Remel Uni-Yeast-Tek	6
Microscan	1
API 20C AUX	46
Antifungal Susceptibility*	
YeastOne -Yeast	26
YeastOne - Mold	3
Etest	3
Disk diffusion	1
Vitek2	1
Others† - Yeast	3
Others† - Mold	3
Cryptococcal antigen	
Immuno-Mycologics	10
Meridien Diagnostics	49
Remel	10

*Include multiple systems used by some laboratories
†Include laboratories using CLSI Microbroth dilution method

MOLD DESCRIPTIONS

M-1. *Acremonium* species

Source: Nail / Tissue / Sinus

CLINICAL SIGNIFICANCE: *Acremonium* spp. cause onychomycosis, keratitis, endophthalmitis, endocarditis, meningitis, peritonitis, and osteomyelitis especially in the immunocompromised patients.

COLONY: *Acremonium* sp. grew at moderate rate (Figure 1). The colonies were powdery to velvety, white to pale pink, reverse pale to yellowish on Sabouraud's dextrose agar, 25°C.

MICROSCOPY: Lactophenol - Cotton blue mount showed hyaline, narrow, septate hyphae often in form of fascicle (bundles). Phialides were unbranched and solitary. Oblong to ovoid, unicellular conidia were born at the apices of the phialides (Figure 1).

DIFFERENTIATION: *Acremonium* spp. can be confused with non-macroconidia producing species of *Fusarium* and *Verticillium* strains, which produce solitary phialides. Both *Fusarium* and *Verticillium* spp. grow faster than *Acremonium* and produce deeply woolly colonies. *Acremonium* spp. can be distinguished from *Lecythophora* and *Phialemonium* spp. by the presence of septa between the base of phialides and hyphae. *Gliomastix* spp. are different from *Acrmonium* spp. by their olive-green to greenish-black colonies and chains or balls of dark conidia.

MOLECULAR TEST: Internal transcribed spacer (ITS) regions can be used for the identification of *Acremonium* spp.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Acremonium strictum* isolate 27 (GenBank accession no. HM016899.1).

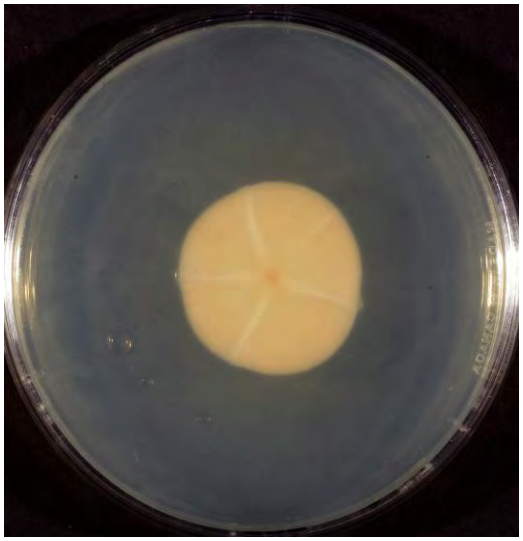
ANTIFUNGAL SUSCEPTIBILITY: *Acremonium* spp. are susceptible to amphotericin B, caspofungin, voriconazole, and posaconazole, but resistant to fluconazole, 5-flucytosine, and itraconazole.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	63
Laboratories with incorrect ID:	03
(*Fusarium solani* species complex)	(1)
(*Fusarium* spp.)	(1)
(*Verticillium* spp.)	(1)

Illustrations:

FIGURE 1. (Upper panel) Seven-day-old, powdery to velvet, white to pinkish colony of *Acremonium* sp. on Sabouraud's dextrose agar; the reverse of colony appears pale to yellow. Microscopic morphology of *Acremonium* sp. showing septate, rope-like hyphae, unbranched phialides, and unicellular conidia accumulated in heads at the apices of the phialides (bar = 10 μm; lower panel).



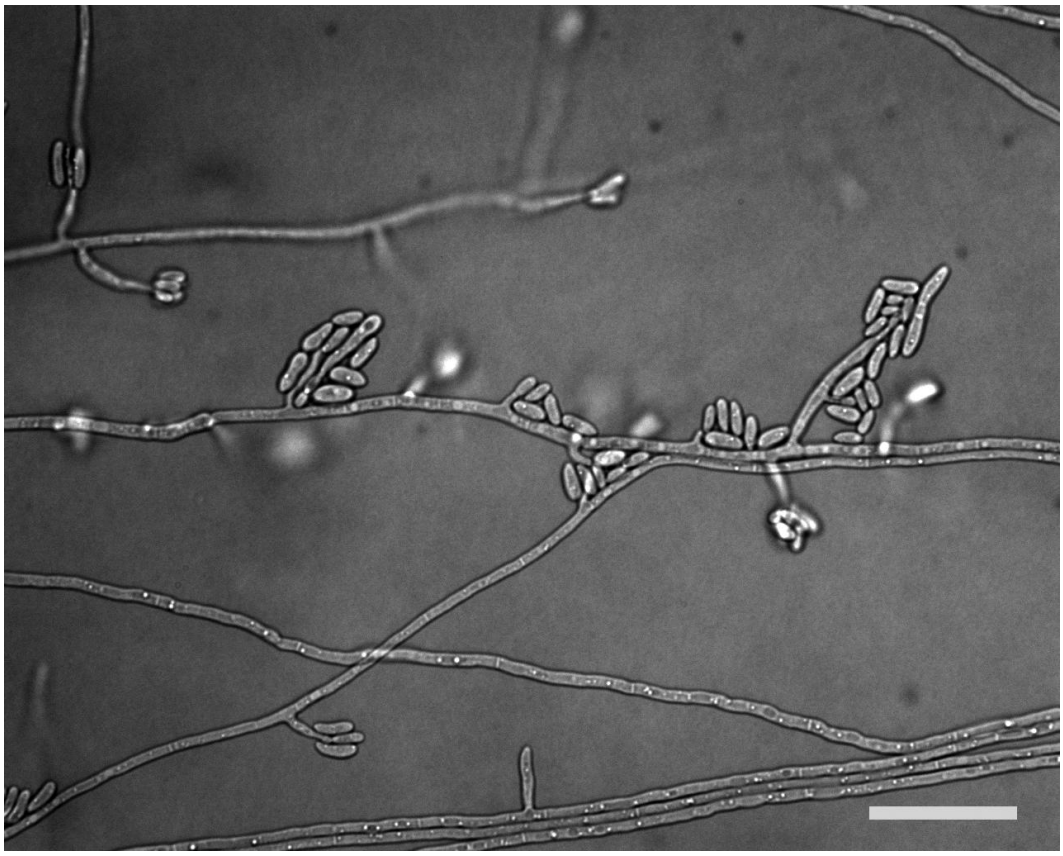
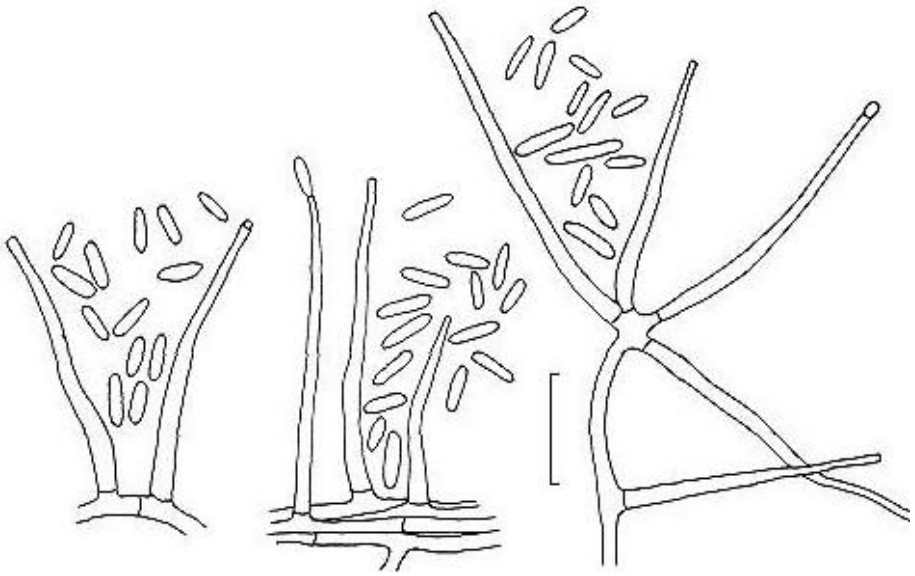


FIGURE 1A. Line drawing to highlight characteristic microscopic features of *Acremonium strictum*.



(http://www.mycobank.org/Biolomics.aspx?Table=Mycobank&MycoBankNr_=308201)

Further reading:

Chang YH, Huang LM, Hsueh PR, Hsiao CH, Peng SF, Yang RS, Lin KH. 2005. *Acremonium pyomyositis* in a pediatric patient with acute leukemia. *Pediatr Blood Cancer*. 44: 521-524.

Creti A, Esposito V, Bocchetti M, Baldi G, De Rosa P, Parrella R, Chirianni A. 2006. Voriconazole curative treatment for *Acremonium* species keratitis developed in a patient with concomitant *Staphylococcus aureus* corneal infection: a case report. *In Vivo.* 20: 169-171.

Doczi I, Dosa E, Varga J, Antal Z, Kredics L, Nagy E. 2004. Etest for assessing the susceptibility of filamentous fungi. *Acta Microbiol Immunol Hung.* 51: 271-81.

Foell JL, Fischer M, Seibold M, Borneff-Lipp M, Wawer A, Horneff G, Burdach S. 2007. Lethal double infection with *Acremonium strictum* and *Aspergillus fumigatus* during induction chemotherapy in a child with ALL. *Pediatr Blood Cancer.* 49: 858-861.

Garcia-Effron G, Gomez-Lopez A, Mellado E, Monzon A, Rodriguez-Tudela JL, Cuenca-Estrella M. 2004. *In vitro* activity of terbinafine against medically important non-dermatophyte species of filamentous fungi. *J Antimicrob Chemother*. 53: 1086-1089.

Guarro J, Del Palacio A, Gené J, Cano J, González CG. 2009. A case of colonization of a prosthetic mitral valve by *Acremonium strictum*. *Rev Iberoam Micol.* 26: 146-148.

Joe SG, Lim J, Lee JY, Yoon YH. 2010. Case report of *Acremonium* intraocular infection after cataract extraction. *Korean J Ophthalmol*. 24: 119-122.

Pastorino AC, Menezes, UP, Marques HH, Vallada MG, Cappellozi, VL, Carnide EM, Jacob CM. 2005. *Acremonium kiliense* infection in a child with chronic granulomatous disease. *Braz J Infect Dis*. 9: 529-534.

Perdomo H, Sutton DA, García D, Fothergill AW, Cano J, Gené J, Summerbell RC, Rinaldi MG, Guarro J. 2011. Spectrum of clinically relevant *Acremonium* species in the United States. *J Clin Microbiol*. 49: 243-56.

Summerbell RC, Gueidan C, Schroers H-J, de Hoog GS, Starink M, Arocha Rosete Y, Guarro J, Scott JA. 2011. *Acremonium* phylogenetic overview and revision of *Gliomastix*, *Sarocladium*, and *Trichothecium*. *Stud Mycol*. 68: 139–162.

M-2 *Alternaria* species

Source: Chest / Toenail

CLINICAL SIGNIFICANCE: *Alternaria* spp. cause onychomycosis, ulcerated cutaneous infection, and keratitis. They are also important causal agents of occupational respiratory allergy.

COLONY: *Alternaria* colony was fast growing, pale gray or dark olive-green, with white fringe, wooly texture on Sabouraud's dextrose agar, after 7 days at 25°C. The reverse was brown to black (Figure 2).

MICROSCOPY: Lactophenol-- Cotton blue mount showed darkly pigmented, septate hyphae. Conidiophores were brown, septate, simple or branched, and darkly pigmented. Conidia were large, smooth or rough, and had both horizontal and transverse septa termed "muriform"(brick-wall pattern). They were club-shaped or elliptical and produced in chain described as "beaked" (Figure 2).

DIFFERENTIATION: *Alternaria* spp. have dark brown or dark olive green colony with a white fringe, and large club-shaped muriform conidia produced in chains. This makes it readily distinguishable from other molds.

MOLECULAR TEST: PCR diagnostic tests targeting the ribosomal DNA internal transcribed spacer (ITS) regions of *Alternaria* spp., have been described. *Alternaria alternata* major allergen Alt a**1** has been produced as a recombinant protein for standardization of antigen for skin testing.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Alternaria alternata* isolate MY9_3 (GenBank accession no. JQ697510.1).

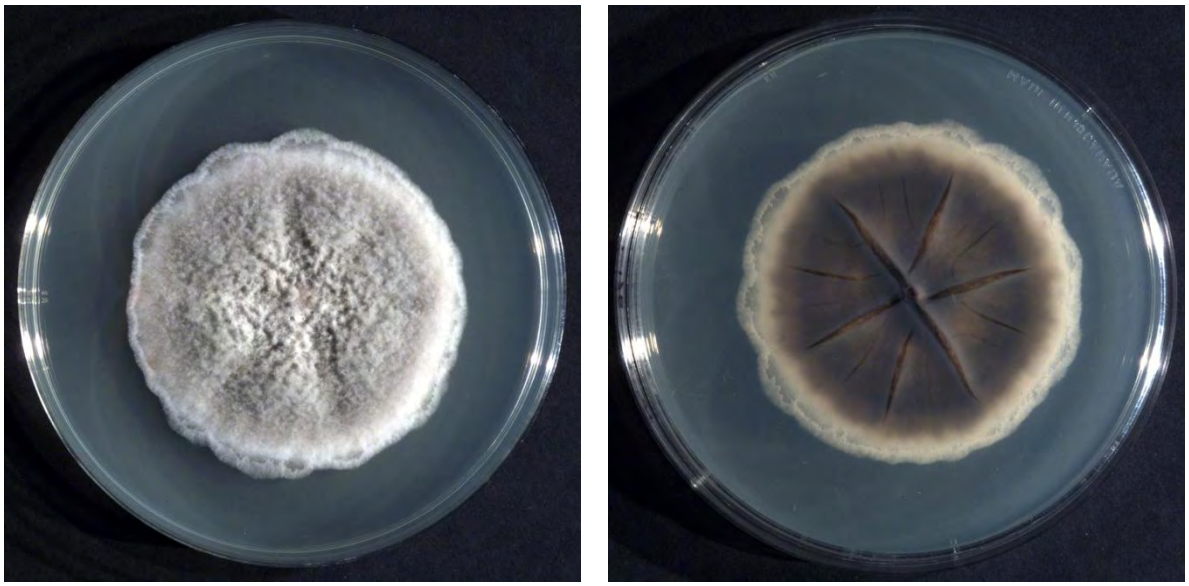
ANTIFUNGAL SUSCEPTIBILITY: In general, *Alternaria* species are susceptible to miconazole and ketoconazole, but the activities of itraconazole, amphotericin B, and fluconazole are variable among different strains. All of the isolates are resistant to flucytosine.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	65
Laboratories with incorrect ID:	01
(*Ulocladium* sp.)	(1)

Illustrations:

FIGURE 2. Seven-day-old, pale gray colony of *Alternaria* sp. with white fringe and wooly surface, on Sabouraud's dextrose agar, 25°C; the reverse is pale to black (Upper panels). Microscopic morphology of *Alternaria* sp. showing septate hyphae, darkly pigmented. club-shaped conidia with both horizontal and transverse septa in chain (bar = 10 μm; lower panel).



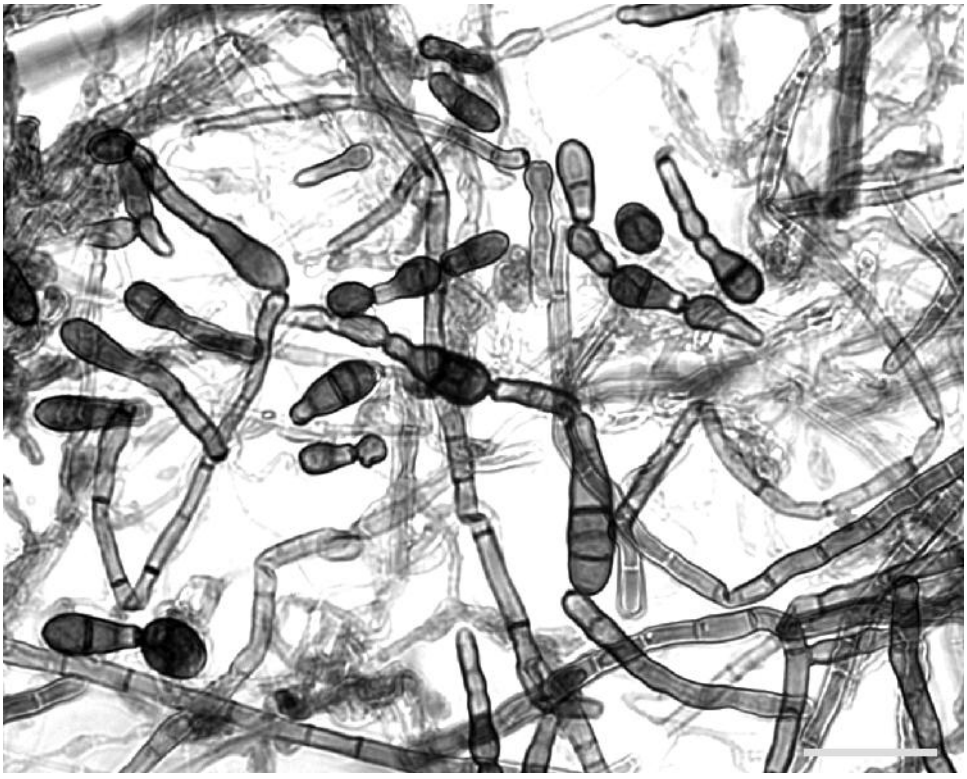
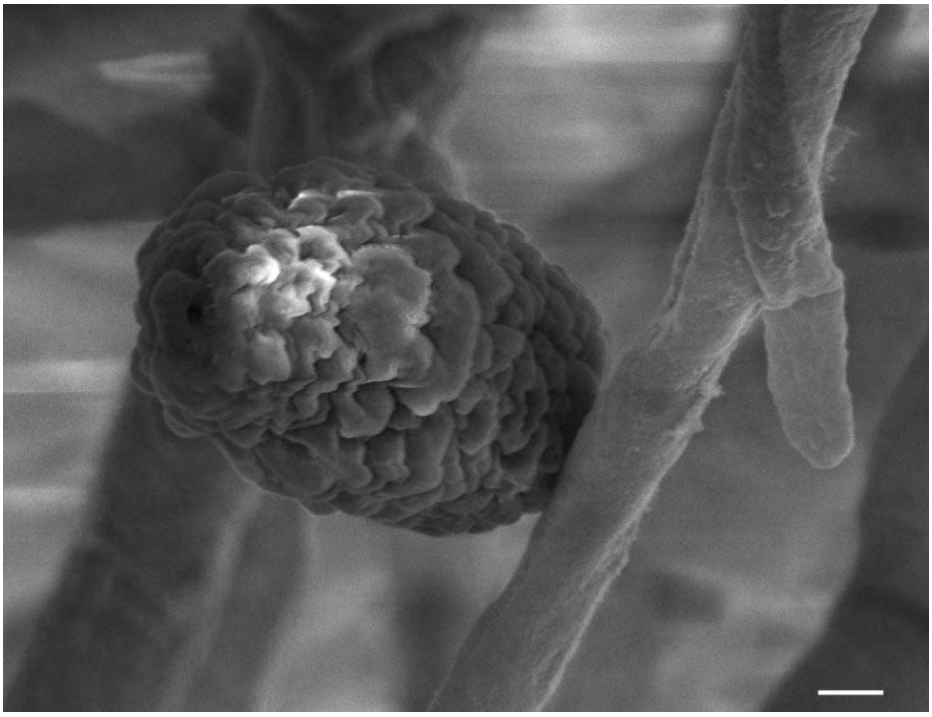
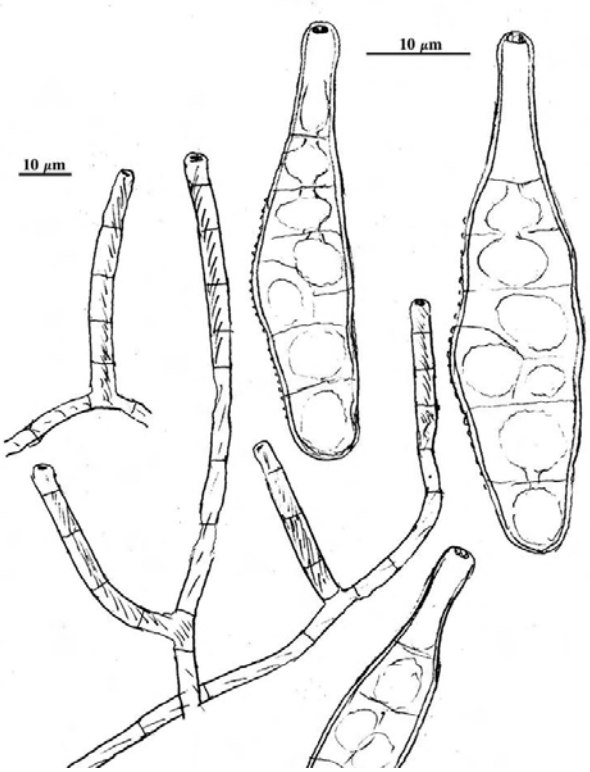


FIGURE 2A. Scanning electron micrograph of conidia and conidiophores of *Alternaria alternata* on Sabouraud's dextrose agar; (Bar = 1 μm; upper panel). Line drawings of *Alternaria alternata* (lower panel).





http://www.mycobank.org/BioloMICS.aspx?Table=Mycobank&Rec=900&Fields=All

Further reading:

Alastruey-Izquierdo A, Cuesta I, Ros L, Mellado E, Rodriguez-Tudela JL. 2011. Antifungal susceptibility profile of clinical *Alternaria* spp. identified by molecular methods. *J Antimicrob Chemother*. 66: 2585-2587.

Benito N, Moreno A, Puig J, Rimola A. 2001. Alternariosis after liver transplantation. *Transplantation* 72: 1840-1843.

Boyce RD, Deziel PJ, Otley CC, Wilhelm MP, Eid AJ, Wengenack NL, Razonable RR. 2010. Phaeohyphomycosis due to *Alternaria* species in transplant recipients. *Transpl Infect Dis.* 12: 242-250.

Downs SH, Mitakakis TZ, Marks GB, Car NG, Belousova EG, Leuppi JD, Xuan W, Downie SR, Tobias A, Peat JK. 2001. Clinical importance of *Alternaria* exposure in children. *Am. J. Respir. Crit. Care Med.*164: 455-459.

Ferrer C, Munoz G, Alio JL, Abad JL, Colomm, F. 2002. Polymerase chain reaction diagnosis in fungal keratitis caused by *Alternaria alternata. Am J Ophthalmol.*133: 398-399.

de Hoog GS, Horré R. 2002. Molecular taxonomy of the *Alternaria* and *Ulocladium* species from humans and their identification in the routine laboratory. *Mycoses*. 45: 259-276.

Neoh CF, Leung L, Vajpayee RB, Stewart K, Kong DC. 2011. Treatment of *Alternaria* keratitis with intrastromal and topical caspofungin in combination with intrastromal, topical, and oral voriconazole. *Ann Pharmacother*. 45: e24.

Ozbek Z, Kang S, Sivalingam J, Rapuano CJ, Cohen EJ, Hammersmith KM. 2006. Voriconazole in the management of *Alternaria keratitis. Cornea.* 25: 242-244.

Rammaert B, Aguilar C, Bougnoux ME, Noël N, Charlier C, Denis B, Lecuit M, Lortholary O. 2011. Success of posaconazole therapy in a heart transplanted patient with *Alternaria infectoria* cutaneous infection. *Med Mycol.* [Epub ahead of print]

Robert T, Talarmin JP, Leterrier M, Cassagnau E, Pape PL, Danner-Boucher I, Malard O, Brocard A, Gay-Andrieu F, Miegeville M, Morio F. 2012. Phaeohyphomycosis due to *Alternaria infectoria*: a single-center experience with utility of PCR for diagnosis and species identification. *Med Mycol.* [Epub ahead of print]

M-3 *Cladophialophora boppii*

Source: Skin / Wound

CLINICAL SIGNIFICANCE: *Cladophialophora* spp. are causative agents of phaeohyphomycosis, chromoblastomycosis, and mycetoma. *Cladophialophora boppii* usually cause skin lesions.

COLONY: *Cladophialophora boppii* grew slowly on Sabouraud's dextrose agar, after 20 days at 30°C (Figure 3). The colony was grey to black with 'downy' appearance and olivaceous to black reverse.

MICROSCOPY: Lactophenol - Cotton blue mount showed dark brown, septate hyphae. Conida fromed in long, unbranched chains (Figure 3).

DIFFERENTIATION: *Cladophialophora boppii* is differentiated from other *Cladophialophora* species by production of long chains of globose conidia, no growth at 37°C.

MOLECULAR TEST: PCR targeting ribosomal DNA internal transcribed spacer regions was used for rapid and more specific identification of different species of *Cladophialophora.*

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Cladophialophora boppii* isolate ATCC MYC-4778 (GenBank accession no. JN882312.1).

ANTIFUNGAL SUSCEPTIBILITY: *C. boppii* is susceptible to amphotericin B, itraconazole, ketoconazole, but resistant to fluconazole.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	37
Other acceptable answers:	24
Cladophialophora carrionii species complex	03
Cladophialophora species	21
Laboratories with incorrect ID:	05
(*Cladosporium* spp.)	(5)

Illustrations:

FIGURE 3. Twenty-day-old, black, 'downy' colony of *Cladophialophora boppii* on Sabouraud's dextrose agar, 25°C; the reverse is dark-olive to black (Upper panel). Microscopic morphology of *Cladophialophora boppii* showing conida in chain (bar = 10 μm; lower panel).

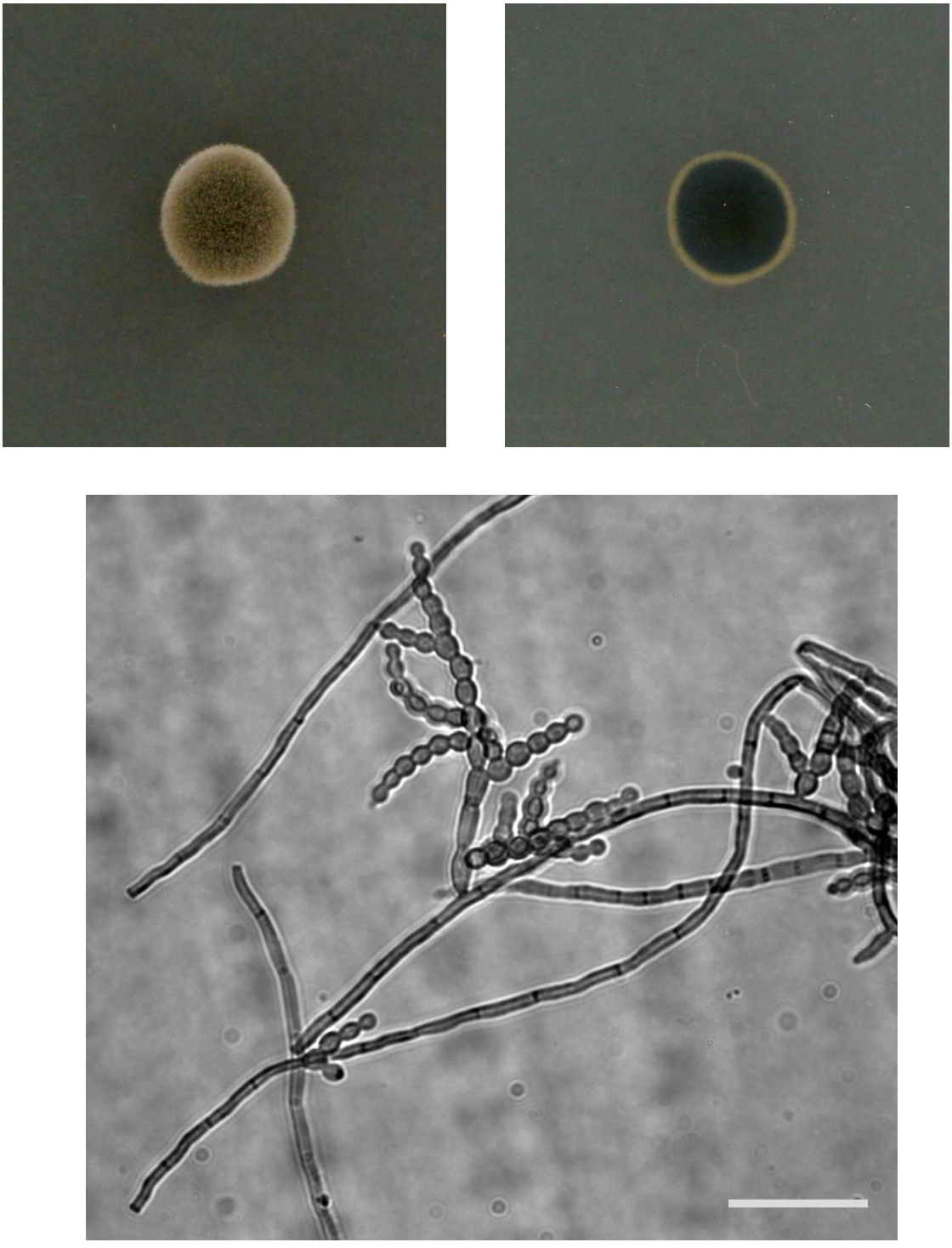
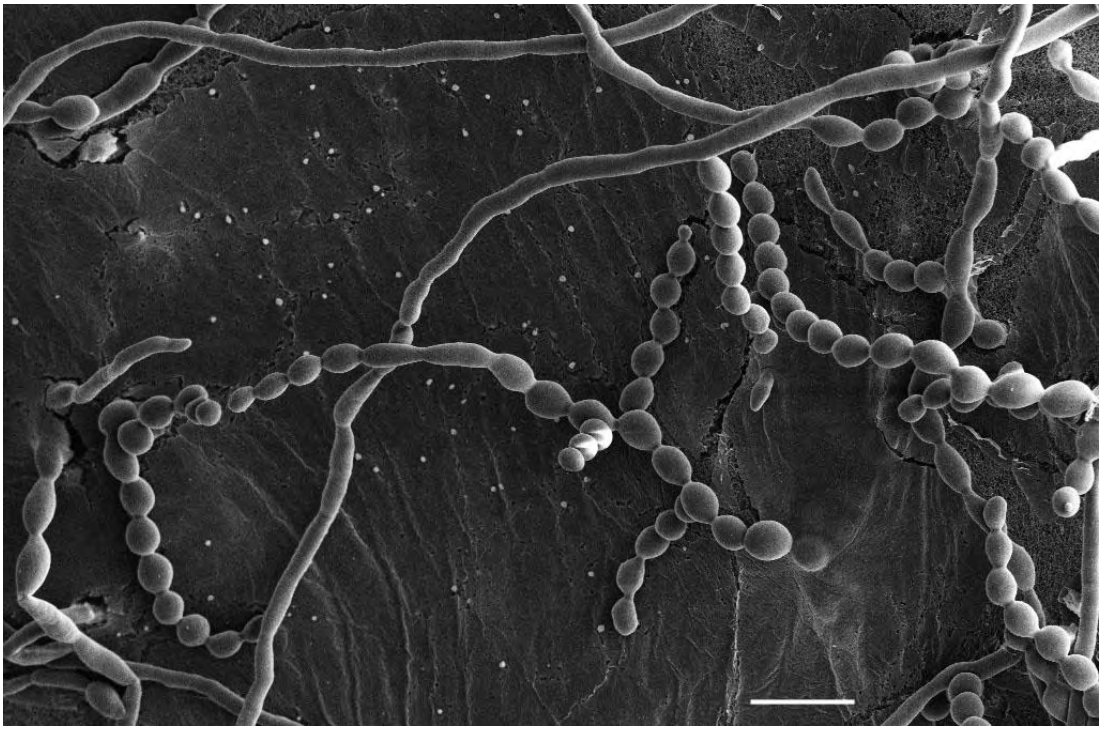
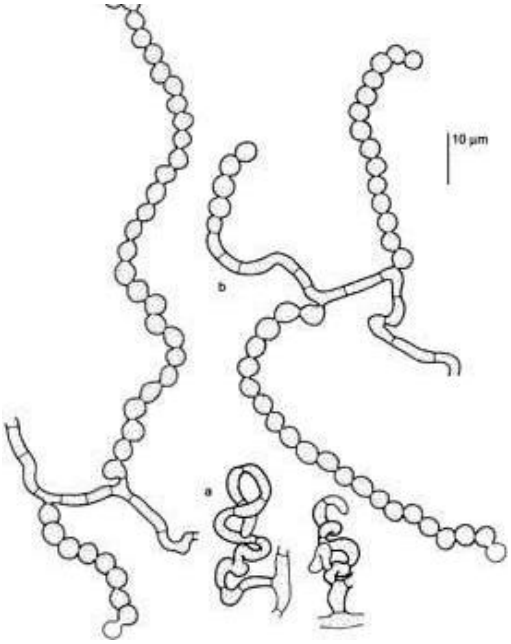


FIGURE 3A. Scanning electron micrograph of *Cladophialophora boppii* with conidia and conidiophores (bar = 10 μm; upper panel). Line drawing with details of *Cladophialophora boppii* (lower panel).





http://www.mycobank.org/Biolomics.aspx?Table=Mycobank&MycoBankNr_=412792

Further reading:

Brasch J, Dressel S, Müller-Wening K, Hügel R, von Bremen D, de Hoog GS. 2011. Toenail infection by *Cladophialophora boppii*. *Med Mycol*. 49: 190-193.

Lastoria C, Cascina A, Bini F, Di Matteo A, Cavanna C, Farina C, Carretto E, Meloni F. 2009. Pulmonary *Cladophialophora boppii* infection in a lung transplant recipient: case report and literature review. *J Heart Lung Transplant.* 28: 635-637.

Pereira RR, Nayak CS, Deshpande SD, Bhatt KD, Khatu SS, Dhurat RS. 2010. Subcutaneous phaeohyphomycosis caused by *Cladophialophora boppii*. *Indian J Dermatol Venereol Leprol*. 76: 695-698.

Saunte DM, Tarazooie B, Arendrup MC, de Hoog GS. 2012. Black yeast-like fungi in skin and nail: it probably matters. *Mycoses.* 55: 161-167.

M-4 *Mucor* species

Source: Sputum / Foot

CLINICAL SIGNIFICANCE: *Mucor* spp. are widely dispersed in nature, but rare cause of human disease. A number of species namely *M. circinelloides, M. ellipsoideus, M. indicus*, *M. hiemalis, M. ramosissimus* and *Mucor velutinosus* are reported as causal agents of cutaneous and systemic mycoses.

COLONY: *Mucor* sp. grew rapidly on Sabouraud's dextrose agar. After 5 days at 25°C, the colony was grayish on surface, very wooly, covering the entire Petri dish (Figure 4).

MICROSCOPY: Lactophenol - Cotton blue mount showed *Mucor* sp. had hyaline hyphae, which were broad and predominantly aseptate. The long and straight sporangiophores arose irregularly from the hyphae - branched or unbranched. Sporangia with columellas lacked apophyses. Rhizoids and stolons were absent (Figure 4).

DIFFERENTIATION: *Mucor* differs from *Rhizopus* and *Rhizomucor* by absence of rhizoids, and from *Absidia* by absence of an apophysis beneath the sporangium. The maximum temperature for growth in *Mucor* is less than 37°C, but *Rhizomucor* can grow at about 54°C.

MOLECULAR TEST: Internal transcribed spacers (ITS1 and ITS2) sequences were used for molecular identification of *Mucor* spp. and other closely related zygomycetes.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Mucor velutinosus* isolate ATCC MYA-4766 (GenBank accession no. JN882307.1).

ANTIFUNGAL SUSCEPTIBILITY: None of the triazoles were active against *Mucor* spp. (MIC_{50}>8 μg/ml), but some species of *Mucor* were reported to be susceptible to Amphotericin B.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	47
Other acceptable answers:	15
Mucor circinelloides	09
Mucor racemosus	06
Laboratories with incorrect ID:	04
(*Cunninghamella* spp.)	(3)
(*Rizomucor* spp.)	(1)

Illustrations:

FIGURE 4. Five-day-old, grayish and very wooly colony of *Mucor* sp. on Sabouraud's dextrose agar, 25°C; the reverse of the colony appears pale yellow (Upper panel). Microscopic morphology of *Mucor* sp. showing hyaline aseptate hyphae. Sporangia with columella that lack apophyses (bar = 10 μm; lower panel)

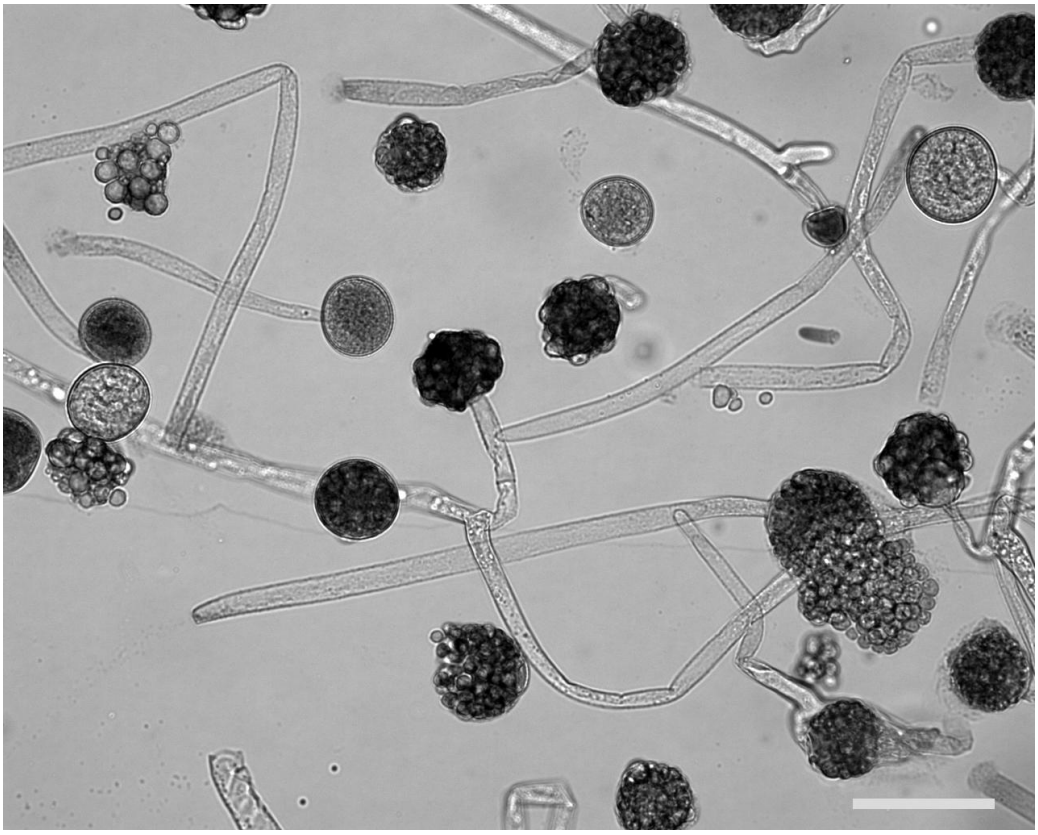
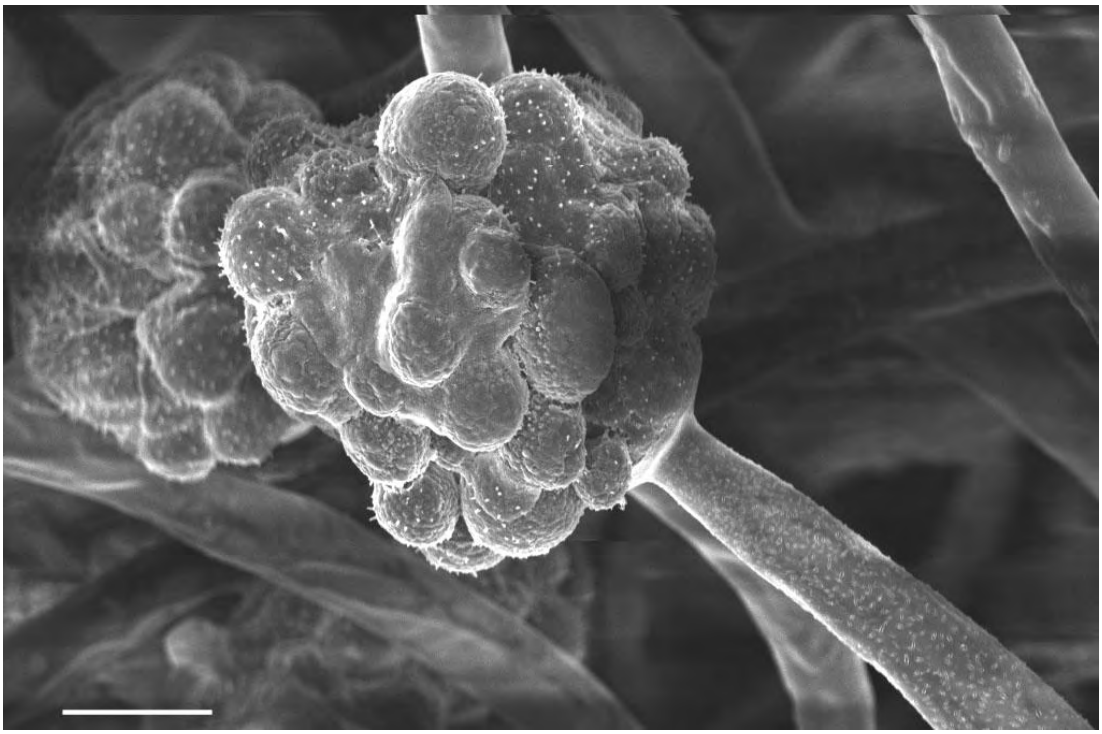


FIGURE 4A. Scanning electron micrograph of *Mucor velutinosus* highlighting characteristic sporangium and hypha (bar = 10 μm).



Further reading:

Alvarez E, Cano J, Stchigel AM, Sutton DA, Fothergill AW, Salas V, Rinaldi MG, Guarro J. 2011. Two new species of *Mucor* from clinical samples. *Med Mycol.* 49:62-72.

Deja M, Wolf S, Weber-Carstens S, Lehmann TN, Adler A, Ruhnke M, Tintelnot K. 2006. Gastrointestinal zygomycosis caused by *Mucor indicus* in a patient with acute traumatic brain injury. *Med Mycol.* 44: 683-687.

Iwen PC, Sigler L, Noel RK, Freifeld AG. 2007. *Mucor circinelloides* was identified by molecular methods as a cause of primary cutaneous zygomycosis. *J Clin Microbiol.* 45: 636-640.

Martín-Moro JG, Calleja JM, García MB, Carretero JL, Rodríguez JG. 2008. Rhinoorbitocerebral mucormycosis: a case report and literature review. *Med Oral Patol Oral Cir Bucal.* 13: E792-5.

de Repentigny L, St-Germain G, Charest H, Kokta V, Vobecky S. 2008. Fatal zygomycosis caused by *Mucor indicus* in a child with an implantable left ventricular assist device. *Pediatr Infect Dis J*. 27: 365-369.

Page RL 2nd, Schwiesow J, Hilts A. 2007. Posaconazole as salvage therapy in a patient with disseminated zygomycosis: case report and review of the literature. *Pharmacotherapy.* 27: 290-298.

Sedlacek M, Cotter JG, Suriawinata AA, Kaneko TM, Zuckerman RA, Parsonnet J, Block CA. 2008. Mucormycosis peritonitis: more than 2 years of disease-free follow-up after posaconazole salvage therapy after failure of liposomal amphotericin B. *Am J Kidney Dis.* 51: 302-306.

Sims CR, Ostrosky-Zeichner L. 2007. Contemporary treatment and outcomes of zygomycosis in a non-oncologic tertiary care center. *Arch Med Res.* 38: 90-93.

Sugui JA, Christensen JA, Bennett JE, Zelazny AM, Kwon-Chung KJ. 2011. Hematogenously disseminated skin disease caused by Mucor velutinosus in a patient with acute myeloid leukemia. *J Clin Microbiol.* 49:2728-32.

Tehmeena W, Hussain W, Zargar HR, Sheikh AR, Iqbal S. 2007. Primary cutaneous mucormycosis in an immunocompetent host. *Mycopathologia.* 164: 197-199.

M-5 *Curvularia* species

Source: Scalp / Blood

CLINICAL SIGNIFICANCE: *Curvularia* spp. are an infrequent cause of sinusitis, keratitis, endocarditis, mycetoma, and cerebral abscess. Few cases of disseminated infection have been reported in immunocompromised patients.

COLONY: *Curvularia* sp. grew fast on Sabouraud's dextrose agar, 25°C. Colonies were initially white, later becoming brownish black, wooly in texture after 5 days (Figure 5).

MICROSCOPY: Lactophenol - Cotton blue mount showed brown septate hyphae, conidiophores with brown, geniculate with poroconidia. Poroconidia are holoblastic, produced through a pore or channel in the cell wall of the conidiophore or conidiogenous cell. The conidia were slightly curved, brown with 3-4 transverse septations; the central cell is larger and darker than the other cells (Figure 5).

DIFFERENTIATION: *Curvularia* species could be easily differentiated from other dark, muriform fungi by its rapid growth. Microscopically, multicellular, subtly curved conidia with large and dark central cell are characteristic. In *Bipolaris* species and *Drechslera* species, the conidia are distoseptate (multicellular conidia in which the cells are contained within sacks rather than separated by septa), while the conidia are transversely septate in *Curvularia* spp.

MOLECULAR TEST: Analysis of genes coding for small subunit rRNA sequences of dematiaceous fungal pathogens provided means for assessing relationships of pathogenic and non-pathogenic forms, and accurate identification. Electrophoretic karyotyping of *Curvularia lunata* demonstrated that there are 12 chromosomes ranging in size from 1.4 to 4.0 Mb.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Curvularia lunatus* (*Cochliobolus lunatus*) isolate CATAS-CL01 (GenBank accession no. GQ169765.1).

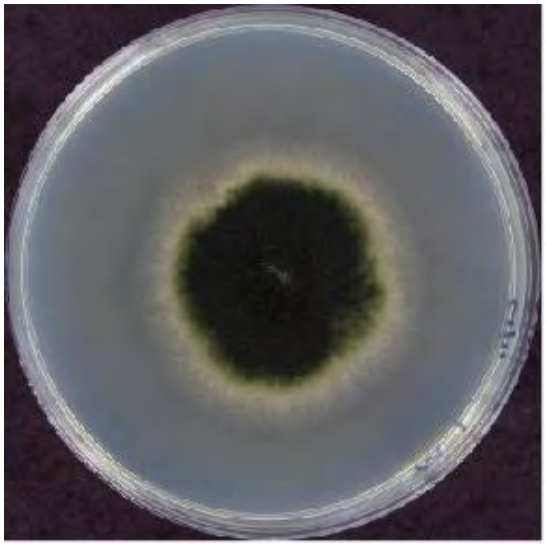
ANTIFUNGAL SUSCEPTIBILITY: Most clinical isolates are susceptible to amphotericin B, itraconazole, miconazole and ketoconazole, but resistant to flucytosine and fluconazole.

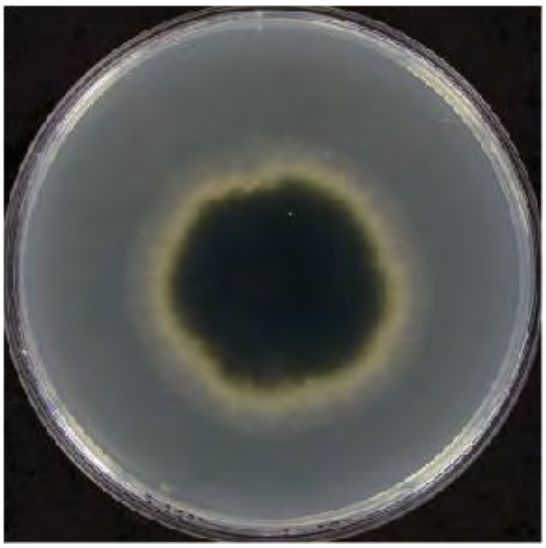
PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	66
Laboratories with incorrect ID:	0

Illustrations:

FIGURE 5. Three-day-old, wooly, olive green to black colony of *Curvularia* sp. on Sabouraud's dextrose agar, 25°C; the reverse of the colony is white to pale (upper panel). Microscopic morphology of *Curvularia* sp. showing hyphae and poroconidia formed sympodially; the condia are slightly curved with transverse septations (bar = 10 μm; lower panel).





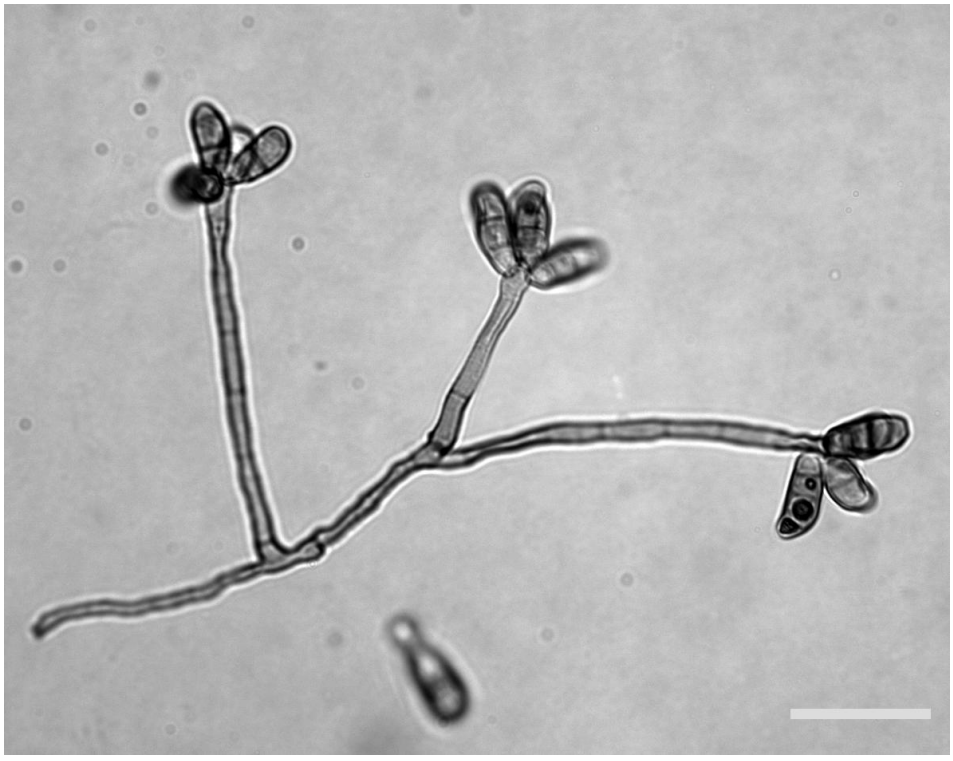


FIGURE 5A. Scanning electron micrograph of *Curvularia lunata* (*Cochliobolus lunatus*) showing poroconidia (bar = 2 μm).

Further reading:

Carter E, Boudreaux C. 2004. Fatal cerebral phaeohyphomycosis due to *Curvularia lunata* in an immunocompetent patient. *J Clin Microbiol*. 42: 5419-5423.

Fan YM, Huang WM, Li SF, Wu GF, Li W, Chen RY. 2009. Cutaneous phaeohyphomycosis of foot caused by *Curvularia clavata*. *Mycoses*. 52: 544-546.

Hiromoto A, Nagano T, Nishigori C. 2008. Cutaneous infection caused by *Curvularia* species in an immunocompetent patient. *Br J Dermatol.* 158: 1374-1375.

Pfaller MA, Messer SA, Boyken L, Hollis RJ, Diekema DJ. 2003. *In vitro* susceptibility testing of filamentous fungi: comparison of Etest and reference M38-A microdilution methods for determining posaconazole MICs. *Diagn Microbiol Infect Dis*. 45: 241-244.

Pimentel JD, Mahadevan K, Woodgyer A, Sigler L, Gibas C, Harris OC, Lupino M, Athan E. 2005. Peritonitis due to *Curvularia inaequalis* in an elderly patient undergoing peritoneal dialysis and a review of six cases of peritonitis associated with other *Curvularia* spp. *J Clin Microbiol*. 43: 4288-4292.

Tessari G, Forni A, Ferretto R, Solbiati M, Faggian G, Mazzucco A, Barba A. 2003. Lethal systemic dissemination from a cutaneous infection due to *Curvularia lunata* in a heart transplant recipient. *J Eur Acad Dermatol Venereol.* 17: 440-442.

Thomas PA. 2003. Fungal infections of the cornea. *Eye*. 17: 852-862.

Vachharajani TJ, Zaman F, Latif S, Penn R, Abreo KD. 2005. *Curvularia geniculata* fungal peritonitis: a case report with review of literature. *Int Urol Nephrol.* 37: 781-784.

YEAST DESCRIPTIONS

Y-1 Candida guilliermondii

Source: Chest / Urine

CLINICAL SIGNIFICANCE: *Candida guilliermondii* is a frequent causal agent of nosocomial fungemia in immunosuppressed patients. It is an infrequent casual agent of urinary tract infections, brain abscess, and ocular infections.

COLONY: *C. guilliermondii* colony was flat, smooth, cream-yellow on Sabouraud's dextrose agar after 7 days at 25°C (Figure 6).

MICROSCOPY: *C. guilliermondii* showed few short pseudohyphae with clusters of blastoconidia on Corn meal agar with Tween 80 (Figure 6).

DIFFERENTIATION: *C. guilliermondii* is the anamorph (asexual form) of *Pichia guilliermondii/ Kodamaea ohmeri*. It ferments glucose, sucrose, and trehalose, grows at 37°C, and on media containing cycloheximide. It does not form pink pigment thereby differentiating it from *Rhodotorula* species. It does not produce true hyphae, which differentiates it from *Candida ciferrii* and *Trichosporon beigelii*. Unlike *Candida lusitaniae*, it is unable to grow at 45°C.

MOLECULAR TEST: Primers for large ribosomal subunit DNA sequences were used in PCR to differentiate *C. guilliermondii* from *C. famata/ Debaryomyces hansenii* complex. Isolates of *C. guilliermondii* were identified using PCR to amplify ribosomal DNA, followed by restriction digestion of the PCR product.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Candida guilliermondii* (*Pichia guilliermondii*) isolate SMB (GenBank accession no. GU385845.1).

ANTIFUNGAL SUSCEPTIBILITY: Most clinical isolates are susceptible to amphotericin B, 5-flucytosine, and azoles such as fluconazole, ketocoanzole, itraconazole and caspofungin. A few isolates are reported to have high MIC to azoles.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	52
Laboratories with incorrect ID:	06
(*Candida famata*)	(5)
(*Candida* sp.)	(1)

Illustrations:

FIGURE 6. *Candida guilliermondii,* flat, smooth, creamish colony on Sabouraud's dextrose agar, 5 days, 25°C. Microscopic morphology on corn meal agar with Tween 80, showing short pseudohyphae with clusters of blastoconidia (bar = 10 μm).

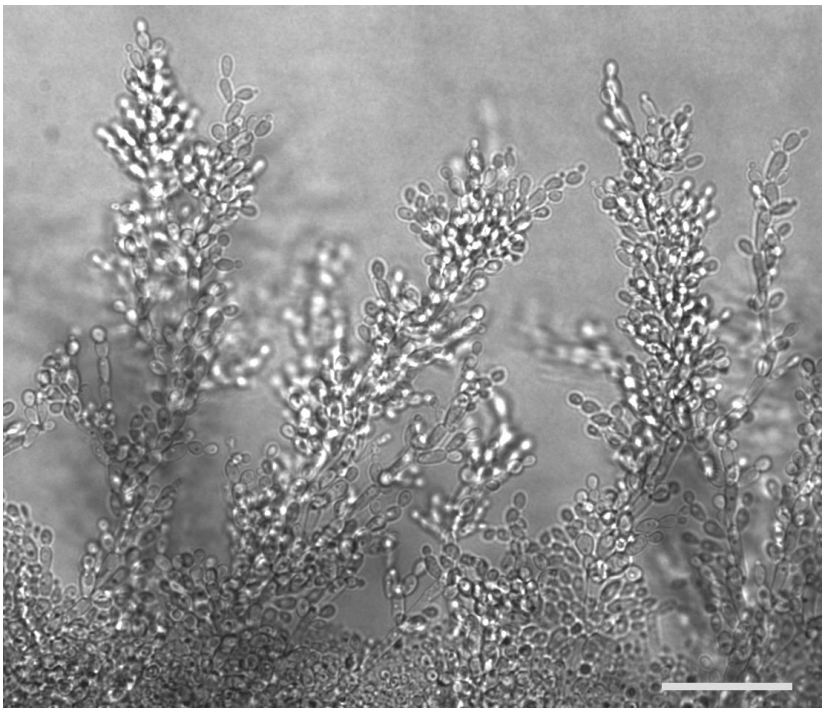
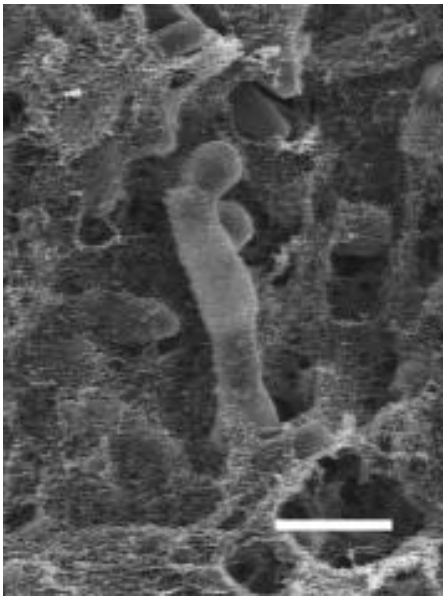


FIGURE 6A. Scanning electron micrograph of *Candida guilliermondii* (*Pichia guilliermondii*) illustrates pseudohyphae and blastoconidia (bar = 2 μm)



Further reading:

Kabbara N, Lacroix C, de Latour RP, Socié G, Ghannoum M, Ribaud P. 2008. Breakthrough *C. parapsilosis* and *C. guilliermondii* blood stream infections in allogeneic hematopoietic stem cell transplant recipients receiving long-term caspofungin therapy. *Haematologica*. 93: 639-640.

Lee GW, Kim TH, Son JH. 2012. Primary *Candida guilliermondii* infection of the knee in a patient without predisposing factors. *Case Report Med*. 2012:375682. Epub 2012 Feb 28.

Macêdo DP, Oliveira NT, Farias AM, Silva VK, Wilheim AB, Couto FM, Neves RP. 2010. Esophagitis caused by *Candida guilliermondii* in diabetes mellitus: first reported case. *Med Mycol.* 48: 862-865.

Mardani M, Hanna, HA, Girgawy, E, Raad, I. 2000. Nosocomial *Candida guilliermondii* fungemia in cancer patients. *Infect Control Hosp. Epidemiol.* 21: 336-337.

Pemán J, Bosch M, Cantón E, Viudes A, Jarque I, Gómez-García M, García-Martínez JM., Gobernado M. 2008. Fungemia due to *Candida guilliermondii* in a pediatric and adult population during a 12-year period. *Diagn Microbiol Infect Dis*. 60: 109-112.

Pfaller MA, Boyken L, Hollis RJ, Messer SA, Tendolkar S, Diekema DJ. 2006. *In Vitro* Susceptibilities of *Candida* spp. to Caspofungin: Four Years of Global Surveillance. *J. Clin. Microbiol.* 44: 760-763.

Savini V, Catavitello C, Onofrillo D, Masciarelli G, Astolfi D, Balbinot A, Febbo F, D'Amario C, D'Antonio D. 2011. What do we know about *Candida guilliermondii*? A voyage throughout past and current literature about this emerging yeast. *Mycoses.* 54:434-41.

Y-2 *Candida lusitaniae*

Source: Blood / Lung / Urine

CLINICAL SIGNIFICANCE: *Candida lusitaniae* causes fungemia and sepsis in immunocompromised and debilitated patients with cancer, diabetes, or asthma, and also neonates in intensive care units. The comon clinical samples are blood, urine, and respiratory tract secretions.

COLONY: *C. lusitaniae* colony was white to creamish, shiny, slightly raised in the center on Sabouraud's dextrose agar, after 7 days at 25°C (Figure 7).

MICROSCOPY: *C. lusitaniae* produced many short, branched ("bushy") pseudohyphae. Along the length of the pseudohyphae, elongated blastoconidia formed in short chains on Corn Meal Agar with Tween 80 (Figure 7).

DIFFERENTIATION: *C. lusitaniae* is able to ferment and assimilate cellobiose, which differentiates it from *C. parapsilosis*.

MOLECULAR TEST: Specific nucleic acid probes targeting the large subunit rRNA genes have been developed for identification of *C. lusitaniae*. Three pulsed-field electrophoretic methods and a random amplified polymorphic DNA (RAPD) method were also reported to delineate strains of *C. lusitaniae*.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Candida lusitaniae* (*Clavispora lusitaniae*) isolate F47819-04 (GenBank accession no. HQ693785.1).

ANTIFUNGAL SUSCEPTIBILITY: Some *C. lusitaniae* strains are reported to be inherently resistant to amphotericin B. Amphotericin B susceptible strains are also known to develop resistance during the course of treatment with this drug. *C. lusitaniae* is reported as more susceptible to voriconazole than fluconazole.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	57
Laboratories with incorrect ID:	01
(*Candida famata*)	(1)

Illustrations:

FIGURE 7. *Candida lusitaniae*, white, smooth colony of on Sabouraud's dextrose agar, 4 days, 25°C. Microscopic morphology on corn meal agar showing pesudohyphae and blastoconidia (bar = 10 μm).

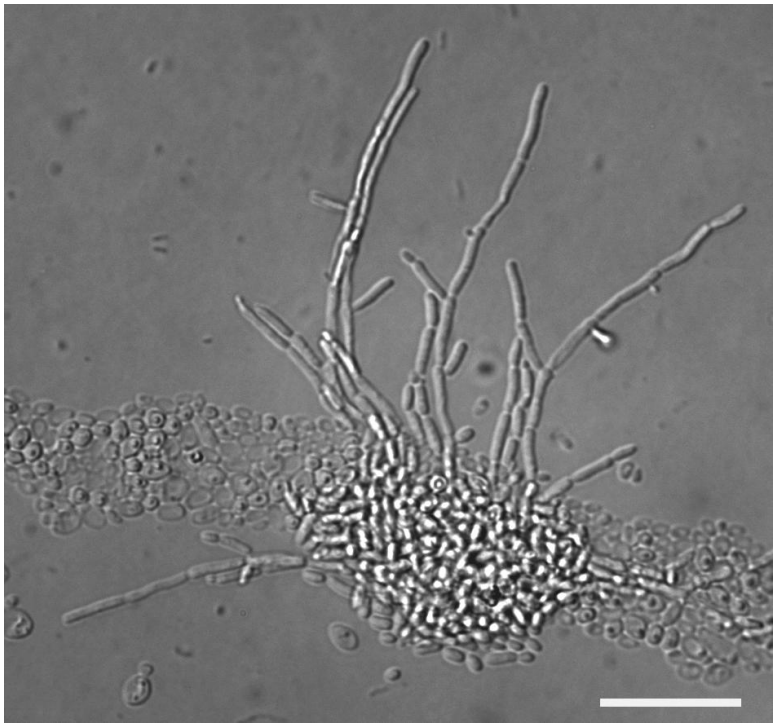
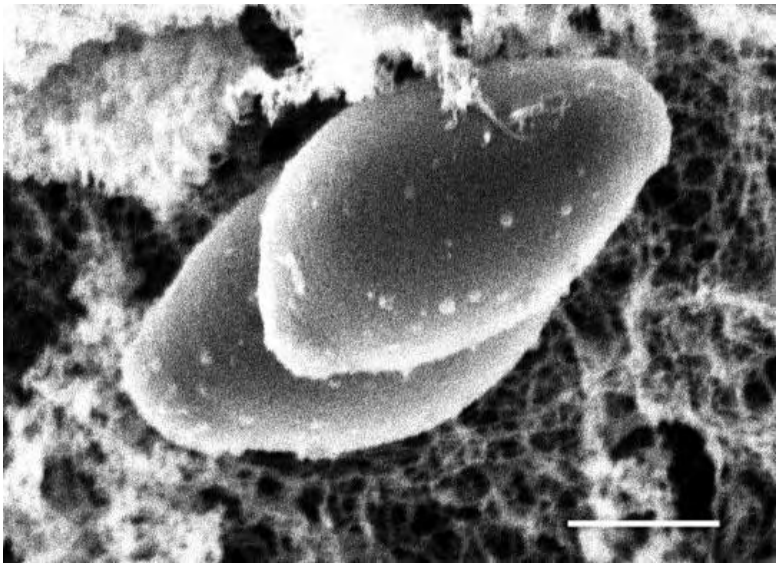


FIGURE 7A. Scanning electron micrograph illustrates blastoconidia (bar = 2 μm).



Further reading:

Alberth M, Majoros L, Kovalecz G, Borbas E, Szegedi I, J Marton I, Kiss C. 2006. Significance of oral *Candida* infections in children with cancer. *Pathol Oncol Res*. 12: 237-241.

Atkinson BJ, Lewis RE, Kontoyiannis DP. 2008. *Candida lusitaniae* fungemia in cancer patients: risk factors for amphotericin B failure and outcome. *Med Mycol.* 46: 541-546.

Bariola JR, Saccente M. 2008. *Candida lusitaniae* septic arthritis: case report and review of the literature. *Diagn Microbiol Infect Dis.* 61: 61-63.

De Carolis E, Sanguinetti M, Florio AR, La Sorda M, D'Inzeo T, Morandotti GA, Fadda G, Posteraro B. 2010. *In vitro* susceptibility to seven antifungal agents of *Candida lusitaniae* isolates from an italian university hospital. *J Chemother*. 22: 68-70.

Estrada B, Mancao MY, Polski JM, Figarola MS. 2006. *Candida lusitaniae* and chronic granulomatous disease. *Pediatr Infect Dis J.* 25: 758-759.

McClenny NB, Fei H, Baron EJ, Gales AC, Houston A, Hollis RJ, Pfaller MA. 2002. Change in colony morphology of *Candida lusitaniae* in association with development of amphotericin B resistance. *Antimicrob Agnets Chemother*. 46: 1325-1328.

Michel RG, Kinasewitz GT, Drevets DA, Levin JH, Warden DW. 2009. Prosthetic valve endocarditis caused by *Candida lusitaniae*, an uncommon pathogen: a case report. *J Med Case Reports*. 3: 7611.

Parentin F, Liberali T, Perissutti P. 2006. Polymicrobial keratomycosis in a three-year-old child. *Ocul Immunol Inflamm.* 14: 129-131.

Pfaller MA, Woosley LN, Messer SA, Jones RN, Castanheira M. 2012. Significance of molecular identification and antifungal susceptibility of clinically significant yeasts and moulds in a Global Antifungal Surveillance Programme. *Mycopathologia.* DOI 10.1007/s11046-012-9551-x

Prigitano A, Biraghi E, Pozzi C, Viviani MA, Tortorano AM. 2010. *In vitro* activity of amphotericin B against *Candida lusitaniae* clinical isolates. *J Chemother*. 22: 71-72.

Werner BC, Hogan MV, Shen FH. 2011. *Candida lusitaniae* discitis after discogram in an immunocompetent patient. *Spine J.* 2011 11:e1-6.

Y-3 *Sacharomyces cerevisiae*

Source: Sputum / Urine

CLINICAL SIGNIFICANCE: *Saccharomyces cerevisiae,* the baker's yeast, causes disseminated infections in immunocompromised hosts.

COLONY: *Sacharomyces cerevisiae* colonies appeared creamy, smooth, dull butyrous or buttery texture after 3 – 5 days of incubation on Sabouraud's dextrose agar 25°C (Figure 8).

MICROSCOPY: *Sacharomyces cerevisiae* showed round to oval yeast cells with no pseudohyphae or rudimentary pseudohyphae on Corn meal agar with Tween 80, characteristic ascospores encased in asci were seen (Figure 8).

DIFFERENTIATION: *Saccharomyces cerevisiae* ferments glucose, maltose and sucrose, does not grow on the media containing cycloheximide, and grows at 37°C. On the API 20C AUX, a specific assimilation biocode is obtained for identification of this organism.

MOLECULAR TEST: *Saccharomyces cerevisiae* is the most intensely studied model organism also being the first eukaryote to have its entire genome sequenced and mapped.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Saccharomyces cerevisiae* isolate D3C (GenBank accession no. JF715201.1).

ANTIFUNGAL SUSCEPTIBILITY: Most isolates are susceptible to amphotericin B, 5-FC, and to azoles like fluconazole, miconazole, voriconazole. etc.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	58
Laboratories with incorrect ID:	0

Illustrations:

FIGURE 8. *Sacchromycetes cerevisiae*, creamy, smooth, dull butyrous colony on Sabouraud's dextrose agar, 5-day, 25°C. Microscopic morphology showing round to oval blastoconidia on Corn meal agar with Tween 80 (bar = 10 μm).

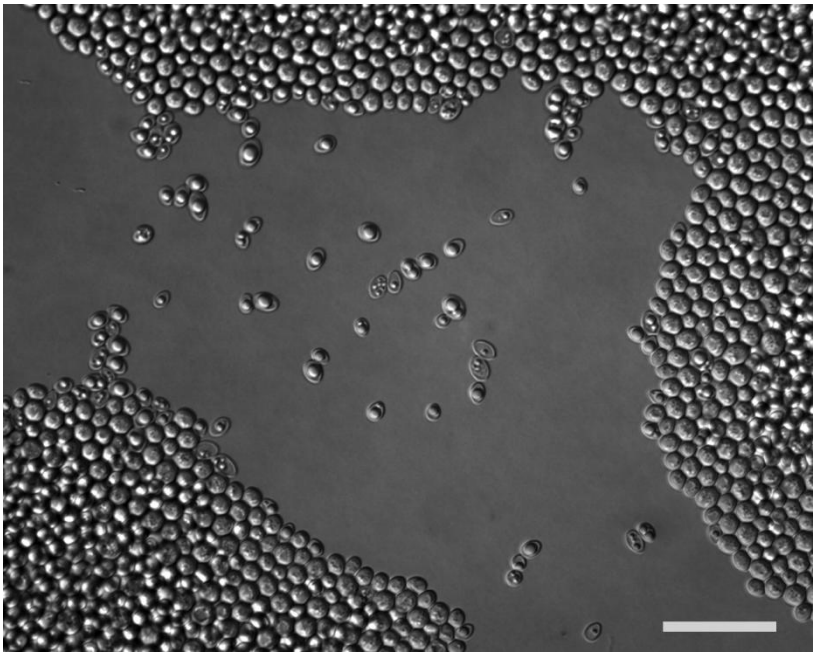
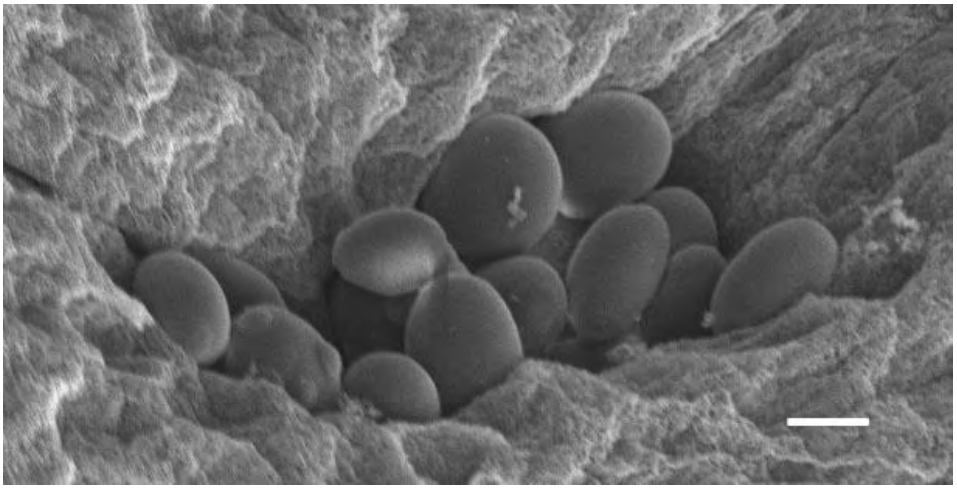


FIGURE 8A. Scanning electron micrograph illustrating oval blastoconidia (bar = 2 μm).



Further reading:

Buchta V, Vejsova M, Vale-Silva LA. 2008. Comparison of disk diffusion test and Etest for voriconazole and fluconazole susceptibility testing. *Folia Microbiol (Praha).* 53: 153-160.

Cherifi S, Robberecht J, Miendje Y. 2004. *Saccharomyces cerevisiae* fungemia in an elderly patient with *Clostridium difficile* colitis. *Acta Clin Belg.* 59: 223-224.

Fiore NF, Conway JH, West KW, Kleiman MB. 1998. *Saccharomyces cerevisiae* infections in children. *Pediatric Infectious Disease J.* 17: 1177 –1179.

Henry S, D'Hondt L, Andre M, Holemans X, Canon JL. 2004 *Saccharomyces cerevisiae* fungemia in a head and neck cancer patient: a case report and review of the literature. *Acta Clin Belg.* 59: 220-222.

Hamoud S, Keidar Z, Hayek T. 2011. Recurrent *Saccharomyces cerevisiae* fungemia in an otherwise healthy patient. *Isr Med Assoc J.* 13:575-6.

Konecny P, Drummond FM, Tish KN, Tapsall JW. 1999. *Saccharomyces cerevisiae* oesophagitis in an HIV –Infected patient. *International J STD & AIDS.* 10: 821 –822.

Ren P, Sridhar S, Chaturvedi V. 2004. Use of paraffin-embedded tissue for identification of *Saccharomyces cerevisiae* in a baker's lung nodule by fungal PCR and nucleotide sequencing. *J Clin Microbiol.* 42: 2840 - 2842.

Munoz P, Bouza E, Cuenca-Estrella M, Eiros JM, Perez MJ, Sanchez-Somolinos M, Rincon C, Hortal J, Pelaez T. 2005. *Saccharomyces cerevisiae* fungemia: an emerging infectious disease. *Clin Infect Dis.* 40: 1625-1634.

Savini V, Catavitello C, Manna A, Talia M, Febbo F, Balbinot A, D'Antonio F, Di Bonaventura G, Celentano C, Liberati M, Piccolomini R, D'Antonio D. 2008. Two cases of vaginitis caused by itraconazole-resistant *Saccharomyces cerevisiae* and a review of recently published studies. *Mycopathologia*. 166: 47-50.

Y-4 *Candida pelliculosa*

Source: Wound / Sinus / Urine

CLINICAL SIGNIFICANCE: *Candida pelliculosa* is an infrequently encountered pathogen causing nosocomial infections. Several cases of fungemia in neonates, and endocarditis in immunosuppressed patients, are reported in the literature.

COLONY: *Candida pelliculosa* colony was smooth, creamy, and soft on Sabouraud's dextrose agar 5 days at 25°C (Figure 9).

MICROSCOPY: *C. pelliculosa* showed blastoconidia and limited pseudohyphae on Corn meal agar with Tween 80 (Figure 9)

DIFFERENTIATION: *Candida pelliculosa* is the anamorph (asexual form) of *Pichia anomala*. It does not grow on media containing cycloheximide, or at 42°C. It assimilates nitrate but is urease-negative.

MOLECULAR TEST: PCR amplification of a specific fragment of 18S rDNA and heteroduplex mobility assays were performed to detect and distinguish *C. pelliculosa* from other clinically important yeasts. Phylogenetic analysis of domain sequences found four new species in the *C. pelliculosa* clade.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Candida pelliculosa* (*Pichia anomala*) isolate M10 (GenBank accession no. FJ865436.1).

ANTIFUNGAL SUSCEPTIBILITY: *C. pelliculosa* is susceptible to amphotericin B, 5-flucytosine, and azoles such as fluconazole, clotrimazole, and itraconazole.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	57
Laboratories with incorrect ID:	01
(*Candida* sp.)	(1)

Illustrations:

FIGURE 9. *Candida pelliculosa*, smooth, creamy, soft colony on Sabouraud's dextrose agar, 4 days, 25°C. Microscopic morphology showing pseudohyphae on Corn meal agar with Tween 80 (bar = 10 μm).

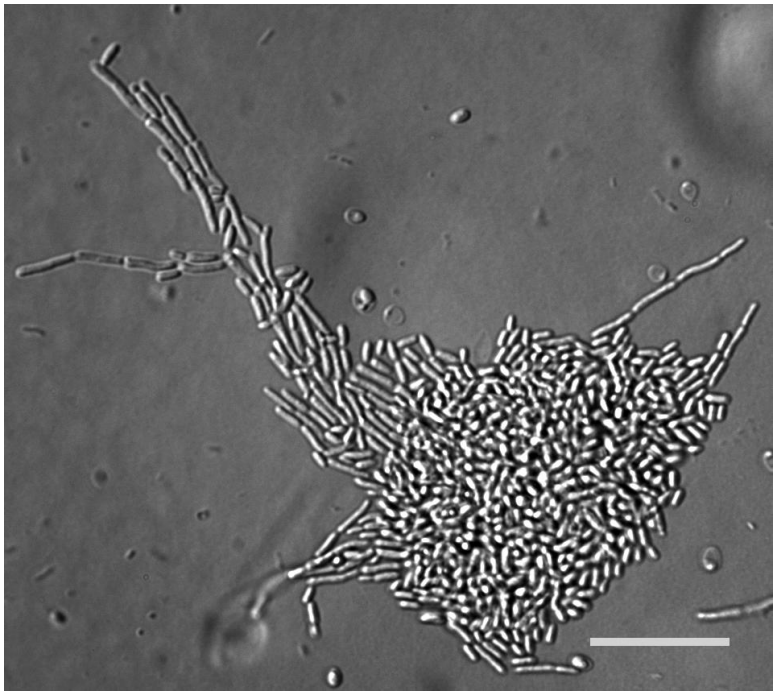
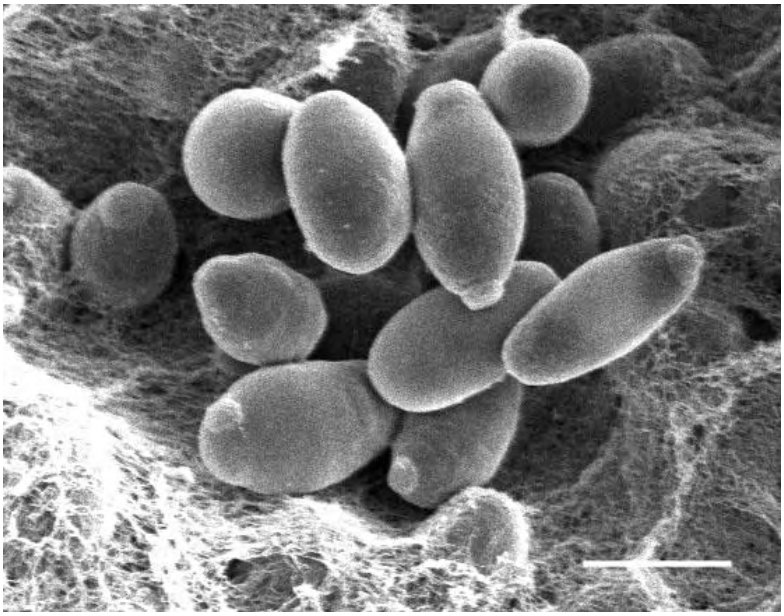


FIGURE 9A. Scanning electron micrograph illustrating blastoconidia (bar = 2 μm).



Further reading:

Barchiesi F, Tortorano AM, Di Francesco LF, Rigoni A, Giacometti A, Spreghini E, Scalise G, Viviani MA. 2005. Genotypic variation and antifungal susceptibilities of *Candida pelliculosa* clinical isolates. *J Med Microbiol.* 54(Pt 3): 279-285.

Hanzen J, Krcmery V. 2002. Polyfungal candidaemia due to *Candida rugosa* and *Candida pelliculosa* in a haemodialyzed neonate. *Scand J Infect Dis*. 34: 555.

Kalkanci A, Dizbay M, Turan O, Fidan I, Yalçin B, Hirfanoğlu I, Kuştimur S, Aktaş F, Sugita T. 2010. Nosocomial transmission of Candida pelliculosa fungemia in a pediatric intensive care unit and review of the literature. *Turk J Pediatr*. 52: 42-49.

Krcmery V, Kisac P, Liskova A. 2009. Voriconazole and posaconazole resistant *Candida pelliculosa* fungemia after cardiac surgery. *Pediatr Infect Dis J*. 28: 75-76.

Neumeister B, Rockemann M, Marre R. 1992. Fungaemia due to *Candida pelliculosa* in a case of acute pancreatitis. *Mycoses.* 35: 309-310.

Passoth V, Olstorpe M, Schnürer J. 2011. Past, present and future research directions with *Pichia anomala*. *Antonie Van Leeuwenhoek.* 99:121-5

Ratcliffe L, Davies J, Anson J, Hales S, Beeching NJ, Beadsworth MB. 2011. *Candida pelliculosa* meningitis as an opportunistic infection in HIV: the first reported case. *Int J STD AIDS.* 22: 54-56.

Y-5 *Prototheca wickerhamii*

Source: Bronchial wash / Vagina / Urine

CLINICAL SIGNIFICANCE: *Prototheca wickerhamii* is a yeast-like alga, which causes protothecosis in humans. The common manifestations are as cutaneous and subcutaneous lesions termed bursitis, but rarely, *P. wickerhamii* causes systemic infections. The infection is acquired through traumatic implantation of alga in the subcutaneous tissue.

COLONY: *Prototheca wickerhamii* colony was moist, cream-colored on Sabouraud's dextrose agar 7 days at 25°C (Figure 10).

MICROSCOPY: *Prototheca wickerhamii* showed sporangia of various sizes, some filled with sporangiospores (endospores) on corn meal agar with Tween 80. There was no budding, no hyphae (Figure 10).

DIFFERENTIATION: *P. wickerhamii* requires thiamine for growth, does not grow on media containing cycloheximide, grows well at 25°C and 37°C. The cells of *P. wickerhamii* are smaller than those of *P. zopfii*. On the API 20C AUX, a specific assimilation biocode is obtained to differentiate it from other *Prototheca* species. The isolates of *P. zopfii* are resistant to 50-µg clotrimazole disk at 37°C while *P. wickerhamii* isolates produces a zone of inhibition.

MOLECULAR TEST: Sequence analysis of the mitochondrial small subunit rRNA from *P. wickerhamii* showed higher homology with mitochondrial sequence from plants.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Prototheca wickerhamii* isolate ATCC 30395 (GenBank accession no. JN869303.1).

ANTIFUNGAL SUSCEPTIBILITY: Almost all isolates of *P. wickerhamii* are susceptible to amphotericin B and voriconazole, but resistant to fluconazole and 5 FC, variably susceptible to itraconazole and ketoconazole.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	57
Other acceptable answer:	01
Prototheca sp.	01
Laboratories with incorrect ID:	0

Illustrations:

FIGURE 10. *Prototheca wickerhamii* colony moist, cream-colored on Sabouraud's dextrose agar, 7 days, 25°C. Microscopic morphology of *Prototheca wickerhamii* showing sporangia of various sizes, some filled with sporangiospores (endospores) on Corn meal agar with Tween 80 (bar = 10 μm).

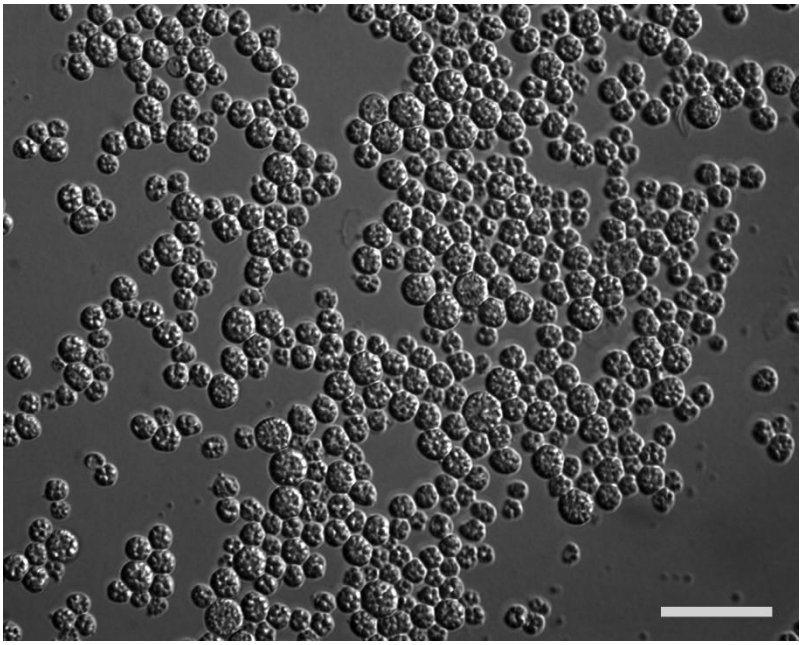
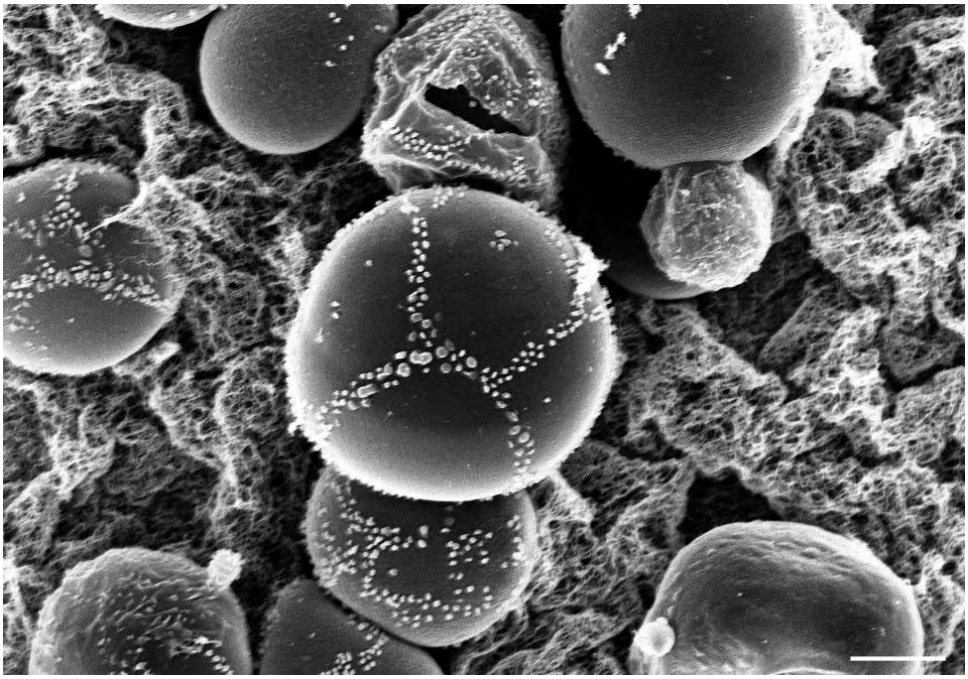


FIGURE 10A. Scanning electron micrograph with sporangiospores (bar = 2 μm).



Further reading:

Hariprasad SM, Prasad A, Smith M, Shah GK, Grand MG, Shepherd JB, Wickens J, Apte RS, Liao RS, Van Gelder R. 2005. Bilateral choroiditis from *Prototheca wickerhamii* algaemia. *Arch Ophthalmol.* 123: 1138-1141.

Lass-Flore C, Mayer A. 2007. Human protothecosis. *Clin. Microbiol. Rev*. 20: 230-242.

Lee JS, Moon GH, Lee NY, Peck KR. 2008. Case Report: Protothecal Tenosynovitis. *Clin Orthop Relat Res*. 466: 3143-3146.

Leimann BC, Monteiro PC, Lazera M, Candanoza ER, Wanke B. 2004. Protothecosis. *Med Mycol.* 42: 95-106.

Linares MJ, Solis F, Casal M.2005. *In vitro* activity of voriconazole against *Prototheca wickerhamii*: comparative evaluation of Sensititre and NCCLS M27-A2 methods of detection. *J Clin Microbiol*. 43: 2520-2522.

Mohd Tap R, Sabaratnam P, Salleh MA, Abd Razak MF, Ahmad N. 2012. Characterization of *Prototheca wickerhamii* isolated from disseminated algaemia of kidney transplant patient from Malaysia. *Mycopathologia.* 2012 173: 173-178.

Narita M, Muder RR, Cacciarelli TV, Singh N. 2008. Protothecosis after liver transplantation. *Liver Transpl*. 14: 1211-1215.

Nwanguma V, Cleveland K, Baselski V. 2011. Fatal *Prototheca wickerhamii* bloodstream infection in a cardiac allograft recipient. J Clin Microbiol. 49: 4024; author reply 4025.

Solky AC, Laver NM, Williams J, Fraire A. 2011. *Prototheca wickerhamii* infection of a corneal graft. *Cornea.* 30: 1173-1175.

Zaitz C, Godoy AM, Colucci FM, de Sousa VM, Ruiz LR, Masada AS, Nobre MV, Muller H, Muramatu LH, Arrigada GL, Heins-Vaccari EM, Martins JE. 2006. Cutaneous protothecosis: report of a third Brazilian case. *Int J Dermatol.* 45: 124-126.

DIRECT DETECTION (*Cryptococcus neformans* ANTIGEN TEST)

INTRODUCTION: In early 1960s, a simple, sensitive latex test, capable of detecting the capsular polysaccharide of *C. neoformans* in serum, was described and proven to be superior in sensitivity to the India ink mount. Clinical studies established the prognostic value of the test, and showed it to be a valuable aid in establishing a diagnosis when culture was negative. Paired serum and CSF specimens allowed detection of antigen in confirmed cases.

In early 1990s, an enzyme immunoassay based upon monoclonal antibody against capsular polysaccharide, was described. More recently, a lateral flow immunoassay was described for point-of-care testing of cryptococcosis from serum.

MATERIALS & METHODS: Sixty-four laboratories participated in the February 1, 2012 direct detection antigen test event. Two positive serum samples (Cn-Ag-1 and Cn-Ag-3) with the titer of 1:128 and 1:16 respectively for cryptococcal antigen were included. Titers within ± 2 dilutions of the reference and/or consensus results were the acceptable results for this event.

RESULTS: Overall, the performance of 64 laboratories was satisfactory in this test event. The consensus results for specimens Cn-Ag-2, Cn-Ag-4, and Cn-Ag-5 were negative as expected. Cn-Ag-1 and Cn-Ag-3 were reported positive by all the participating laboratories with the acceptable titer ranges 1:32 – 1:512 and 1:4 – 1:64 respectively. One laboratory reported false positive result for specimen Cn-Ag-2. One laboratory reported lower titer than the acceptable range for specimen Cn-Ag-1 (Table 1).

Table 1. Summary of laboratory performance for semi-quantitative detection of cryptococcal antigen

Method		Cn-Ag-1 Titers						
No. laboratories		16	32	64	80	128	256	512
EIA	3	1		2				
Latex Agglutination	61		4	14	2	23	15	3
Immuno-Mycologics	8		1	3		2	1	1
Meridien Diagnostic	44		3	9	2	16	12	2
Remel	9			2		5	2	
Total	64	1	4	16	2	23	15	3

Method		Cn-Ag-3 Titers					
No. laboratories		4	8	10	16	32	64
EIA	3	1	2				
Latex Agglutination	61	2	12	1	27	15	4
Immuno-Mycologics	8	1	1		4	2	
Meridien Diagnostic	44	1	10	1	18	10	4
Remel	9		1		5	3	
Total	64	3	14	1	27	15	4

Further Reading:

Bennett JE, Hasenclever HF, Tynes BS. 1964. Detection of cryptococcal polysaccharide in serum and spinal fluid: value in diagnosis and prognosis. *Trans Assoc Am Physicians*. 77: 145-150.

Bloomfield N, Gordon MA, Elmendorf DF, Jr. 1963. Detection of *Cryptococcus neoformans* antigen in body fluids by latex particle agglutination. *Proc Soc Exp Bio Med.* 114: 64-67.

Diamond D, Bennett E. 1974. Prognostic factors in cryptococcal meningitis. *Ann Int Med.* 80: 176-181.

Goodman JS, Kaufman L, Koening MG. 1971. Diagnosis of cryptococcal meningitis: Value of immunologic detection of cryptococcal antigen. *New Eng J Med*. 285: 434-436.

Gordon MA, Vedder DK. 1966. Serologic tests in diagnosis and prognosis of cryptococcosis. *JAMA.* 197: 961-967.

Kaufman L, Blumer S. 1968. Value and interpretation of serological tests for the diagnosis of cryptococcosis. *Appl. Microbial.* 16: 1907-1912.

Gray LD, Roberts GD. 1988. Experience with the use of pronase to eliminate interference factors in the latex agglutination test for cryptococcal antigen. *J Clin Microbiol* 26: 2450-2451.

Singh N, Alexander BD, Lortholary O, Dromer F, Gupta KL, John GT, del Busto R, Klintmalm GB, Somani J, Lyon GM, Pursell K, Stosor V, Muñoz P, Limaye AP, Kalil AC, Pruett TL, Garcia-Diaz J, Humar A, Houston S, House AA, Wray D, Orloff S, Dowdy LA, Fisher RA, Heitman J, Wagener MM, Husain S. 2008. Pulmonary cryptococcosis in solid organ transplant recipients: clinical relevance of serum cryptococcal antigen. *Clin Infect Dis.* 46: e12-18

Gade W, Hinnefeld SW, Babcock LS, Gilligan P, Kelly W, Wait K, Greer D, Pinilla M, Kaplan RL. 1991. Comparison of the PREMIER cryptococcal antigen enzyme immunoassay and the latex agglutination assay for detection of cryptococcal antigens. *J Clin Microbiol*. 29: 1616-1619.

Lindsley MD, Mekha N, Baggett HC, Surinthong Y, Autthateinchai R, et al. 2011. Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. *Clin Infect Dis*. 53: 321-325.

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR YEASTS

INTRODUCTION: Clinical laboratories perform susceptibility testing of pathogenic yeasts to determine their in vitro resistance to antifungal drugs. This test is also useful in conducting surveillance for evolving patterns of antifungal drug resistance in a healthcare facility. The results are likely to facilitate the selection of appropriate drugs for treatment. Clinical Laboratory Standards Institute (CLSI) documents of M27-A3, M27-S3 and M44-A, describe the current standard methods for antifungal susceptibility testing of pathogenic yeasts. Another resource for standardized method is the EUCAST Definitive Document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. The FDA approved devices for antifungal susceptibility testing of yeasts include Sensititre YeastOne Colorimetric Panel (Trek Diagnostic Systems Inc. Cleveland, OH) and Etest (bioMérieux, Inc., Durham, NC). The following ten drugs are included in the Mycology Proficiency Test Program - amphotericin B, anidulafungin, caspofungin, flucytosine (5-FC), fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. The participating laboratories are allowed to select any number of antifungal drug(s) from this test panel based upon practices in their facilities.

MATERIALS: *Candida glabrata* (S-1) was the analyte in the February 1, 2012 antifungal proficiency testing event. Thirty-two laboratories participated in this event.

The interpretation of MIC values for antifungal susceptibility testing of yeasts and molds is in a state of constant change. These changes are necessitated by new information emerging from clinical trials and laboratory susceptibility testing. NYSDOH Mycology Laboratory uses latest CLSI and EUCAST documents to score proficiency testing results. However, the participating laboratories are advised to regularly consult these organizations for the latest version of their standard documents.

Adapted from M-27S3 Vol. 28 No. 15, February 2010

Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Third Informational Supplement

Table 2. Interpretative Guidelines for *In Vitro* Susceptibility Testing of *Candida* spp.

Antifungal Agent	Susceptible (**S**)	Susceptible- dose dependent (**S-DD**)	Intermediate (**I**)	Resistant (**R**)	Nonsusceptible (**NS**)
Anidulafungin	**≤2**	-	-	-	**>2**
Caspofungin	**≤2**	-	-	-	**>2**
Fluconazole	**≤8**	**16-32**	-	**≥64**	-
Flucytosine	**≤4**	-	**8-16**	**≥32**	-
Itraconazole	**≤0.125**	**0.25-0.5**	-	**≥1**	-
Micafungin	**≤2**	-	-	-	**>2**
Voriconazole	**≤1**	**2**	-	**≥4**	-

Note: Please consult relevant CLSI publications for further details about these guidelines. No recommended guideline is currently available for the interpretation of MIC values for ketocoanzole and posaconazole.

***Candida* spp. EUCAST Antifungal Clinical Breakpoint Table v. 4.1, valid from 2012-03-05**

Antifungal agent	MIC breakpoint (mg/L)													
	C. albicans		*C. glabrata*		*C. krusei*		*C. parapsilosis*		*C. tropicalis*		*C. guillermondii*		Non-species related breakpoints[1]	
	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >
Amphotericin B	1	1	1	1	1	1	1	1	1	1	IE	IE	IE	IE
Anidulafungin	0.03	0.03	0.06	0.06	0.06	0.06	-	-	0.06	0.06	IE[2]	IE[2]	IE	IE
Caspofungin	Note[3]	Note[3]	Note[3]	Note[3]	Note[3]	Note[3]	-	-	Note[3]	Note[3]	IE[2]	IE[2]	IE	IE
Fluconazole	2	4	IE[2]	IE[2]	-	-	2	4	2	4	IE[2]	IE[2]	2	4
Itraconazole	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP
Micafungin	IP	IP	IP	IP	IP	IP	-	-	IP	IP	IE[2]	IE[2]	IP	IP
Posaconazole	0.06	0.06	IE[2]	IE[2]	IE[2]	IE[2]	0.06	0.06	0.06	0.06	IE[2]	IE[2]	IE	IE
Voriconazole	0.12[4]	0.12[4]	IE	IE	IE	IE	0.12[4]	0.12[4]	0.12[4]	0.12[4]	IE[2]	IE[2]	IE	IE

IE Insufficient Evidence IP In preparation

Notes

1. Non-species related breakpoints have been determined mainly on the basis of PK/PD data and are independent of MIC distributions of specific species. They are for use only for organisms that do not have specific breakpoints.

2. The ECOFFs for these species are in general higher than for *C. albicans*.

3. Due to significant inter-laboratory variation in MIC ranges for caspofungin, EUCAST breakpoints have not yet been established.

4. Strains with MIC values above the S/I breakpoint are rare or not yet reported. The identification and antimicrobial susceptibility tests on any such isolate must be repeated and if the result is confirmed the isolate sent to a reference laboratory. Until there is evidence regarding clinical response for confirmed isolates with MIC above the current resistant breakpoint (in italics) they should be reported resistant.

COMMENTS: Acceptable results were MICs +/-2 dilutions of the reference laboratory results for any single drug. Only 5 of the 32 laboratories participating in this test event tested all 10 antifungal drugs. The reported results were as follows: itraconazole (30 laboratories), flucytosine (26 laboratories), amphotericin B (23 laboratories), caspofungin (22 laboratories), posacoanazole (18 laboratories), anidulafungin (17 laboratories), and micafungin (17 laboratories), ketocoanzole (5 laboratories). Fluconazole was the only drug tested by all 32 laboratories, but three laboratories failed to achieve acceptable results for this antifungal. Voriconazole was not validated since 6 out of 24 (25%) laboratories reported MIC value less and equal to 1 with the interpretation of 'susceptible'. Eight laboratories did not report any interpretation for amphotericin B and six laboratories had no interpretation for posaconazole MIC.

Table 3. Laboratory Performance

S- 1: *Candida glabrata* (M956)

Drug	Laboratories with acceptable responses / Total Laboratories (% acceptable responses)
Amphotericin B	23/23 (100%)
Anidulafungin	17/17 (100%)
Caspofungin	22/22 (100%)
Flucytosine (5-FC)	26/26 (100%)
Fluconazole	29/32 (91%)
Itraconazole	28/30 (93%)
Ketoconzole	5/5 (100%)
Micafungin	17/17 (100%)
Posaconazole	17/18 (94%)
Voriconazole	18/24 (75%)

Table 4. Antifungal MICs (µg/ml) Reported by the Participating Laboratories

S-1: *Candida glabrata* (M956)

Drug	No. labs	MIC (µg/ml)															
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256
Amphotericin B	23					2	10	10	1								
Anidulafungin	17		10	7													
Caspofungin	22				7	12	2		1								
Flucytosine (5-FC)	26			8	16	2											
Fluconazole	31*									1				2	6	10	12
Itraconazole	29*						1	1	6	5	1		14	1			
Ketoconazole	4*							1		1	1	1					
Micafungin	17	11	5	1													
Posaconazole	18							1		4		12		1			
Voriconazole	24						1	1	4	12	4	2					

* One laboratory used disk diffusion method. No MIC value was reported.

Colors represent the testing method used:

- CLSI microdilution method
- YeastOne Colorimetric method
- Etest
- Both CLSI microdilution and Vitek II methods
- Both CLSI microdilution and YeastOne Colorimetric methods
- Both YeastOne Colorimetric and Etest methods
- CLSI microdilution, YeastOne Colorimetric, and Etest methods

Table 5. Antifungal Susceptibility Interpretations Reported by the Participating Laboratories

S-1: *Candida glabrata* (M956)

Drug	No. laboratories	Susceptible	Susceptible-dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	23	15					8
Anidulafungin	17	17					
Caspofungin	22	22					
Flucytosine	26	26					
Fluconazole	32	1	1	1	29		
Itraconazole	30		2		28		
Ketoconazole	5	1	1				3
Micafungin	17	17					
Posaconazole	18		3		9		6
Voriconazole	24	6	12		6		

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR MOLDS

INTRODUCTION: Clinical laboratories perform susceptibility testing of pathogenic molds to determine their *in vitro* resistance to antifungal drugs. This test is also useful in conducting surveillance for evolving patterns of antifungal drug resistance in a healthcare facility. It is not clear at this juncture if the results of mold susceptibility testing have direct relevance in the selection of appropriate drugs for treatment. Clinical Laboratory Standards Institute (CLSI) document of M38-A2 describes the current standard methods for antifungal susceptibility testing of pathogenic yeasts. Another resource for standardized method is the EUCAST Technical Note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia-forming moulds. The following nine drugs are included in the antifungal susceptibility panel - amphotericin B, anidulafungin, caspofungin, fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole.

MATERIALS: *Aspergillus fumigatus* M2039 was used as test analyte; it was obtained from a reference laboratory. Laboratories were free to choose any number of drugs and preferred test method. Three laboratories used CLSI Microdilution method while the remaining three used YeastOne Colorimetric method. Please refer to Table 6 and 7 for summary of performances.

COMMENTS: Six out of thirty-two laboratories, which hold antifungal susceptibility testing for yeasts permit, participated in this test event for molds. Acceptable results were MICs +/-2 dilutions of the reference laboratory results for any single drug. All the participating laboratories reported the MIC values within the acceptable ranges for amphotericin B, fluconazole, ketocoanzole, posaconazole, and voriconazole. One laboratory reported higher MIC value for caspofungin than the acceptable range and one laboratory reported lower MIC value for itraconazole than the acceptable range. Anidulafungin and micafungin were not validated since no consensus MIC ranges were obtained.

Table 6. Mold Antifungal Susceptibility: *Aspergillus fumigatus* M2039.

Drugs	Acceptable MIC (μg/ml) Range	Reference laboratory MIC (μg/ml)	Participating laboratories MIC(μg/ml) range in previous event	Participating laboratories MIC (μg/ml) range in current event
Amphotericin B	0.06 – 1.0	0.25	0.25 – 3.0	0.12 – 1.0
Anidulafungin **(Invalidated)**	0.008 – 0.12	0.03	0.015 – 8.0	0.015 – 8.0
Caspofungin	0.008 – 1.0	0.5	0.03 – 16	0.008 – 8.0
Fluconazole	≥ 64	64	≥ 64	≥ 64
Itraconazole	2.0 – 16	16	4.0 – 128	0.25 – 16
Ketoconzole	4.0 – 64	16	16 – 64	16 – 32
Micafungin **(Invalidated)**	0.008 – 0.12	0.03	0.015 – 8.0	0.008 – 8.0
Posaconazole	0.06 – 1.0	1.0	0.5 – 4.0	0.06 – 1.0
Voriconazole	0.25 – 2.0	2.0	0.5 – 4.0	0.25 – 2.0

Table 7. MIC (μg/ml) Values of Mold Antifungal Susceptibility: *Aspergillus fumigatus* M2039

Drugs (μg/ml)	Total # of labs	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1.0	2.0	8.0	16	32	64	256
Amphotericin B	6					1	1	2	2						
Anidulafungin	4		1	1	1						1				
Caspofungin	5	1		1	1			1			1				
Fluconazole	5													2	3
Itraconazole	6						1			1	1	3			
Ketoconazole	2											1	1		
Micafungin	4	1		1	1						1				
Posaconazole	5				1		1	1	2						
Voriconazole	5						2	1		2					

Colors represent the testing method used:

CLSI microdilution method
YeastOne Colorimetric method
Both CLSI microdilution and YeastOne Colorimetric methods

Further Reading:

Canton E, Peman J, Gobernado M, Alvarez E, Baquero F, Cisterna R, Gil J, Martin-Mazuelos E, Rubio C, Sanchez-Sousa A, Settano C. 2005. Sensititre YeastOne caspofungin susceptibility testing of *Candida* clinical isolates: correlation with results of NCCLS M27-A2 multicenter study. *Antimicrobiol Agents Chemother*. 49: 1604-1607.

Clinical and Laboratory Standards Institute. 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard - Third Edition. CLSI document M27-A3 (ISBN 1-56238-666-2).

Clinical and Laboratory Standards Institute. 2008. Quality Control Minimal Inhibitory Concentration (MIC) Limits for Broth Microdilution and MIC Interpretive Breakpoints; Informational Supplement - Third Edition. CLSI document M27-S3 (ISBN 1-56238-667-0).

Clinical and Laboratory Standards Institute. 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard – Second Edition. CLSI document M38-A2 (1-56238-668-9).

Clinical and Laboratory Standards Institute. 2009. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline – Second Edition. CLSI document M44-A2 (ISBN 1-56238-703-0).

Clinical and Laboratory Standards Institute. 2009. Zone Diameter Interpretive Standards, Corresponding Minimal Inhibitory Concentration (MIC) Interpretive Breakpoints, and Quality Control Limits for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Informational Supplement. CLSI document M44-S3.

Clinical and Laboratory Standards Institute. 2010. Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Approved Guideline. CLSI document M51-A (ISBN 1-56238-725-1).

Clinical and Laboratory Standards Institute. 2010. Performance Standards for Antifungal Disk Diffusion Susceptibility Testing of Filamentous Fungi; Informational Supplement. CLSI document M51-S1 (ISBN 1-56238-725-1).

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST technical note on fluconazole. Clin Microbiol Infect. 14: 193-195.

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST definitive document Edef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. Clin Microbiol Infect. 14: 398-405.

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST technical note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia–forming moulds. Clin Microbiol Infect. 14: 982-984.

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST technical note on voriconazole. Clin Microbiol Infect. 14: 985-987.

Nirav R. Shah, M.D., M.P.H.
Commissioner