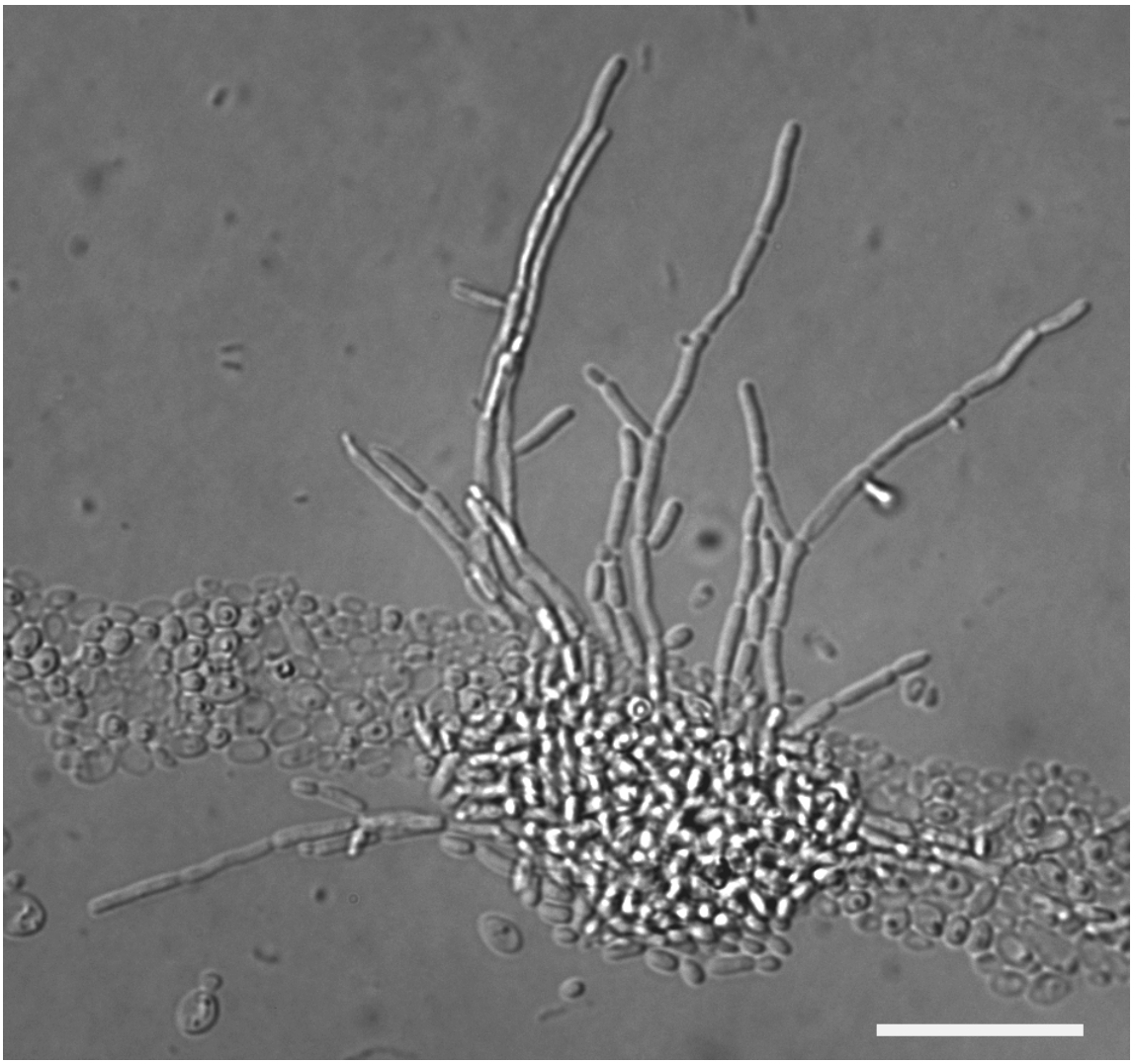
Mycology Proficiency Testing Program



Test Event Critique
May 2015

NEW YORK STATE | Department of Health | Wadsworth Center

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Mycology Laboratory

Mycology Laboratory at the Wadsworth Center, New York State Department of Health (NYSDOH) is a reference diagnostic laboratory for the fungal diseases. The laboratory services include testing for the 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 related environmental surveys. The Fungal Culture Collection of the Mycology Laboratory is an important resource for high quality cultures used in the proficiency-testing program and for the in-house development and standardization 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 Clinical Laboratory Improvement Amendments (CLIA)-compliant Proficiency Testing (Mycology) for clinical laboratories in New York State. All analytes for these test events are prepared and standardized internally. The program also provides continuing educational activities in the form of detailed critiques of test events, workshops and occasional one-on-one training of laboratory professionals.

Mycology Laboratory Staff and Contact Details

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Mycology Proficiency Testing Program (PTP)

CATEGORY DESCRIPTION

COMPREHENSIVE: This category is for the laboratories that examine specimens for the pathogenic molds and yeasts encountered in a clinical microbiology laboratory. These laboratories are expected to identify fungal pathogens to the genus and species level (for detail, please see mold and yeast master lists). 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 the laboratories that restrict their testing to one or more of the following:

> **Identification yeast only:** This category is for laboratories that isolate and identify pathogenic yeasts or yeast-like fungi to genus and species level (for detail, please see yeast master list). Laboratories holding this category may also perform susceptibility testing on yeasts. 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.

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 yeasts*
- Susceptibility testing of yeasts

Antigen Detection

- Antigen detection of *Cryptococcus neoformans**

TEST SPECIMENS & GRADING POLICY

Test Specimens

At least two strains of each mold or yeast species 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 the consensus/reference laboratories' MIC values within +/- 2 dilutions and the interpretation per CLSI (NCCLS) guidelines or related, peer-reviewed publications. One yeast species is to be tested against following drugs: amphotericin B, anidulafungin, caspofungin, flucytosine, 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

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. This list neither includes all yeasts that might be encountered in a clinical laboratory nor is intended to be used for the 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.

It is expected that major pathogenic yeasts listed in the Master List will be completely identified to genus and species levels while those yeasts not listed in the master list will be identified to genus only (i.e. *Candida inconspicua* as *Candida* species). However, the laboratory can elect to provide only genus level identification if it reflects the standard operating procedures (SOP) for patient testing. Please use "species complex" where appropriate, e.g. *Candida parapsilosis* species complex if it is consistent with current reporting format used by the laboratory.

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
Sporobolomyces species
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 tropicalis*	*Candida tropicalis*		108/110 (98%)
Y-2	*Candida dubliniensis*	*Candida dubliniensis*		102/108 (94%)
Y-3	*Candida albicans*	*Candida albicans*		109/110 (99%)
Y-4	Specimen negative for fungi	Specimen negative for fungi	No fungal growth	104/108 (96%)
Y-5	*Candida lusitaniae*	*Candida lusitaniae*		106/108(98%)

Antifungal Susceptibility Testing for Yeast (S-1: *Candida parapsilosis* M957)

Drugs	Acceptable MIC (μg/ml) Range	Acceptable interpretation*	Laboratories with acceptable responses/ Total laboratories (% correct responses)
Amphotericin B	0.25 – 1	Susceptible / No interpretation	21/21 (100%)
Anidulafungin	1 – 2	Susceptible	18/18 (100%)
Caspofungin	0.25 – 2	Susceptible	25/25 (100%)
Flucytosine (5-FC)	0.03 – 0.25	Susceptible / No interpretation	22/22 (100%)
Fluconazole	0.25 - 4	Susceptible / Susceptible-dose dependent	32/32 (100%)
Itraconazole	0.015 – 0.125	Susceptible / No interpretation	25/25 (100%)
Ketoconazole	0.015-0.06	Susceptible / No interpretation	3/3 (100%)
Micafungin	1 – 2	Susceptible	18/18 (100%)
Posaconazole	0.015 – 0.06	Susceptible / No interpretation	17/17 (100%)
Voriconazole	0.008 – 0.125	Susceptible	29/29 (100%)

*Please use interpretations for *Candida* spp. provided in CLSI M27-S4 document. If there is no antifungal agent listed in CLSI M27-S4 document, CLSI M27-S3 document can be used as an alternate guideline.

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	41
BD Phoenix	2
Chrome agar	1
Dade Behring MicroScan Rapid Yeast Identification Panel	4
MALDI-TOF	15
Remel RapID Yeast Plus System	4
Sequencing	3
Vitek2	54
Antifungal Susceptibility*	
Disk diffusion	1
Etest	1
Vitek II	4
YeastOne – Mold	1
YeastOne – Yeast	24
CLSI Microbroth dilution method – Yeast	2
CLSI Microbroth dilution method – Mold	3

*Include multiple systems used by some laboratories

YEAST DESCRIPTIONS

Y-1 *Candida tropicalis*

Source: Urine / Blood / Sputum / Stool

Clinical significance: *Candida tropicalis* causes sepsis, wound infections, and disseminated infections in immunocompromised patients.

Colony: *C. tropicalis* colony is smooth to wrinkled, cream-colored and rapid-growing on Sabouraud's dextrose agar after 7 days of incubation at 25°C, (Figure 1).

Microscopy: *C. tropicalis* shows long true hyphae and pseudohyphae, with either single or small clusters of blastoconidia on cornmeal agar with Tween 80 (Figure 1).

Differentiation: *C. tropicalis* is differentiated from *C. albicans* and *C. dubliniensis* by variable growth on media containing cycloheximide, and by its fermentation of glucose, maltose, sucrose, and trehalose. Occasionally, *C. tropicalis* produces chlamydospores on cornmeal agar.

Molecular test: Reverse-hybridization line probe assay combined with PCR amplification of internal transcribed-spacer (ITS) regions are used for rapid identification of clinically significant fungal pathogens including *C. tropicalis*. The combination of pan-fungal PCR and multiplex liquid hybridization of ITS regions are developed for detection and identification of fungi in tissue specimens.

The ribosomal *ITS1* and *ITS2* regions of the test isolate showed 100 % nucleotide identity with *C. tropicalis* CBL Cd-3 (GenBank accession no. EU924133)

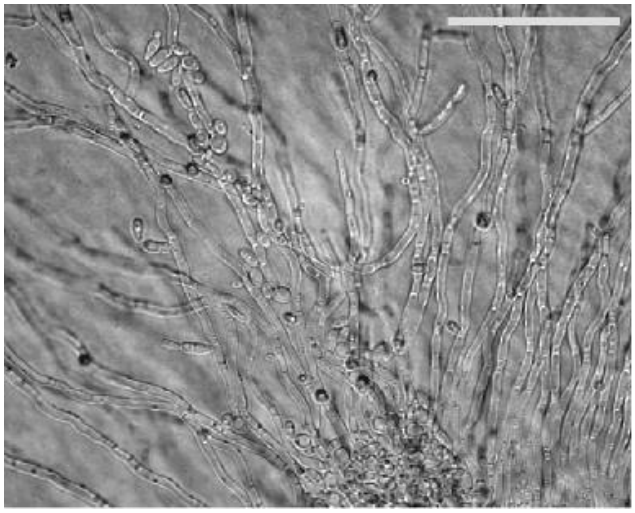
Antifungal susceptibility: *C. tropicalis* is generally susceptible to azoles and echinocandins, but variably susceptible to flucytosine. Few strains of *C. tropicalis* have been reported with high amphotericin B MIC.

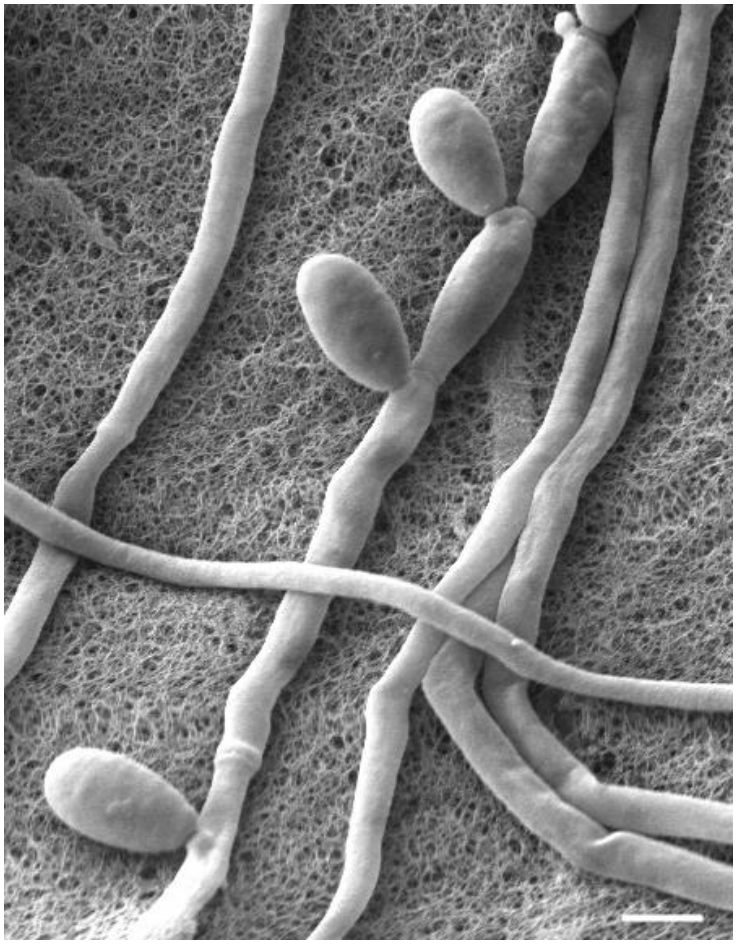
Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	108
Laboratories with incorrect ID:	2
(*Candida guilliermondii*)	(1)
(*Candida parasilosis* species complex)	(1)

Illustrations:

Figure 1. *Candida tropicalis,* smooth-to-wrinkled, creamish colony, Sabouraud's dextrose agar 7-days, 25°C**.** Microscopic morphology on cornmeal agar with Tween 80, showing long true hyphae and pseudohyphae with clusters of blastoconidia (bar = 50 μm). Scanning electron micrograph illustrates true and pseudohyphae (with constrictions) and blastoconidia (bar = 2 μm).





Further reading:

Chai LY, Denning DW, Warn P. 2010. *Candida tropicalis* in human disease. *Crit Rev Microbiol*. 36: 282-98.

de Carvalho Parahym AM, da Silva CM, Leão MP, Macario MC, Filho GA, de Oliveira NT, Neves RP. 2011. Invasive infection in an acute myeloblastic leukemia patient due to triazole-resistant *Candida tropicalis*. *Diagn Microbiol Infect Dis*. 71: 291-293.

Fesharaki SH, Haghani I, Mousavi B, Kargar ML, Boroumand M, Anvari MS, Abbasi K, Meis JF, Badali H. 2013. Endocarditis due to a co-infection of *Candida albicans* and *Candida tropicalis* in a drug abuser. *J Med Microbiol*. 62(Pt 11): 1763-1767.

Hilmioglu S, Ilkit M, Badak Z. 2007. Comparison of 12 liquid media for germ tube production of *Candida albicans* and *C. tropicalis. Mycoses.* 50: 282-285.

Lidder S, Tasleem A, Masterson S, Carrington RW. 2013. *Candida tropicalis*: diagnostic dilemmas for an unusual prosthetic hip infection. *J R Army Med Corps.* 159: 123-125.

Magri MM, Gomes-Gouvêa MS, de Freitas VL, Motta AL, Moretti ML, Shikanai-Yasuda MA. 2012. Multilocus sequence typing of *Candida tropicalis* shows the presence of different clonal clusters and fluconazole susceptibility profiles in sequential isolates from candidemia patients in Sao Paulo, Brazil. *J Clin Microbiol*. 51: 268-277.

Muñoz P, Giannella M, Fanciulli C, Guinea J, Valerio M, Rojas L, Rodríguez-Créixems M, Bouza E. 2011. *Candida tropicalis* fungemia: incidence, risk factors, and mortality in a general hospital. *Clin Microbiol Infect*. 17: 1538-1545.

Negri M, Silva S, Henriques M, Oliveira R. 2011. Insights into *Candida tropicalis* nosocomial infections and virulence factors. *Eur J Clin Microbiol Infect Dis*. DOI. 10.1007/s10096-011-1455-z.
Nucci M, Colombo AL. 2007. Candidemia due to *Candida tropicalis*: clinical, epidemiologic, and microbiologic characteristics of 188 episodes occurring in tertiary care hospitals. *Diagn Microbiol Infect Dis.* 58: 77-82.

Pfaller MA, Castanheira M, Messer SA, Moet GJ, Jones RN. 2010. Variation in *Candida* spp. distribution and antifungal resistance rates among bloodstream infection isolates by patient age: report from the SENTRY Antimicrobial Surveillance Program (2008-2009). *Diagn Microbiol Infect Dis*. 68: 278-283.

Y-2 *Candida dubliniensis*

Source: Wound / Urine / Oral

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 is white to cream, smooth, and soft on Sabouraud's dextrose agar after 7 days of incubation at 25°C (Figure 2). *C. dubliniensis* does not grow at 45°C.

Microscopy: *C. dubliniensis* shows abundant, branched pseudohyphae and true hyphae with blastoconidia. Chlamydospores are single, or in pairs, or in chains, or clusters on cornmeal agar with Tween 80 (Figure 2).

Differentiation: *C. dubliniensis* is practically indistinguishable from *C. albicans* on the basis of many common phenotypic tests. One physiologic feature that does appear to be fairly stable is that *C. dubliniensis* grows poorly at 42°C or does 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:	10
Laboratories with correct ID:	102
Laboratories with incorrect ID:	4
(*Candida albicans*)	(2)
(*Candida zeylanoides*)	(1)
(*Rhodotorula* sp.)	(1)

Illustrations:

Figure 2. *Candida dubliniensis*, white, glossy, and smooth colony on Sabouraud's dextrose agar, 4 days, 25°C. Microscopic morphology on cornmeal agar with Tween 80, showing abundant branched pseudohyphae and true hyphae with blastoconidia (bar = 10 μm).

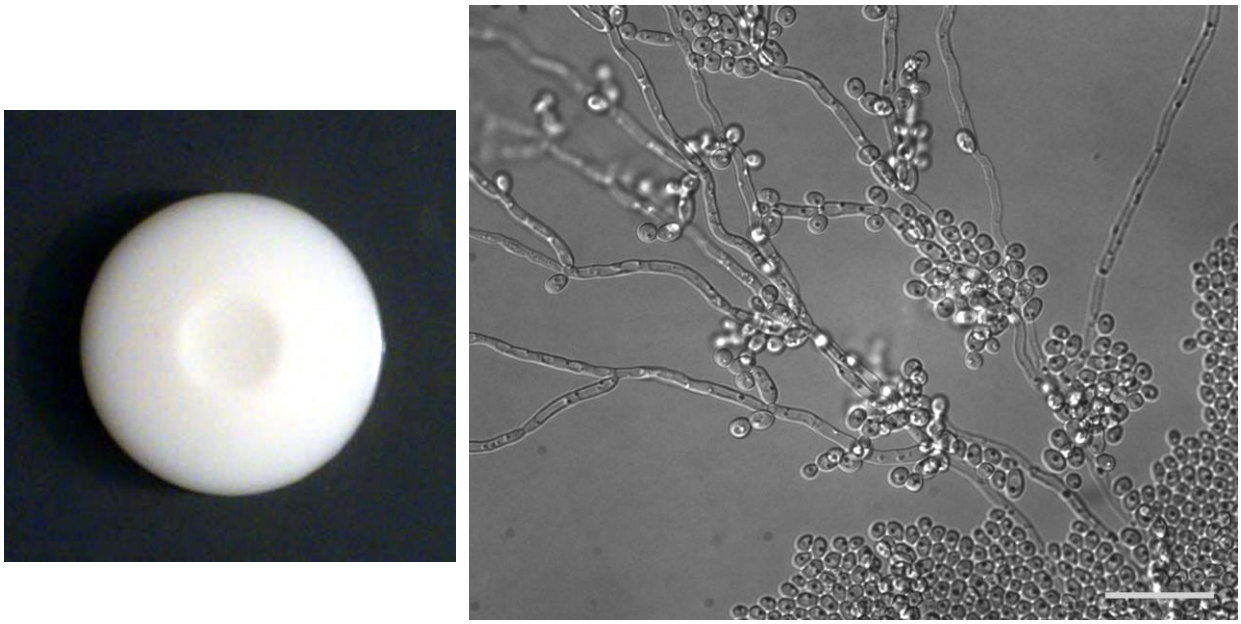
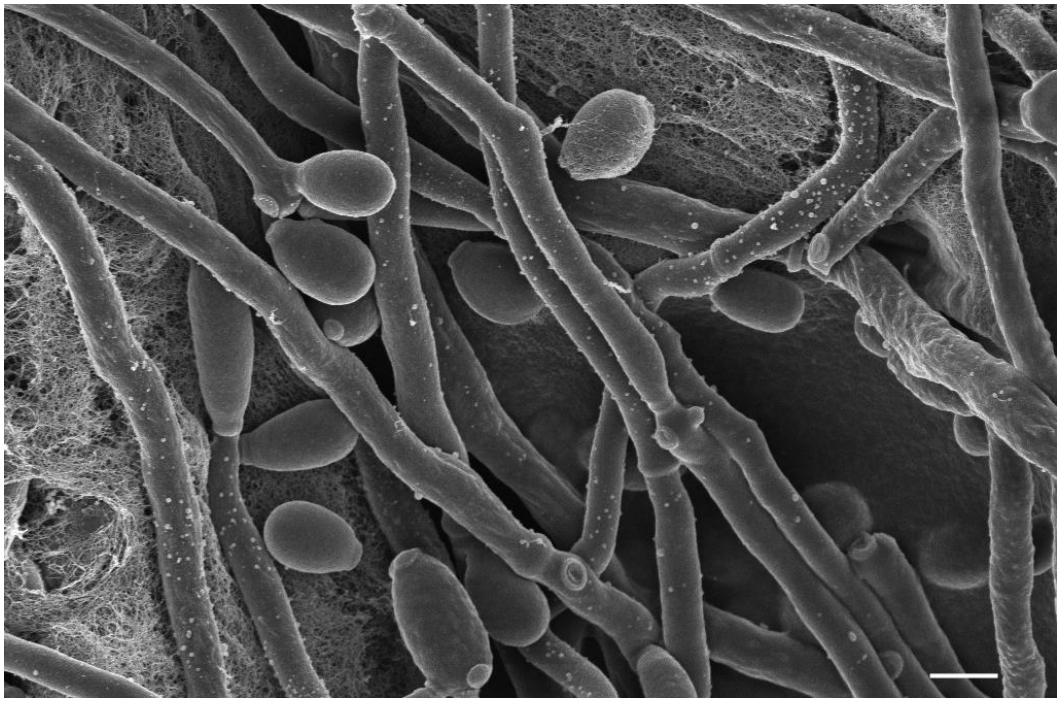


Figure 2A. Scanning electron micrograph of *Candida dubliniensis* illustrates pseudohyphae and blastoconidia (bar = 2 μm)



Further reading:

Bosco-Borgeat ME, Taverna CG, Cordoba S, Isla MG, Murisengo OA, Szusz W, Vivot W, Davel G. 2011. Prevalence of *Candida dubliniensis* fungemia in Argentina: identification by a novel multiplex PCR and comparison of different phenotypic methods. Mycopathologia. 172(5):407-414.

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.

Romeo O, Criseo G. 2009. Molecular epidemiology of *Candida albicans* and its closely related yeasts *Candida dubliniensis* and *Candida africana*. *J Clin Microbiol*. 47: 212-214.

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.

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-3 *Candida albicans*

Source: Eye / Vaginal Swab / Urine

Clinical significance: *Candida albicans* is the most common cause of candidiasis. It is ubiquitous in humans who probably encounter it initially during passage through the birth canal. The serious infections are generally seen in immunocompromised patients.

Colony: *C. albicans* colony is white to creamy, glossy, smooth and soft on Sabouraud's dextrose agar at 25°C for 3 to 5 days (Figure 3).

Microscopy: *C. albicans* yeasts are round blastoconidia bunched together with pseudohyphae on cornmeal agar with Tween 80. Thick walled, mostly terminal chlamydospores are prominent (Figure 3).

Differentiation: By morphological criterion, *C. albicans* is difficult to distinguish from *C. dubliniensis*. However, *C. albicans* grows well at 42°C and 45°C, but *C. dubliniensis* grows poorly or not at all at 42°C or 45°C. *C. dubliniensis* generally produces more abundant chlamydospores than *C. albicans*. If the CHEOMagar is used for diagnosis, bluish green color distinguishes *C. albicans* from dark-green color of *C. dubliniensis*. The positive germ tube test for *C. albicans* distinguishes it from *C. tropicalis*.

Molecular test: Molecular tests are available for identification of *C. albicans*. A large number of DNA typing and nucleotide sequencing methods are available for molecular epidemiology of *C. albicans* strains.

The ribosomal *ITS1* and *ITS2* regions of the test isolate showed 100 % nucleotide identity with *Candida albicans* strain CS-KW8723 (GenBank accession no. KC176533.1).

Antifungal susceptibility: *C. albicans* is sensitive to amphotericin B, anidulafungin, caspofungin, micafungin, fluconazole, and posaconazole. Fluconazole-resistant isolates of *C. albicans* are also reported.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	109
Laboratories with incorrect ID:	1
(*Sacchromycetes cerevisiae*)	(1)

Illustrations:

Figure 3. *Candida albicans*, glossy and smooth colony on Sabouraud's dextrose agar, 25°C. *Candida albicans* on corn meal agar with Tween 80 showing pseudohyphae with blastoconidia (bar = 25 μm).

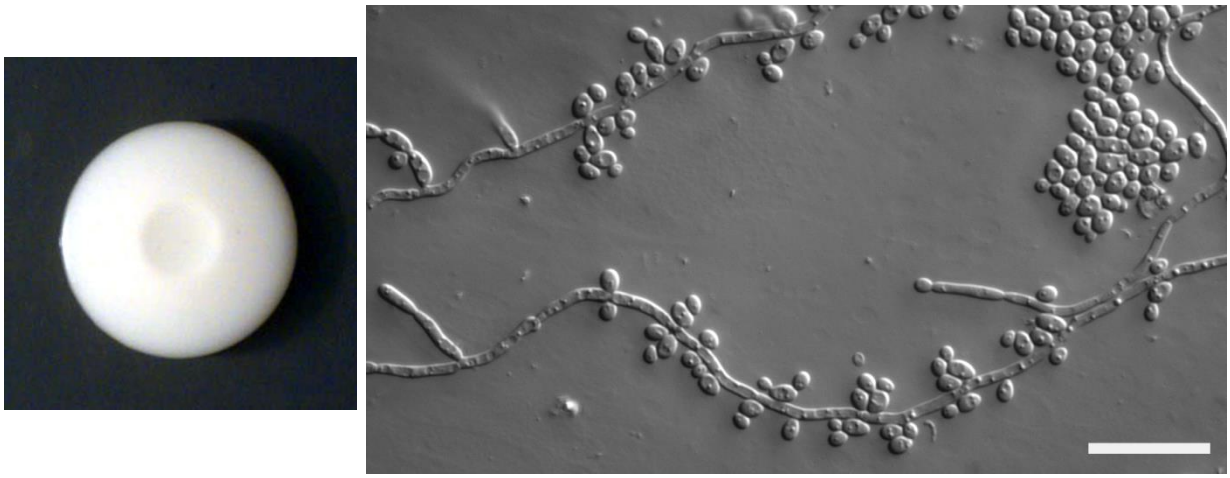
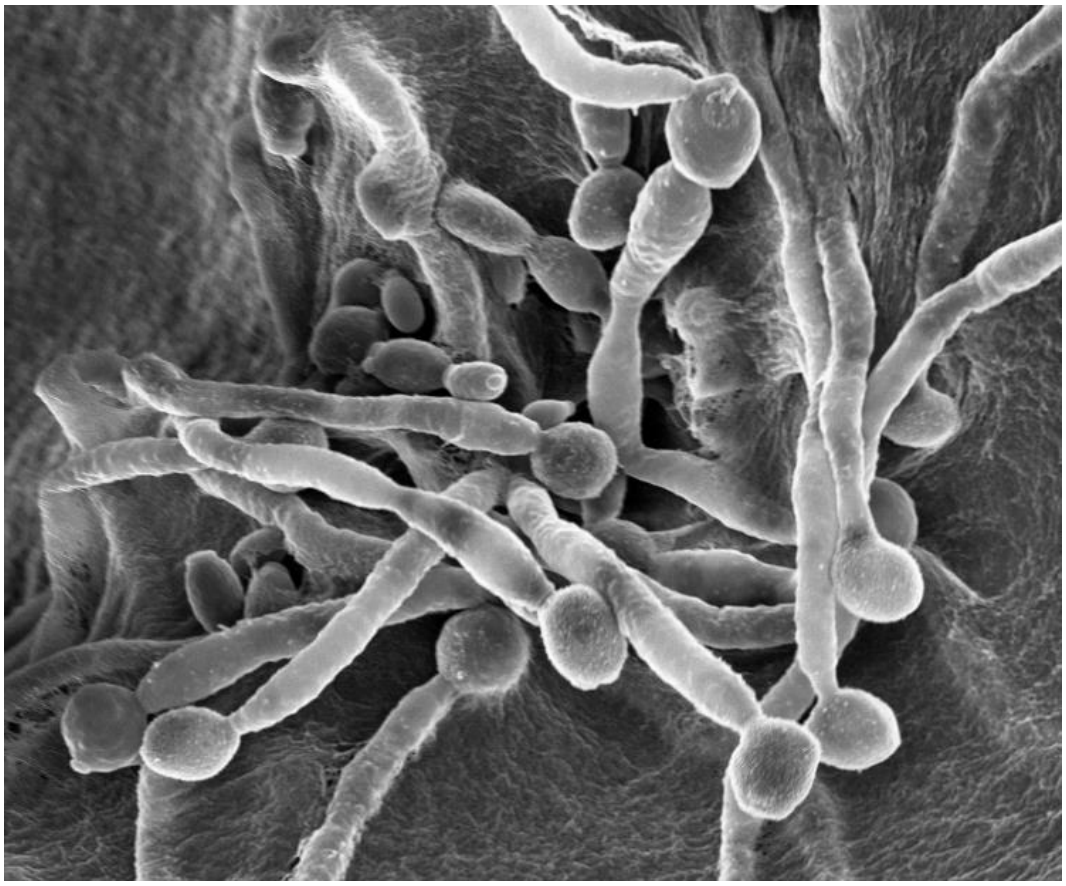


Figure 3A. Scanning electron micrograph illustrating pseudohyphae with blastoconidia of *Candida albicans*.



Further reading:

Bartie KL, Williams DW, Wilson MJ, Potts AJ, Lewis MA. 2001. PCR fingerprinting of *Candida albicans* associated with chronic hyperplastic candidosis and other oral conditions. *J Clin Microbiol.* 39: 4066-4075.

Chi HW, Yang YS, Shang ST, Chen KH, Yeh KM, Chang FY, Lin JC. 2011. *Candida albicans* versus non-albicans bloodstream infections: The comparison of risk factors and outcome. *J Microbiol Immunol Infect*. 44: 369-375.

Donelli G. 2006. Vascular catheter-related infection and sepsis. *Surg Infect (Larchmt).* 7 Suppl 2:S25-7.

Eraso E, Moragues MD, Villar-Vidal M, Sahand IH, Gonzalez-Gomez N, Ponton J, Quindos G. 2006. Evaluation of the new chromogenic medium *Candida* ID 2 for isolation and identification of *Candida albicans* and other medically important *Candida* species. *J Clin Microbiol.* 44: 3340-3345.

Kim D, Shin W-S, Lee K-H, Kim K, Park JY. 2002. Rapid differentiation of *Candida albicans* from other *Candida* species using its unique germ tube formation at 39°C. *Yeast* 19: 957-962.

Krcmery V, Huttova M, Mateicka F, Laho L, Jurga L, Ondrusova A, Tarekova Z, Kralinsky K, Hanzen J, Liskova A, Mrazova M, Sabo A, Pisarcikova M, Kovacicova G, Chovancova D, Szovenyiova Z. 2001. Breakthrough fungaemia in neonates and infants caused by *Candida albicans* and *Candida parapsilosis* susceptible to fluconazole *in vitro*. *J Antimicrob Chemother*. 8: 521-525.

Liguori G, Di Onofrio V, Gallé F, Lucariello A, Albano L, Catania MR, Guida M. 2010. *Candida albicans* identification: comparison among nine phenotypic systems and a multiplex PCR. *J Prev Med Hyg*. 51: 121-124.

Manfredi R, Sabbatani S. 2006. Severe *Candida albicans* panophthalmitis treated with all available and potentially effective antifungal drugs: Fluconazole, liposomal amphotericin B, caspofungin, and voriconazole. *Scand J Infect Dis*. 38: 950-951.

Mean M, Marchetti O, Calandra T. 2008. Bench-to-bedside review: *Candida* infections in the intensive care unit. *Crit Care.* 12: 204.

Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. 2006. A one-enzyme PCR-RFLP assay for identification of six medically important *Candida* species. *Nippon Ishinkin Gakkai Zasshi.* 47: 225-229.

Moudgal V, Sobel J. 2010. Antifungals to treat *Candida albicans*. *Expert Opin Pharmacother.* 2010 11: 2037-2048.

Odds FC. 2010. Molecular phylogenetics and epidemiology of *Candida albicans*. *Future Microbiol*. 5: 67-79.

Patel M, Shackleton JT, Coogan MM. 2006. Effect of antifungal treatment on the prevalence of yeasts in HIV-infected subjects. *J Med Microbiol.* 55: 1279-1284.

Rautemaa R, Richardson M, Pfaller MA, Perheentupa J, Saxén H. 2008. Activity of amphotericin B, anidulafungin, caspofungin, micafungin, posaconazole, and voriconazole against *Candida albicans* with decreased susceptibility to fluconazole from APECED patients on long-term azole treatment of chronic mucocutaneous candidiasis. *Diagn Microbiol Infect Dis.* 62:182-185.

Spiess B, Seifarth W, Hummel M, Frank O, Fabarius A, Zheng C, Mörz H, Hehlmann R, Buchheidt D. 2007. DNA microarray-based detection and identification of fungal pathogens in clinical samples from neutropenic patients. *J Clin Microbiol.* 45: 3743-3753.

Y-4 Specimen negative for fungal

Source: Lung / Vaginal / Throat

Only *Actinomyces neuii* was included in this specimen. So no fungus (neither yeast nor mold) should be recovered. Identification of *Actinomyces* species is not required.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	105
Laboratories with incorrect ID:	3
(*Candida albicans*)	(1)
(*Candida guilliermondii*)	(1)
(*Malassezia furfur*)	(1)

Y-5 *Candidda lusitaniae*

Source: Body fluid / Bronchial lavage / Skin

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 common clinical samples are blood, urine, and respiratory tract secretions.

Colony: *C. lusitaniae* colony is white to creamish, shiny, and slightly raised in the center on Sabouraud's dextrose agar, after 7 days of incubation at 25°C (Figure 4).

Microscopy: *C. lusitaniae* produced many short, branched ("bushy") pseudohyphae. Along the length of the pseudohyphae, elongated blastoconidia formed in short chains on cornmeal agar with Tween 80 (Figure 4).

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:	106
Laboratories with incorrect ID:	1
(*Trichosporon* sp.)	(1)

Illustrations:

Figure 4. *Candida lusitaniae*, white, smooth colony of on Sabouraud's dextrose agar, 4 days, 25°C. Microscopic morphology on corn meal agar showing bushy pesudohyphae and blastoconidia (bar = 10 μm). Scanning electron micrograph illustrates pseudohyphae and blastoconidia (bar = 2 μm).

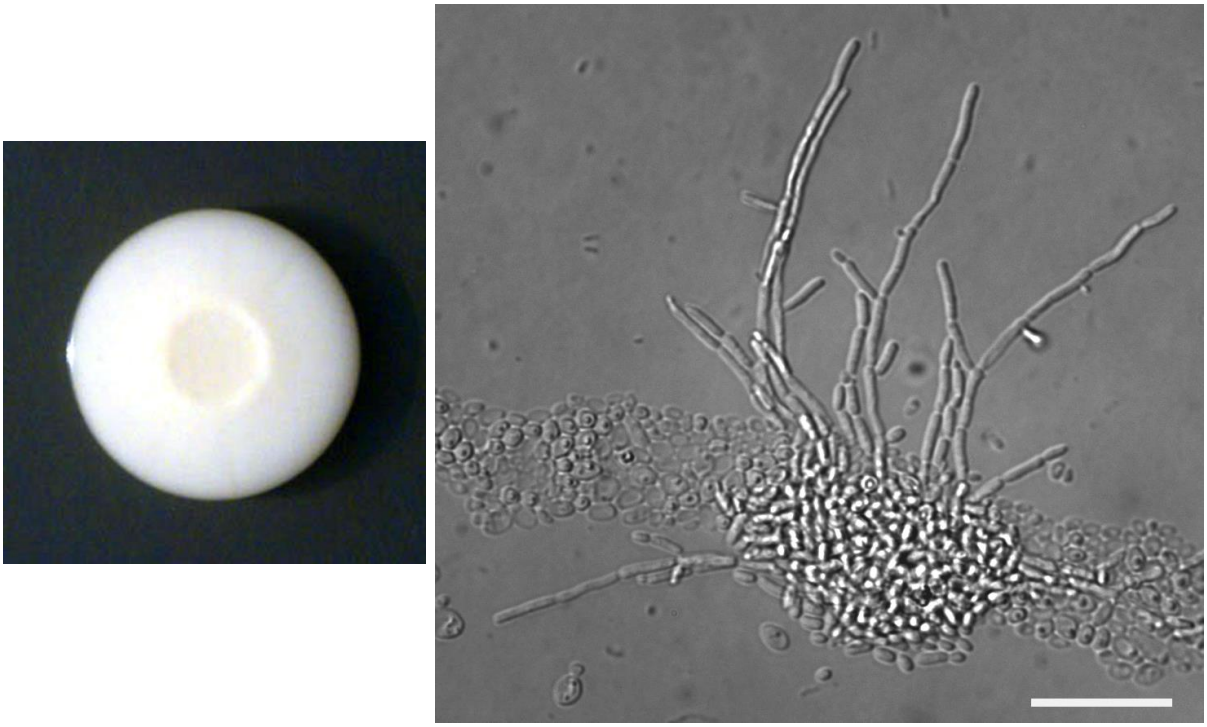
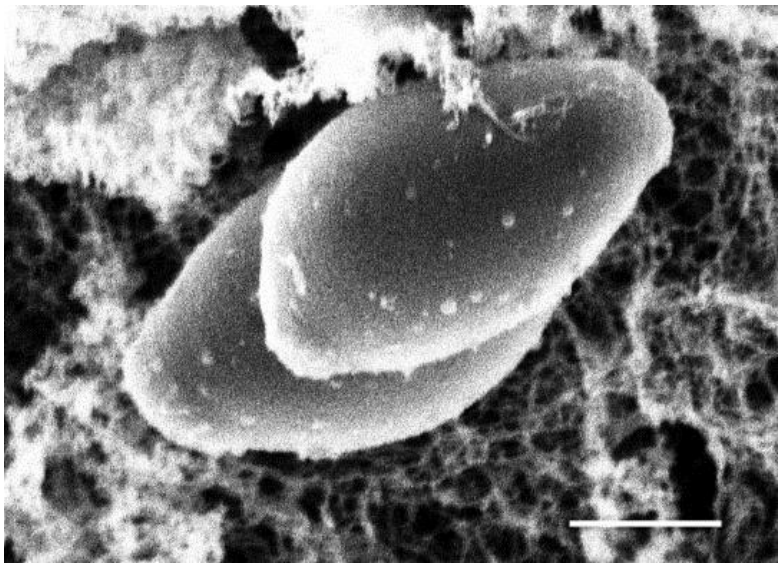


Figure 4A. Scanning electron micrograph illustrating pseudohyphae with blastoconidia of *Candida lusitaniae*.



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.* 11: e1-6.

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, M27-S4, 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 parapsilosis* (S-1) was the analyte in the May 27, 2015 antifungal proficiency testing 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.

Comments: Acceptable results were MICs +/-2 dilutions of the reference laboratory results for any single drug. Only 2 of the 32 laboratories participating in this test event tested all 10 antifungal drugs. The reported results were as follows: voriconazole (29 laboratories), caspofungin and itraconazole (25 laboratories respectively), flucytosine (22 laboratories), amphotericin B (21 laboratories), anidulafungin and micafungin (18 laboratories respectively), posacoanazole (17 laboratories), and ketoconazole (3 laboratories). Fluconazole was the only drug tested by all 32 laboratories.

Table 3. Antifungal MICs (µg/ml) Reported by the Participating Laboratories

S-1: *Candida parapsilosis* (M957)

Drug	No. labs	MIC (µg/ml)									
		0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4
Amphotericin B	21						1	19	1		
Anidulafungin	18								2	16	
Caspofungin	25						3	19	2	1	
Flucytosine (5-FC)	22			1	10	10	1				
Fluconazole	32*						1	7	21	1	1
Itraconazole	25*		1	3	11	9					
Ketoconazole	3*		1		1						
Micafungin	18								2	16	
Posaconazole	17		2	7	8						
Voriconazole	29	1	19	2	2	4					

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

Colors represent the testing method used:

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

Table 4. Antifungal Susceptibility Interpretations Reported by the Participating Laboratories

S-1: *Candida parapsilosis* (M957)

Drug	No. laboratories	Susceptible	Susceptible-dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	21	4					17
Anidulafungin	18	18					
Caspofungin	25	24					1
Flucytosine	22	15					7
Fluconazole	32	31	1				
Itraconazole	25	18					7
Ketoconazole	3	1					2
Micafungin	18	18					
Posaconazole	17	7					10
Voriconazole	29	29					

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR MOLDS (EDUCATIONAL)

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 molds. 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* M2040 was used as a test analyte; it was obtained from a reference laboratory. Participating laboratories volunteered to perform the test and they were free to choose any number of drugs and a test method. Three laboratories used CLSI broth microdilution method while the remaining one laboratories used TREK YeastOne Colorimetric method.

Comments: Four out of thirty-two laboratories, which hold antifungal susceptibility testing for yeasts permit, voluntarily participated in this test event for molds. Please refer to Table 5 for summary of performances. Since too few laboratories have participated in this test, no consensus data could be generated.

Table 5. MIC (µg/ml) Values of Mold Antifungal Susceptibility: *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	4.0	8.0	16	32	64	256
Amphotericin B	4							2	1	1						
Anidulafungin	4		2	1	1											
Caspofungin	4		2		1	1										
Fluconazole	3														2	1
Itraconazole	5								1				3			
Ketoconazole	1												1			
Micafungin	4	1			2						1					
Posaconazole	4							3	1							
Voriconazole	4							1	2	1						

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).

Clinical and Laboratory Standards Institute. 2012. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Fourth Informational Supplement. CLSI document M27-S4 (ISBN 1-56238-863-0).

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.