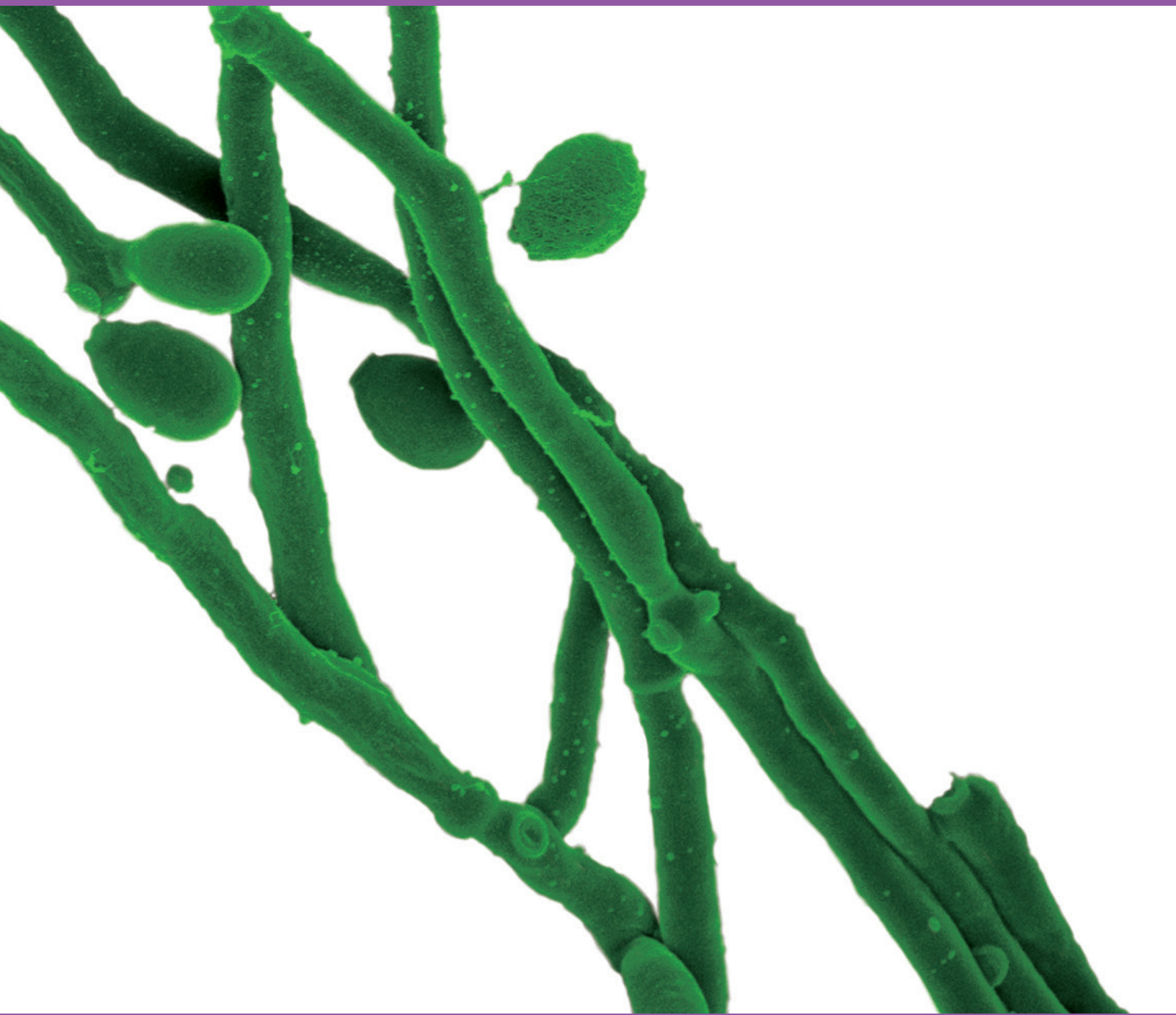
Mycology Proficiency Testing Program



Test Event Critique
May 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 morphologic features are matched with molecular identification using PCR and nucleotide sequencing of ribosomal ITS1 – ITS2 regions. The strain that best demonstrates the morphologic and physiologic characteristics of the proposed test analyte, and has high nucleotide sequence homology with reference strains in the genome databases, is selected as final test specimen.

Grading Policy

A laboratory's response for each sample is compared with the responses that reflect 80% agreement of 10 referee laboratories and/or responses from 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 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) and EUCAST guidelines. 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 usual 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'.

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 – Yeast Only

	Specimen key	Validated specimen	Other acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
Y-1	*Candida dubliniensis*	(Not Validated)		
Y-2	*Cryptococcus uniguttulatus*	*Cryptococcus uniguttulatus*		118/119 (99%)
Y-3	*Blastoschizomyces capitatus*	*Blastoschizomyces capitatus*	*Geotrichum capitatum* *Geotrichum* sp.	102/119 (86%)
Y-4	*Cryptococcus albidus*	*Cryptococcus albidus*	*Cryptococcus saitoi* *Cryptococcus* sp.	116/119 (97%)
Y-5	*Candida rugosa*	*Candida rugosa*		115/119 (97%)

Antifungal Susceptibility Testing for Yeast (S-1: *Candida krusei* M2559)

Drugs	Acceptable MIC (µg/ml) Range	Acceptable interpretation	Laboratories with acceptable responses/ Total laboratories (% correct responses)
Amphotericin B	0.12 – 1.0	Susceptible / No interpretation	21/21 (100%)
Anidulafungin	0.008 – 0.12	Susceptible	16/16 (100%)
Caspofungin	0.06 – 1.0	Susceptible	22/22 (100%)
Flucytosine (5-FC)	2.0 – 32	Susceptible / Intermediate / Resistant	25/25 (100%)
Fluconazole*	8.0 - 128	Resistant / No interpretation	(Not Validated)
Itraconazole*	0.06 – 0.5	Susceptible / Susceptible-Dose Dependent	(Not Validated)
Ketoconzole	0.12 – 2.0	No interpretation	5/5 (100%)
Micafungin	0.03 – 0.5	Susceptible	16/16 (100%)
Posaconazole	0.06 – 1.0	Susceptible / No interpretation	16/16 (100%)
Voriconazole	0.06 – 1.0	Susceptible	24/24 (100%)

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

Antifungal SusceptibilityTesting for Mold (MS-1: *Aspergillus fumigatus* M2040)

Drugs	Acceptable MIC (μg/ml) Range	Laboratories within MIC range / Total laboratories (%)
Amphotericin B	0.12 – 2.0	5/5 (100%)
Anidulafungin	0.004 – 0.06	3/3 (100%)
Caspofungin	0.008 – 0.25	4/4 (100%)
Fluconazole	≥ 64	4/4 (100%)
Itraconazole*	4.0 – 64	(Not Validated)
Ketoconzole	4.0 – 64	2/2 (100%)
Micafungin	0.004 – 0.12	3/3 (100%)
Posaconazole	0.06 – 1.0	4/4 (100%)
Voriconazole	0.12 – 2.0	4/4 (100%)

*This analyte is not validated as no consensus on MIC values by different devices/methods used for antifungal susceptibility testing.

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	85
AMS Vitek	5
Vitek2	65
Remel Rapid ID Yeast Plus System	9
Microscan	5
Sequencing	5
MALDI-TOF	1
Antifungal Susceptibility*	
YeastOne -Yeast	26
YeastOne - Mold	2
Etest	3
Disk diffusion	1
Others† - Yeast	3
Others† - Mold	3

*Include multiple systems used by some laboratories

†Include laboratories using CLSI Microbroth dilution method

YEAST DESCRIPTIONS

Y-1 *Candida dubliniensis*

Source: Wound / Bronchial wash / Urine

CLINICAL SIGNIFICANCE: *Candida dubliniensis* was initially recovered from the oral cavities of HIV infected individuals and AIDS patients causing erythematous and/or pseudomembranous oral candidiasis or angular cheilitis. *C. dubliniensis* has also been isolated from other body sites including lungs, vagina, blood, and feces.

COLONY: *C. dubliniensis* colony was white to cream, smooth, and soft on Sabouraud's dextrose agar after 7 days at 25°C (Figure 1). This isolate of *C. dubliniensis* did not grow at 42°C.

MICROSCOPY: *C. dubliniensis* showed abundant, branched pseudohyphae and true hyphae with blastoconidia. Chlamydospores were single, in pairs, chains, or clusters on Corn meal agar with Tween 80 (Figure 1).

DIFFERENTIATION: *C. dubliniensis* is practically indistinguishable from *C. albicans* on the basis of many common phenotypic test. One physiologic feature that does appear to be fairly stable is that *C. dubliniensis* grows poorly at 42°C or not at all at 45°C while *C. albicans* grows well at both of these temperatures. In addition, *C. dubliniensis* is able to assimilate glycerol, but not xylose or trehalose as opposed to observations in *C. albicans*. Some commercial yeast identification kits such as the API 20C AUX, VITEK2, or the ID 32C have biocodes for *C. dubliniensis* included in the databases. These two closely related yeasts can also be distinguished by molecular methods.

MOLECULAR TEST: Genetically, *C. dubliniensis* has been found to be distinct from *C. albicans* in DNA fingerprinting studies even though the two species are closely related phylogenetically. Several *C. dubliniensis* molecular probes are available in reference laboratories.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Candida dubliniensis* isolate CD36 (GenBank accession no. FM992695.1).

ANTIFUNGAL SUSCEPTIBILITY: Several isolates of *C. dubliniensis* have been found to have higher resistance to fluconazole than other pathogenic species of *Candida*, and the resistance to fluconazole may be induced in some originally sensitive strains. This fact may have serious implications for immunocompromised individuals prescribed fluconazole for prolonged periods.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	8
Laboratories with correct ID:	92
Laboratories with incorrect ID:	27
(*Candida albicans*)	(26)
(*Cryptococcus neoformans*)	(1)

Illustrations:

FIGURE 1. *Candida dubliniensis,* white, glossy, and smooth colony on Sabouraud's dextrose agar, 4 days, 25°C. Microscopic morphology on corn meal agar with Tween 80, showing abundant branched pseudohyphae and true hyphae with blastoconidia (bar = 10 μm).

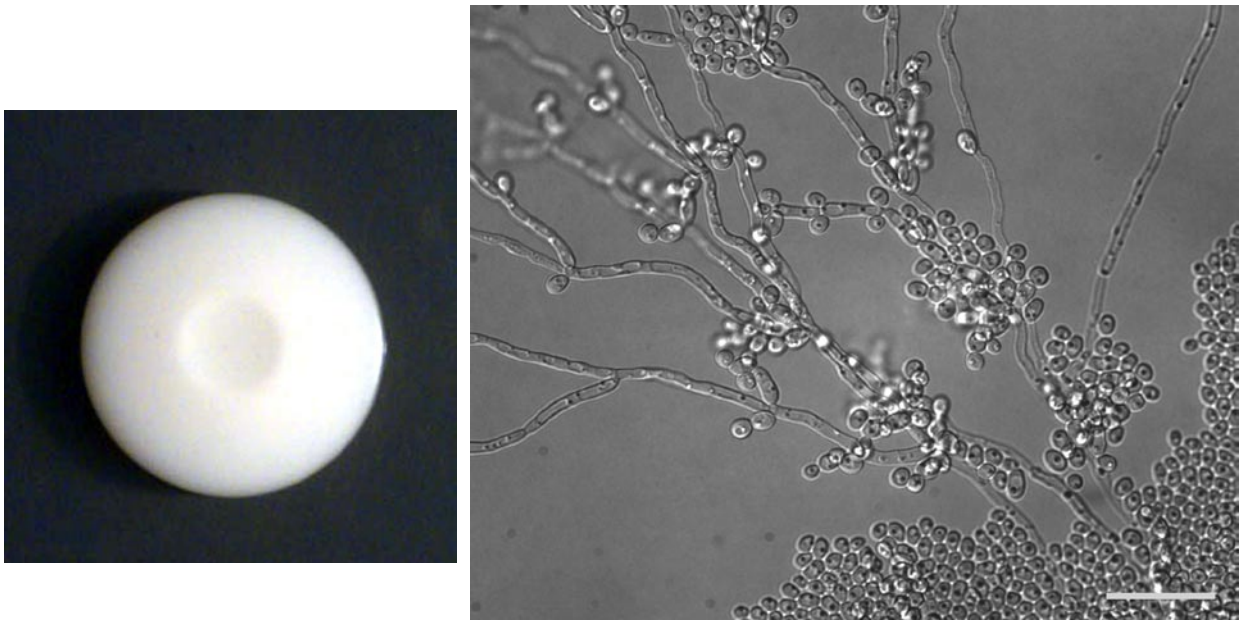
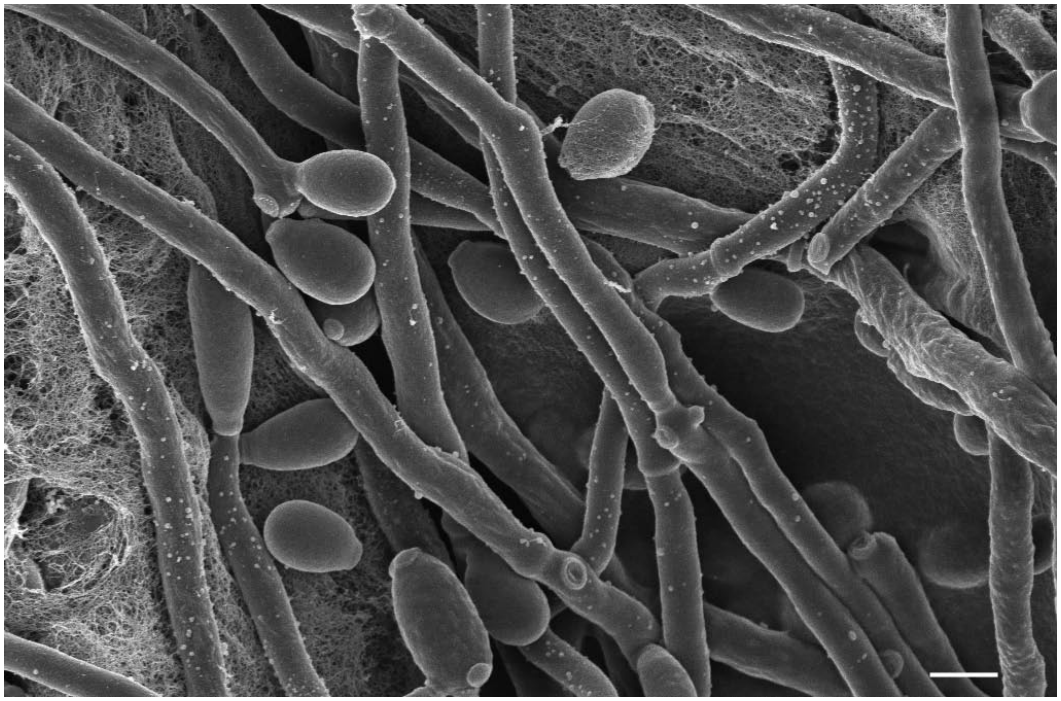


FIGURE 1A. Scanning electron micrograph of *Candida dubliniensis* illustrates pseudohyphae and blastoconidia (bar = 2 μm)



Further reading:

Cardenes-Perera CD, Torres- Lana A, Alonso-Vargas R, Moragues-Tosantas MD, Emeterio JP, Quindos-Andres G, Arevalo-Morales MP. 2004. Evaluation of API ID 32C® and Vitek-2® to identify *Candida dubliniensis*. *Diagn Microbiol & Infect Dis*. 50: 219 – 221.

Ellepola AN, Khan ZU. 2012. Rapid Differentiation of *Candida dubliniensis* from *Candida albicans* by Early D-Xylose Assimilation. *Med Princ Pract.* 21: 375-378.

Espinosa-Heidmann DG, McMillan BD, Lasala PR, Stanley J, Larzo CR. 2012. *Candida dubliniensis* endophthalmitis: first case in North America. *Int Ophthalmol*. 32: 41-45.

Khan Z, Ahmad S, Chandy R, Joseph L. 2012. A simple xylose-based agar medium for the differentiation of *Candida dubliniensis* and *Candida albicans*. *Diagn Microbiol Infect Dis*. 72: 285-287.

Khan Z, Ahmad S, Joseph L, Chandy R. 2012. *Candida dubliniensis*: an appraisal of its clinical significance as a bloodstream pathogen. *PLoS One.* 7:e32952.

Mirhendi H, makimura K, Zomorodian K, Maeda N, Ohshima T, Yamaguchi H. 2005. Differentiation of *Candida albicans* and *Candida dubliniensis* using a single enzyme PCR-RFLP method. *Jpn J Infect Dis.* 58: 235 – 237.

Nunn MA, Schäfer SM, Petrou MA, Brown JRM. 2007. Environmental source of *Candida dubliniensis*. *Emerg Infect Dis* [serial on the Internet]. Available from http://www.cdc.gov/EID/content/13/5/747.htm

Salgado-Parreno FJ, Alcoba-Florez J, Arias A, Moragues MD, Quindos G, Ponton J, Arevalo MP. 2006. *In vitro* activities of voriconazole and five licensed antifungal agents against *Candida dubliniensis*: comparison of CLSI M27-A2, Sensititre YeastOne, disk diffusion, and Etest methods. *Microb Drug Resist*. 12: 246-51.

Schabereiter-Gurtner C, Selitsch B, Rotter ML, Hirschl AM, Willinger B. 2007. Development of novel real-time PCR assays for detection and differentiation of eleven medically important *Aspergillus* and *Candida* species in clinical specimens. *J Clin Microbiol.* 45: 906-914.

Scheid LA, Mario DA, Kubiça TF, Santurio JM, Alves SH. 2012. *In vitro* activities of antifungal agents alone and in combination against fluconazole-susceptible and -resistant strains of *Candida dubliniensis. Braz J Infect Dis*. 16: 78-81.

Sullivan DJ, Moran GP, Pinjon E, Al-Mosaid A, Stokes C, Vaughan C, Coleman DC. 2004. Comparison of the epidemiology, drug resistance mechanisms and virulence of *Candida dubliniensis* and *Candida albicans*. *FEMS Yeast Research*. 4: 369 – 376.

Tsuruta R, Oda Y, Mizuno H, Hamada H, Nakahara T, Kasaoka S, Maekawa T. 2007. *Candida dubliniensis* isolated from the sputum of a patient with end-stage liver cirrhosis. *Intern Med*. 46: 597-600.

Us E, Cengiz SA. 2007. Prevalence and phenotypic evaluation of *Candida dubliniensis* in pregnant women with vulvovaginal candidosis in a university hospital in Ankara. *Mycoses.* 50: 13-20.

Yu N, Kim HR, Lee MK. 2012. The First Korean Case of Candidemia due to *Candida dubliniensis*. *Ann Lab Med*. 32: 225-228.

Y-2 *Cryptococcus uniguttulatus*

Source: Catheter / Urine

CLINICAL SIGNIFICANCE: *Cryptococcus uniguttulatus* ventriculitis was documented in a case report in 2001.

COLONY: *C. uniguttulatus* colony was smooth, dull, cream colored on Sabouraud's dextrose agar, after 7 days at 25°C (Figure 2).

MICROSCOPY: *C. uniguttulatus* produced round blastoconidia on corn meal agar with Tween-80 (Figure 2). No pseudo- or true hyphae were formed.

DIFFERENTIATION: *C. uniguttulatus* does not ferment any carbohydrate, does not grow at 37°C or on the media containing cycloheximide. It produces urease enzyme. It does not form brown colonies on caffeic seed agar, thus differentiating it from *C. neoformans*. It does not assimilate nitrate, differentiating from *C. albidus. C. laurentii* assimilates lactose and dulcitol, but *C. uniguttulatus* does not assimilate these carbohydrates.

MOLECULAR TEST: Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA was reported to differentiate several *Cryptococcus* species including *C. uniguttulatus*.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Filobasidium uniguttulatum* (*Cryptococcus uniguttulatus*) isolate YA07-b (GenBank accession no. DQ668348.1).

ANTIFUNGAL SUSCEPTIBILITY: A single clinical isolate was susceptible to amphotericin B and itraconazole.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	118
Laboratories with incorrect ID:	1
(*Cryptococcus* sp.)	(1)

Illustrations:

FIGURE 2. *Cryptococcus uniguttulatus*, smooth, creamy colored colony of on Sabouraud's dextrose agar, 7 days, 25°C. Microscopic morphology on corn meal agar showing round blastoconidia (bar = 10 μm).

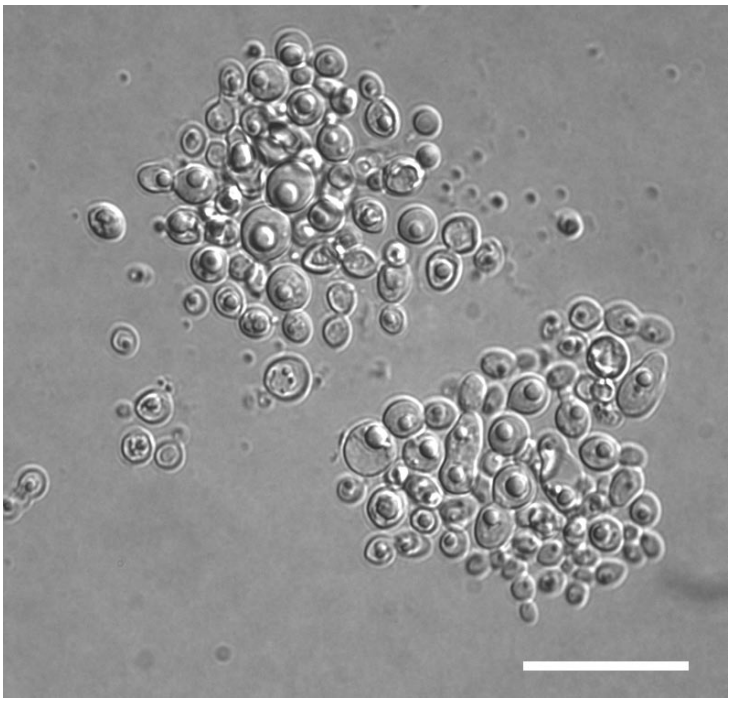
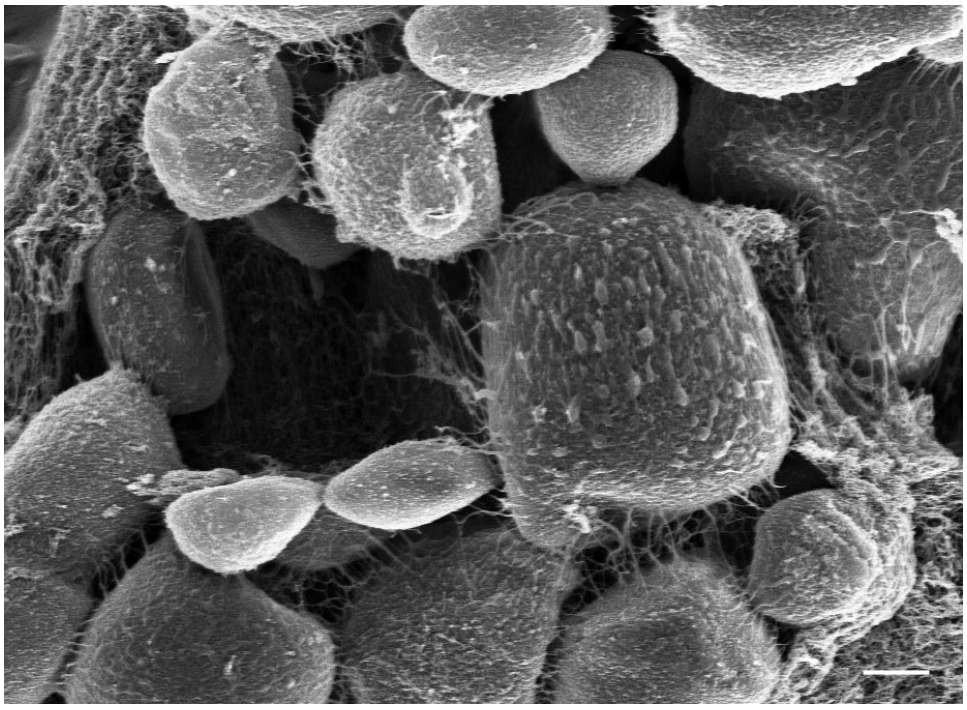


FIGURE 2A. Scanning electron micrograph illustrates blastoconidia (bar = 1 μm).



Further reading:

Kwon-Chung KJ, Hill WB, Bennett JE. 1981. New, special stain for histopathological diagnosis of cryptococcosis. *J. Clin. Microbiol*. 13: 383-387.

Manzano-Gayosso P, Hernández-Hernández F, Méndez-Tovar LJ, Palacios-Morales Y, Córdova-Martínez E, Bazán-Mora E, López-Martinez R. 2008. Onychomycosis incidence in type 2 diabetes mellitus patients. *Mycopathologia.* 166: 41-45.

McCurdy LH, Morrow JD. 2001. Ventriculitis due to *Cryptococcus uniguttulatus. South Med. J.* 94: 65-66.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol*. 172: 4238-4246.

Bernal-Martinez L, Gomez-Lopez A, Castelli MV, Mesa-Arango AC, Zaragoza O, Rodriguez-Tudela JL, Cuenca-Estrella M. 2010. Susceptibility profile of clinical isolates of non-*Cryptococcus neoformans*/non-*Cryptococcus gattii* species and literature review. *Med Mycol.* 48: 90-96.

Y-3 *Blastoschizomyces capitatus*

Source: Stool / Bone lesions / Urine

CLINICAL SIGNIFICANCE: *Blastoschizomyces capitatus* is an opportunistic pathogen in neutropenic patients.

COLONY: *B. capitatus* colony was smooth to wrinkled, raised, and hyaline on Sabouraud's dextrose agar for 7 days at 25°C (Figure 3).

MICROSCOPY: On corn meal agar with Tween 80, true hyphae were produced. Annelloconidia emerged from the annellides. Annellides became longer and narrower with the production of each new conidium (Figure 3). The resulting conidia simulated the appearance of arthroconidia as seen in *Trichsporon* spp. and *Geotrichum* spp.

DIFFERENTIATION: *B. capitatus* can be differentiated from *G. candidum* by the lack of growth on a medium containing D-xylose as a carbon source. It can be differentiated from *T. beigelii* by lack of urease and its growth at 45°C. *B. capitatus* is included in the database of commercial yeast identification systems.

MOLECULAR TEST: Primers for large ribosomal subunit DNA sequences were used in PCR to differentiate between *C. famata* and *C. guilliermondii*. The amplification of 340 bp of the large rDNA led to rapid and specific identification of *C. famata*. RAPD-PCR analysis was applied to identify *C. famata* in dairy product.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Dipodascus capitatus (Geotrichum capitatus)* isolate wb410 (GenBank accession no. AF455443.1).

ANTIFUNGAL SUSCEPTIBILITY: *B. capitatus* is susceptible to amphotericin B; fluconazole resistant strains have been reported from cancer patients.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	8
Laboratories with correct ID:	102
Laboratories with incorrect ID:	24
(*Geotrichum candidum*)	(4)
(*Candida lipolytica*)	(3)
(*Candida kefyr*)	(1)
(Candida krusei)	(1)
(*Candida* sp.)	(1)

Illustrations:

FIGURE 3. White, smooth to slightly wrinkled, raised colony of *Blastoschizomyces captitatus* on Sabouraud's dextrose agar 7-day, 25°C. Microscopic morphology showing annelloconidia formed from true hyphae on Corn meal agar with Tween 80 (bar = 10 μm).

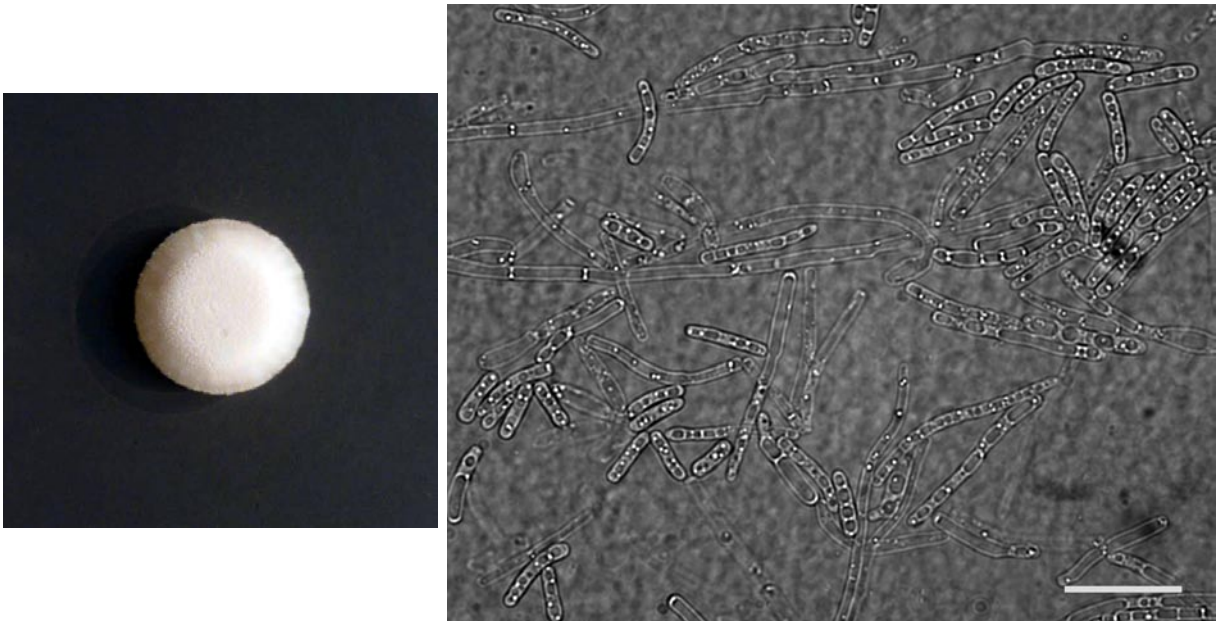
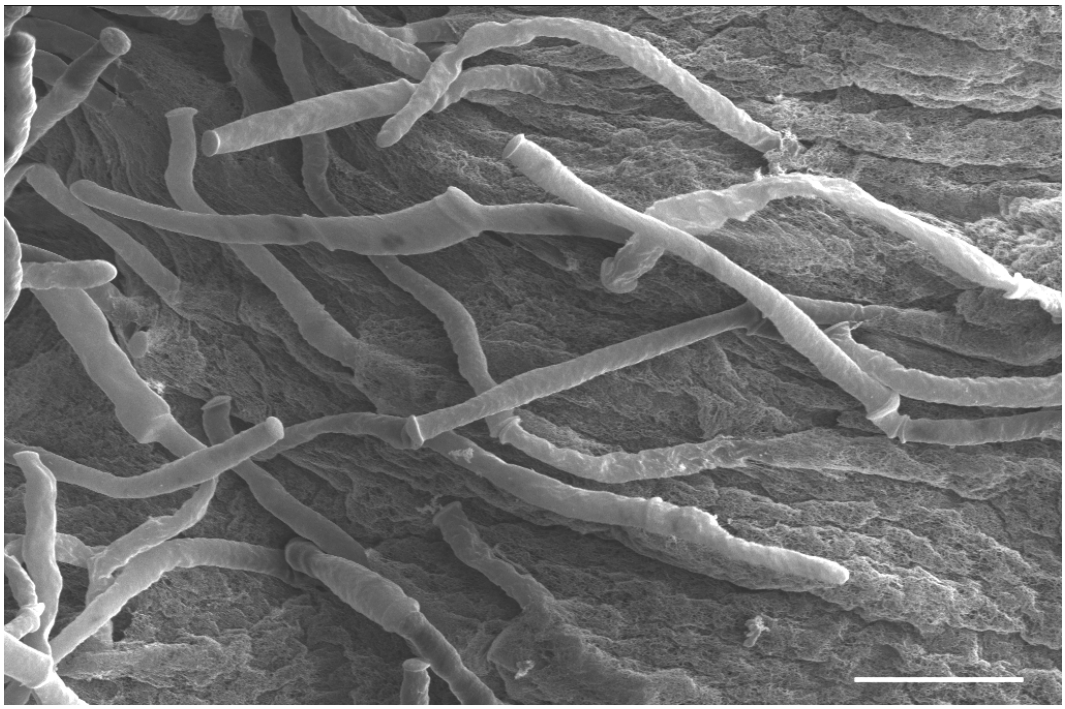


FIGURE 3A. Scanning electron micrograph illustrating true hyphae (bar = 10 μm).



Further reading:

Adami F, Scarin M, Pescarini L, Binotto G, Pavan L, Sgarabotto D, Semenzato G. 2011. Successful control of *Blastoschizomyces capitatus* infection in three consecutive acute leukaemia patients despite initial unresponsiveness to liposomal amphotericin B. *Mycoses.* 54: 365-369.

Birrenbach T, Bertschy S, Aebersold F, Mueller NJ, Achermann Y, Muehlethaler K, Zimmerli S. 2012. Emergence of *Blastoschizomyces capitatus* yeast infections, Central Europe. *Emerg Infect Dis*. 18: 98-101.

Celik AD, Ozaras R, Kantarcioglu S, Mert A, Tabak F, Ozturk R. 2009. Spondylodiscitis due to an emergent fungal pathogen: *Blastoschizomyces capitatus*, a case report and review of the literature. *Rheumatol Int.* 29: 1237-1241.

Chittick P, Palavecino EL, Delashmitt B, Evans J, Peacock JE Jr. 2009. Case of fatal *Blastoschizomyces capitatus* infection occurring in a patient receiving empiric micafungin therapy. *Antimicrob Agents Chemother*. 53: 5306-5307.

Gill PK, Gill JS. 2011. *Blastoschizomyces capitatus* pneumonia in an immuno-competent female. *Indian J Tuberc*. 58: 88-89.

Gurgui M, Sanchez F, March F, Lopez-Contreras J, Martino R, Cotura A, Galvez ML, Roig C, Coll P. 2011. Nosocomial outbreak of *Blastoschizomyces capitatus* associated with contaminated milk in a haematological unit. *J Hosp Infect*. 78: 274-278.

Sreeja S, Banashankari GS, Bhavana MV, Devi DR. 2011. *Blastoschizomyces capitatus* pneumonia: a rare case. *Indian J Pathol Microbiol.* 54: 846-847.

Y-4 *Cryptococcus albidus*

Source: Stool / Eye / Urine

CLINICAL SIGNIFICANCE: *Cryptococcus albidus* is a rare causal agent of sepsis, wound infection, and pneumonia in immunocompromised patients.

COLONY: *C. albidus* colony was soft, mucoid, cream to pink on Sabouraud's dextrose agar 7 days at 25°C (Figure 4).

MICROSCOPY: *C. albidus* showed large, round budding yeast cells on Corn meal agar with Tween 80. No true hyphae or pseudohyphae was seen (Figure 4).

DIFFERENTIATION: *C. albidus* does not grow on media containing cycloheximide, grows poorly at 37°C, produces urease enzyme, and assimilates nitrate. It is differentiated from *C. neoformans* by its inability to form brown colonies on niger seed agar. Although *C. terreus* is also nitrate-positive, it differs from *C. albidus* in assimilation of sorbitol and N-acetylglucosamine.

MOLECULAR TEST: Ribosomal DNA sequence analysis revealed diversity in *C. albidus*.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Cryptococcus saitoi* (a new species distinguished from *Cryptococcus albidus*) strain SN26 (GenBank accession no. FJ515177.1).

ANTIFUNGAL SUSCEPTIBILITY: Almost all isolates are susceptible to amphotericin B, flucytosine, and azoles.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	116
Laboratories with incorrect ID:	5
(*Cryptococcus neoformans*)	(1)
(*Cryptococcus uniguttulatus*)	(1)
(*Cryptococcus laurentii*)	(1)

Illustrations:

FIGURE 4. *Cryptococcus albidus*, mucoid, soft colony on Sabouraud's dextrose agar, 7 days, 25°C. Microscopic morphology showing large, round blastoconidia on Corn meal agar with Tween 80 (BAR = 10 μm).

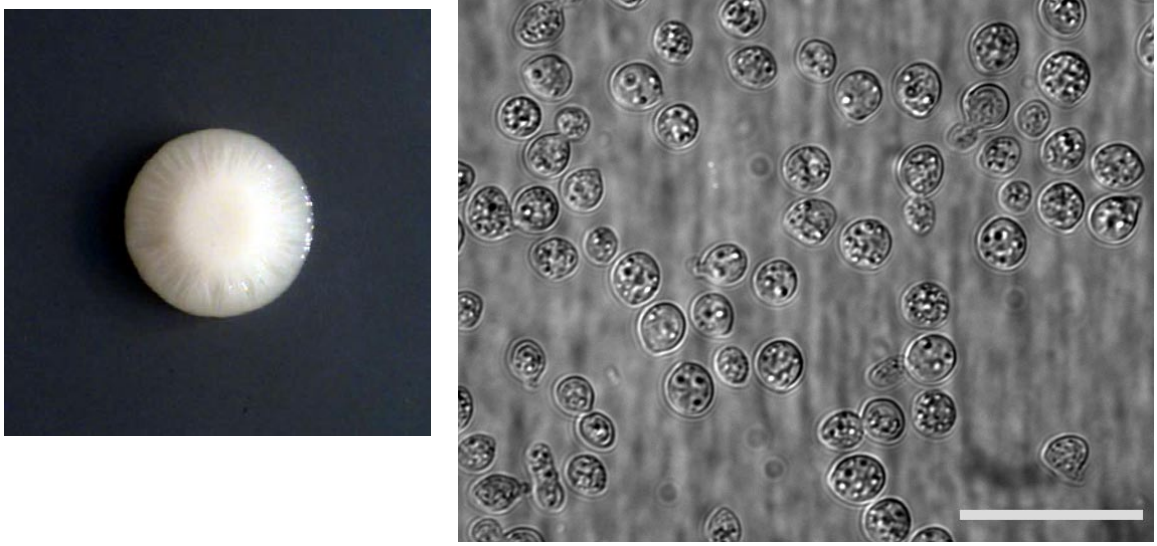
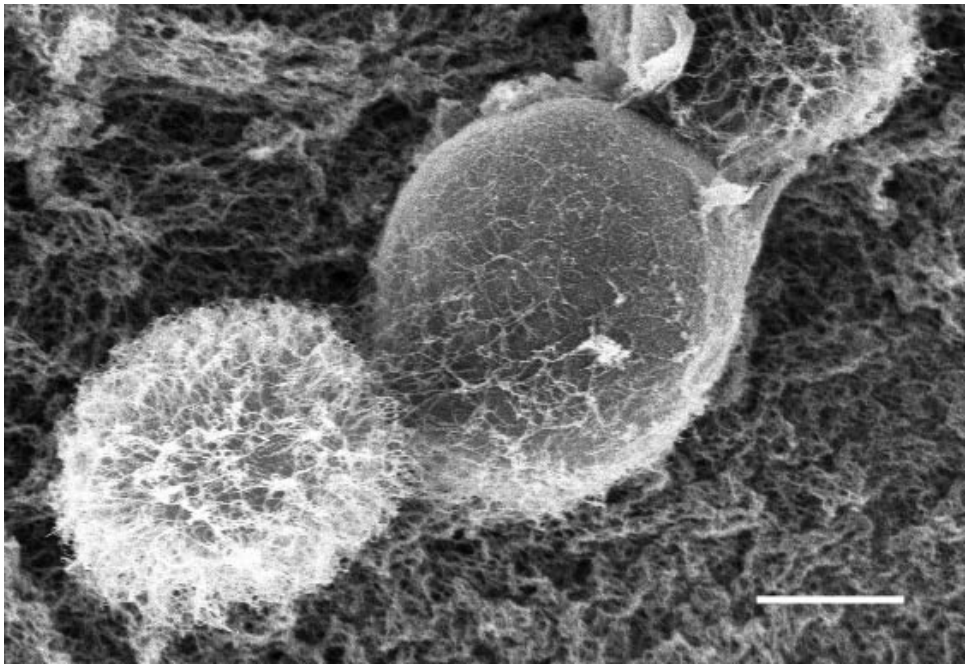


FIGURE 4A. Scanning electron micrograph illustrating blastoconidia (bar = 2 μm).



Further reading:

Burnik C, Altintas ND, Ozkaya G, Serter T, Selçuk ZT, Firat P, Arikan S, Cuenca-Estrella M, Topeli A. 2007. Acute respiratory distress syndrome due to *Cryptococcus albidus* pneumonia: case report and review of the literature. *Med Mycol.* 45: 469-73.

de Castro LE, Sarraf OA, Lally JM, Sandoval HP, Solomon KD, Vroman DT. 2005.*Cryptococcus albidus* keratitis after corneal transplantation. *Cornea*. 24: 882-883.

Endo JO, Klein SZ, Pirozzi M, Pirozzi C, Hull CM. 2011. Generalized *Cryptococcus albidus* in an immunosuppressed patient with palmopustular psoriasis. *Cutis.* 88: 129-132.

Fonseca A, Scorzetti G, Fell JW. 2000. Diversity in the yeast *Cryptococcus albidus* and related species as revealed by ribosomal DNA sequence analysis. *Can. J. Micobiol*. 46: 7-27.

Garelick JM, Khodabakhsh AJ, Lopez Y, Bamji M, Lister M. 2004. Scleral ulceration caused by *Cryptococcus albidus* in a patient with acquired immune deficiency syndrome. *Cornea.* 23: 730-731.

Hoang JK, Burruss J. 2007. Localized cutaneous *Cryptococcus albidus* infection in a 14-year-old boy on etanercept therapy. *Pediatr Dermatol*. 24: 285-288.

Lee YA, Kim HJ, Lee TW, Kim MJ, Lee MH, Lee JH, Ihm CG. 2004. First report of *Cryptococcus albidus*--induced disseminated cryptococcosis in a renal transplant recipient. *Korean J Intern Med.* 19: 53-57.

Manzano-Gayosso P, Hernández-Hernández F, Méndez-Tovar LJ, Palacios-Morales Y, Córdova-Martínez E, Bazán-Mora E, López-Martinez R. 2008. Onychomycosis Incidence in Type 2 Diabetes Mellitus Patients. *Mycopathologia.* 166:41-45

Narayan S, Batta K, Colloby P, Tan CY. 2000. Cutaneous *Cryptococcus* infection due to *C. albidus* associated with Sezary syndrome. *Br. J. Dermatol*. 143: 632-634.

Wells GM, Gajjar A, Pearson TA, Hale KL, Shenep JL. 1998. Pulmonary cryptosporidiosis and *Cryptococcus albidus* fungemia in a child with acute lymphocytic leukemia. *Med Pediatr. Oncol*. 31: 544-546.

Y-5 *Candida rugosa*

Source: Blood / Catheter / Urine

CLINICAL SIGNIFICANCE: *Candida rugosa* is an infrequent causal agent of fungemia in patients with indwelling catheters. Also, it is reported to cause infection in burn patients.

COLONY: *C. rugosa* colony was white to cream, wrinkled on Sabouraud's dextrose agar 7 days at 25°C (Figure 5).

MICROSCOPY: *C. rugosa* showed branched pseudohyphae with chains of elongated blastoconidia (Figure 5).

DIFFERENTIATION: *C. rugosa* ferments only glucose, does not grow on media containing cycloheximide shows variable growth at 42°C, and is urea and nitrate negative. Microscopically, it forms branched pseudohyphae that differentiates it from *C. lusitaniae* and *C. parapsilosis*. It does not form true hyphae, differentiating it from *Trichosporon beigelii*.

MOLECULAR TEST: PCR assay of the ITS1 and ITS2 regions of ribosomal DNA was developed to identify *C. rugosa* in clinical specimens. A repetitive sequence-based PCR technique was developed to characterize the genotypic relatedness among *C. rugosa* isolates. Karyotyping by PFGE was developed as a typing tool for discrimination among strains of *C. rugosa*.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Candida rugosa* isolate ATCC 10571 (GenBank accession no. GU144663.1).

ANTIFUNGAL SUSCEPTIBILITY: Clinical isolates are susceptible to caspofungin, 5-flucytosine, and various azoles such as fluconazole, ketocoanzole, and itraconazole. It is less susceptible to polyene antifungals like amphotericin B and nystatin.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	115
Laboratories with incorrect ID:	4
(*Candida krusei*)	(1)
(*Candida* sp.)	(1)
(*Candida zeylanoides*)	(1)
(*Trichosporon* sp.)	(1)

Illustrations:

FIGURE 5. *Candida rugosa,* cream colored, wrinkled colony on Sabouraud's dextrose agar, 7 days, 25°C. Microscopic morphology of *Candida rugosa* showing branched pseudohyphae with elongated blastoconidia on Corn meal agar with Tween 80 (bar = 10 μm).

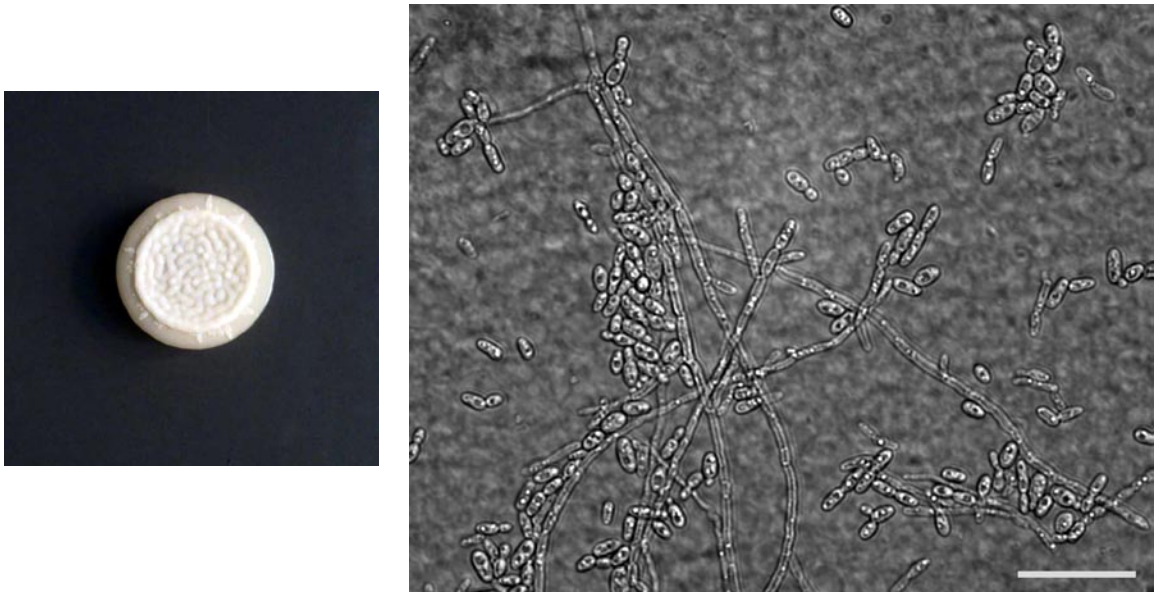
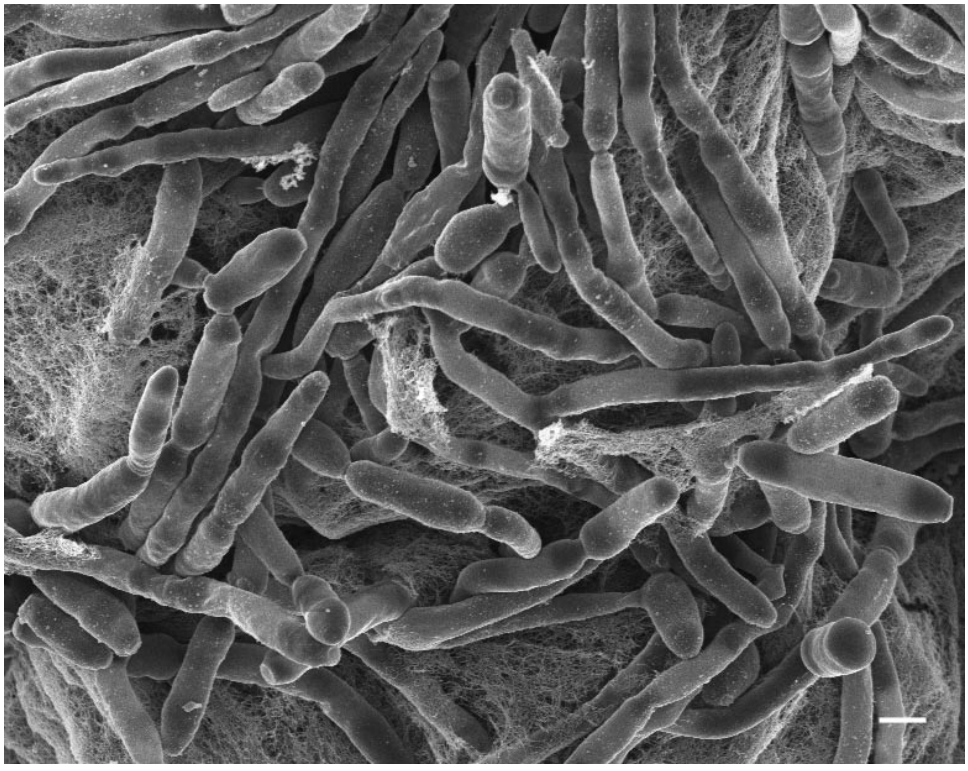


FIGURE 5A. Scanning electron micrograph with pseudohyphae (bar = 2 μm).



Further reading:

Behera B, Singh RI, Xess I, Mathur P, Hasan F, Misra MC. 2010. *Candida rugosa*: a possible emerging cause of candidaemia in trauma patients. *Infection.* 38: 387-393.

Dib JC, Dube M, Kelly C, Rinaldi MG, Patterson JE. 1996. Evaluation of pulsed-field gel electrophoresis as a typing system for *Candida rugosa*: comparison of karyotype and restriction fragment length polymorphisms. *J. Clin. Microbiol*. 34: 1494-1496.

Kocyigit I, Unal A, Sipahioglu MH, Tokgoz B, Oymak O, Utas C. 2010. Peritonitis Due to *Candida rugosa*: the first case report. *Perit Dial Int.* 30: 576-577.

Krcmery V, Barnes AJ. 2002. Non-*albicans Candida* spp. causing fungaemia: pathogenicity and antifungal resistance. *J. Hosp. Infect.* 50: 243-2460.

Lopes Colombo A, Azevedo Melo AS, Crespo Rosas RF, Salomao R, Briones M, Hollis RJ, Messer SA, Pfaller MA. 2003. Outbreak of *Candida rugosa* candidemia: an emerging pathogen that may be refractory to amphotericin B therapy. *Diagn. Microbiol. Infect. Dis*. 46: 253-257.

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.

Pfaller MA, Diekema DJ, Colombo AL, Kibbler C, Ng KP, Gibbs DL, Newell VA. 2006. *Candida rugosa*, an emerging fungal pathogen with resistance to azoles: geographic and temporal trends from the ARTEMIS DISK antifungal surveillance program. *J Clin Microbiol.* 44: 3578-3582.

Redkar RJ, Dube MP, McCleskey FK, Rinaldi MG, del Vecchio VG. 1996. DNA fingerprinting of *Candida rugosa* via repetitive sequence-based PCR. *J. Clin. Microbiol*. 34: 1677-1681.

Shenoy S, Samuga M, Urs S, Anuradha KM, Kurian MM, Augustine A, Anand AR, Prasad A. 1996. Intravenous catheter-related *Candida rugosa* fungaemia. *Trop. Doct*. 26: 31.

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.

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.

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: Adapted from Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Third Informational Supplement M-27S3 Vol. 28 No. 15, February 2010. 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.

MATERIALS: Candida krusei (S-1) was the analyte in the May 30, 2012 antifungal proficiency testing event. Thirty-one laboratories participated in this event.

COMMENTS: Only 3 of the 31 laboratories participating in this test event tested all 10 antifungal drugs for yeast. Overall participation for various drugs was as follows: fluconazole (30 laboratories), itraconazole (29 laboratories), flucytosine (25 laboratories), amphotericin B (21 laboratories), caspofungin (22 laboratories), anidulafungin, micafungin, and posacoanazole (16 laboratories each), and ketocoanzole (5 laboratories).

Fourteen out of 21 laboratories reported a 'Susceptible' interpretation and 7 laboratories reported 'No interpretation' for amphotericin B; the reported range of MIC ≤ 1.0 μg/ml.

Similarly, eleven out of 16 laboratories reported a 'Susceptible' interpretation and 5 laboratories reported 'No interpretation' for posaconazole all with an MIC≤ 0.5 μg/ml. All participating laboratories that tested anidulafungin, caspofungin, micafungin, and/or voriconazole reported acceptable MIC values and interpretation of 'Susceptible'. Of the 25 participants that reported MIC results for 5-FC, 96% obtained a MIC ≤ 8 μg/ml with the interpretation of 'Intermediate' or 'Susceptible'. One laboratory reported MIC ≥ 32 μg/ml with the interpretation of 'Resistant' for this isolate. Itraconazole was not validated for this isolate in this event as less than 80% laboratories reported the interpretation of 'susceptible' or 'susceptible-dose dependent'. One laboratory reported the test isolate for itraconazole as 'Intermediate', which is not defined in CLSI. For fluconazole, 70% laboratories reported an MIC of fluconazole to be ≥ 32 μg/ml, with 1/30 reporting the MIC as < 8 μg/ml. Six participants did not report the MIC value. It is customary in many laboratories to report all *C. krusei* strains as 'Resistant' or 'No interpretation' for fluconazole.

Summary of results for antifungal susceptibility test for S- 1: *Candida krusei* (M2559)

Drug	Laboratories with acceptable responses / Total Laboratories (% acceptable responses)
Amphotericin B	21/21 (100%)
Anidulafungin	16/16 (100%)
Caspofungin	22/22 (100%)
Flucytosine (5-FC)	25/25 (100%)
Fluconazole	23/30 (77%)
Itraconazole	23/29 (79%)
Ketoconzole	5/5 (100%)
Micafungin	16/16 (100%)
Posaconazole	16/16 (100%)
Voriconazole	24/24 (100%)

Range of Reported MICs (µg/ml) for S-1: *Candida krusei* (M2559)

Drug	No. labs	MIC (µg/ml)																
		NA	0.015	0.03	0.06	0.094	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256
Amphotericin B	21						1		14	6								
Anidulafungin	16		2	9	5													
Caspofungin	22						3	9	7	3								
Flucytosine (5-FC)	25											8	16		1			
Fluconazole	29*	6										1		1	16	2	2	1
Itraconazole	28*	1					2	19	4	1	1							
Ketoconazole	4*								3	1								
Micafungin	16				6		10											
Posaconazole	16				1		2	9	4									
Voriconazole	24					1	4	16	3									

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

Colors Scheme for the testing method used:

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

Antifungal Susceptibility Test Interpretations Reported by the Participating Laboratories: S-1: *Candida krusei* (M2559)

Drug	No. laboratories	Susceptible	Susceptible-dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	21	14					7
Anidulafungin	16	16					
Caspofungin	22	22					
Flucytosine	25	8		15	2		
Fluconazole	30	1	4		17		8
Itraconazole	29	2	21	1	4		1
Ketoconazole	5	1	1				3
Micafungin	16	16					
Posaconazole	16	11					5
Voriconazole	24	24					

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 molds. 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* M2040 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 two used YeastOne Colorimetric method.

COMMENTS: Five out of thirty-one 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, anidulafungin, caspofungin, fluconazole, ketocoanzole, micafungin, posaconazole, and voriconazole. Itraconazole was not validated since no consensus MIC ranges were obtained based on different devices/methods used for performing antifungal susceptibility testing.

Mold Antifungal Susceptibility Test: *Aspergillus fumigatus* M2040.

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	0.12 – 2.0	0.5	0.19 – 2.0	0.12 – 1.0
Anidulafungin	0.004 – 0.06	0.015	0.008 – 8.0	0.015 – 0.06
Caspofungin	0.008 – 0.25	0.12	0.008 – 8.0	0.008 – 0.12
Fluconazole	≥ 64	64	≥ 64	≥ 64
Itraconazole	4.0 – 64	16	≥ 8.0	0.25 – 16
Ketoconazole	4.0 – 64	16	8 – 64	16 – 32
Micafungin	0.004 – 0.12	0.015	0.008 – 8.0	0.008 – 0.06
Posaconazole	0.06 – 1.0	0.5	0.25 – 2.0	0.06 – 1.0
Voriconazole	0.12 – 2.0	2.0	0.25 – 4.0	0.12 – 2.0

Reported MIC (µg/ml) Values for Mold Antifungal Susceptibility Test: *Aspergillus fumigatus* M2040

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	5					1		3	1						
Anidulafungin	3		2		1										
Caspofungin	4	1	1			2									
Fluconazole	4													2	2
Itraconazole	5						1				1	3			
Ketoconazole	2											1	1		
Micafungin	3	1		1	1										
Posaconazole	4				1			2	1						
Voriconazole	4					1		1	1	1					

Colors represent the testing method used:

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

Further Reading:

Alastruey-Izquierdo, A., and M. Cuenca-Estrella. 2012. EUCAST and CLSI: How to Assess in Vitro Susceptibility and Clinical Resistance. *Current Fungal Infection Reports*:1-6. DOI 10.1007/s12281-012-0100-3

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., Ph.D.
Commissioner