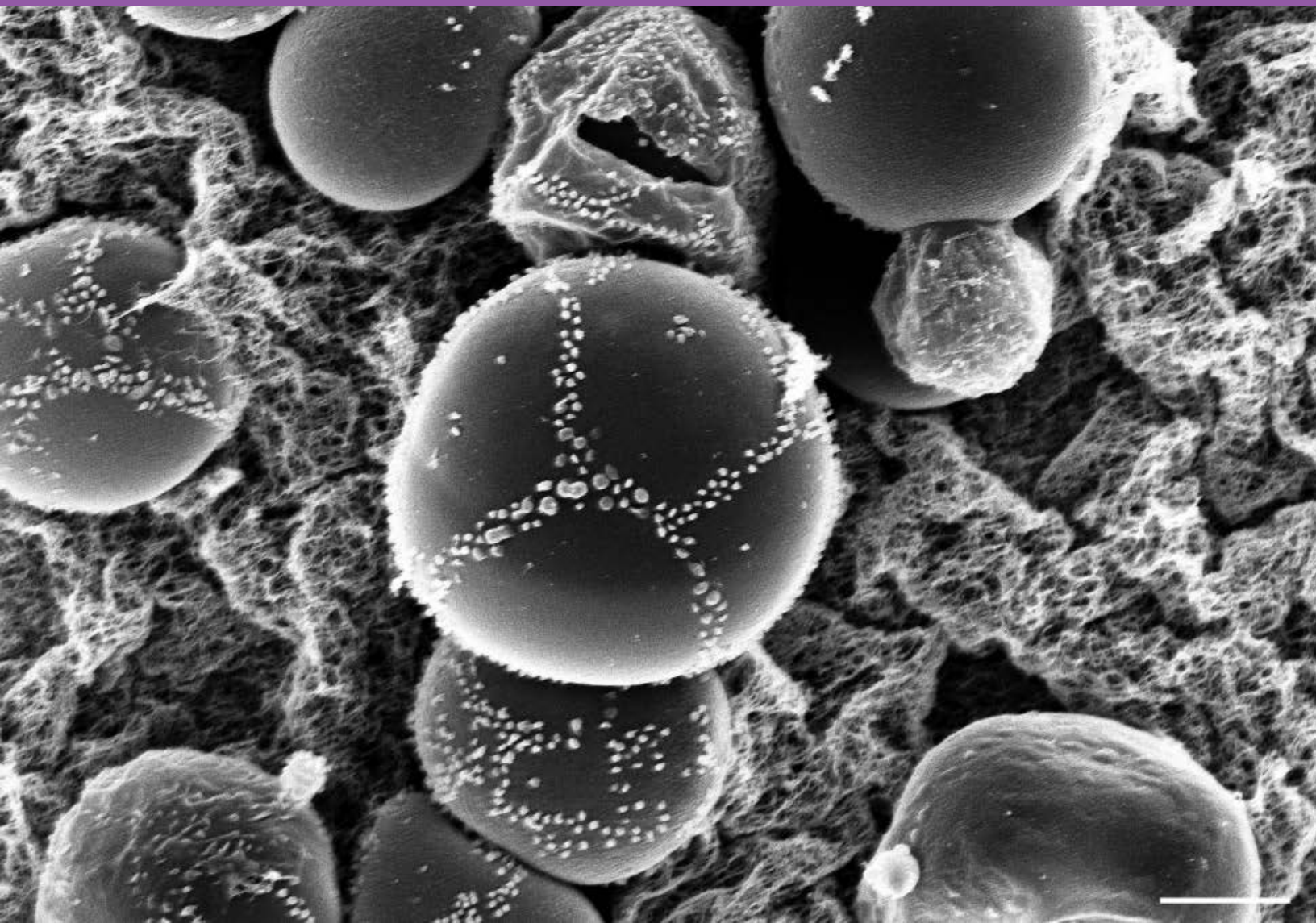


Mycology Proficiency Testing Program



Test Event Critique
May 2014

Wadsworth Center
NEW YORK STATE DEPARTMENT OF HEALTH

Table of Contents

Mycology Laboratory	2
Mycology Proficiency Testing Program	3
Test Specimens & Grading Policy	5
Test Analyte Master Lists	7
Performance Summary	9
Commercial Device Usage Statistics	11
Yeast Descriptions	12
Y-1 <i>Candida parapsilosis</i>	12
Y-2 <i>Candida glabrata</i>	15
Y-3 <i>Rhodotorula minuta</i>	18
Y-4 <i>Trichosporon asahii</i>	21
Y-5 <i>Prototheca wickerhamii</i>	24
Antifungal Susceptibility Testing - Yeast	27
Antifungal Susceptibility Testing - Mold (Educational)	29

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

Name	Responsibility	Phone	Email
Dr. Vishnu Chaturvedi	Director (on leave of absence)	518-474-4177	vishnu.chaturvedi@health.ny.gov
Dr. Sudha Chaturvedi	Deputy Director	518-474-4177	sudha.chaturvedi@health.ny.gov
Dr. Ping Ren	PT Program Coordinator	518-474-4177	mycologypt@wadsworth.org or ping.ren@health.ny.gov
Ms. Xiaojiang Li	Research Scientist (Diagnostic Section)	518-486-3820	mycologydiagnostics@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 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.

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

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.

<i>Blastoschizomyces capitatus</i> (<i>Geotrichum capitatum</i>)	<i>Cryptococcus terreus</i>
<i>Blastoschizomyces</i> species	<i>Cryptococcus uniguttulatus</i>
<i>Candida albicans</i>	<i>Geotrichum candidum</i>
<i>Candida dubliniensis</i>	<i>Geotrichum</i> species
<i>Candida famata</i>	<i>Hansenula anomala</i> (<i>Candida pelliculosa</i>)
<i>Candida glabrata</i>	<i>Malassezia furfur</i>
<i>Candida guilliermondii</i> species complex	<i>Malassezia pachydermatis</i>
<i>Candida kefyr</i>	<i>Malassezia</i> species
<i>Candida krusei</i>	<i>Pichia ohmeri</i> (<i>Kodamaea ohmeri</i>)
<i>Candida lipolytica</i> (<i>Yarrowia lipolytica</i>)	<i>Prototheca</i> species
<i>Candida lusitaniae</i>	<i>Prototheca wickerhamii</i>
<i>Candida norvegensis</i>	<i>Prototheca zopfii</i>
<i>Candida parapsilosis</i> species complex	<i>Rhodotorula glutinis</i>
<i>Candida rugosa</i>	<i>Rhodotorula minuta</i>
<i>Candida</i> species	<i>Rhodotorula mucilaginosa</i> (<i>rubra</i>)
<i>Candida tropicalis</i>	<i>Rhodotorula</i> species
<i>Candida viswanathii</i>	<i>Saccharomyces cerevisiae</i>
<i>Candida zeylanoides</i>	<i>Saccharomyces</i> species
<i>Cryptococcus albidus</i>	<i>Sporobolomyces salmonicolor</i>
<i>Cryptococcus gattii</i>	<i>Sporobolomyces</i> species
<i>Cryptococcus laurentii</i>	<i>Trichosporon asahii</i>
<i>Cryptococcus neoformans</i>	<i>Trichosporon inkin</i>
<i>Cryptococcus neoformans-</i>	<i>Trichosporon mucoides</i>
<i>Cryptococcus gattii</i> species complex	<i>Trichosporon</i> species
<i>Cryptococcus</i> 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	<i>Candida parapsilosis</i> species complex	<i>Candida parapsilosis</i> species complex	<i>Candida parapsilosis</i>	114/114 (100%)
Y-2	<i>Candida glabrata</i>	<i>Candida glabrata</i>		113/114 (99%)
Y-3	<i>Rhodotorula minuta</i>	<i>Rhodotorula minuta</i>	<i>Rhodotorula sloofiae</i>	102/102 (100%)
Y-4	<i>Trichosporon asahii</i>	<i>Trichosporon asahii</i>		105/107 (98%)
Y-5	<i>Prototheca wickerhamii</i>	<i>Prototheca wickerhamii</i>		106/106 (100%)

Antifungal Susceptibility Testing for Yeast (S-1: *Candida albicans* M955)

Drugs	Acceptable MIC (µg/ml) Range	Acceptable interpretation	Laboratories with acceptable responses/ Total laboratories (% correct responses)
Amphotericin B	0.5 – 1	Susceptible / No interpretation	21/21 (100%)
Anidulafungin	0.125 – 0.25	Susceptible	16/16 (100%)
Caspofungin*	0.5 – 4	Susceptible / Intermediate / Resistant	22/22 (100%)
Flucytosine (5-FC)	0.06 – 1	Susceptible / No interpretation	23/23 (100%)
Fluconazole	16 - > 64	Resistant	32/32 (100%)
Itraconazole	0.125 – 0.5	Susceptible / Susceptible-dose dependent / No interpretation	25/27 (93%)
Ketoconazole	1	Susceptible / No interpretation	4/5 (80%)
Micafungin	0.06 – 0.25	Susceptible	16/16 (100%)
Posaconazole	0.06 – 0.5	Susceptible / No interpretation	15/15 (100%)
Voriconazole*	1 – 4	Susceptible / Susceptible-dose dependent / Resistant	25/25 (100%)

*Interpretations were accepted based on either CLSI M27-S3 document or CLSI M27-S4 document for this test event. In future events, 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*	
AMS Vitek	2
API 20C AUX	54
Dade Behring MicroScan Rapid Yeast Identification Panel	4
MALDI-TOF	7
Remel RapID Yeast Plus System	4
Sequencing	4
Vitek2	60
Antifungal Susceptibility*	
Disk diffusion	1
Etest	1
Vitek II	1
YeastOne – Mold	2
YeastOne – Yeast	25
CLSI Microbroth dilution method – Yeast	5
CLSI Microbroth dilution method – Mold	3

*Include multiple systems used by some laboratories

YEAST DESCRIPTIONS

Y-1 *Candida parapsilosis*

Source: Urine / Blood / Eye

Clinical significance: *Candida parapsilosis* is an important bloodstream pathogen. It is commonly implicated in endocarditis, endophthalmitis, fungemia, and infection in burn patients. It is also an important nosocomial pathogen in various hospital outbreaks such as neonatal fungemia and endophthalmitis after cataract surgery. *Candida parapsilosis* is also increasingly prevalent as causative agent of onychomycosis.

Colony: *Candida parapsilosis* colony is white to cream, dull with smooth surface on Sabouraud's dextrose agar after 5 days at 25°C (Figure 1).

Microscopy: *Candida parapsilosis* shows long, multibranched pseudohyphae, together with small elongated blastoconidia on corn meal agar with Tween 80 (Figure 1).

Differentiation: *C. parapsilosis* ferments glucose, but not maltose, sucrose, lactose, or trehalose. It does not grow on media containing cycloheximide, but it grows at 37°C. It assimilates glucose, maltose, and sucrose, but it is urease- and nitrate-negative. Biochemically, *C. lusitanae* is similar to *C. parapsilosis*, but it does not form long pseudohyphae.

Molecular test: PCR assay of ITS regions of rDNA was used to identify *C. parapsilosis* in clinical specimens. Chromosome length polymorphism and RAPD procedures were used to characterize the genetic diversity of this organism.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with a reference strain of *Candida parapsilosis* CBS 604 (Genbank accession no: AY391843).

Antifungal susceptibility: *C. parapsilosis* is susceptible to amphotericin B, 5-flucytosine, caspofungin, and azoles such as fluconazole, ketocoazole, itraconazole, and voriconazole. A few clinical isolates are reported as resistant to fluconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	114
Laboratories with incorrect ID:	0

Illustrations:

Figure 1. *Candida parapsilosis* white to cream, smooth colony on Sabouraud's dextrose agar, 25°C. Microscopic morphology of *Candida parapsilosis* with long, multibranched pseudohyphae together with small cluster of elongated blastoconidia on Corn meal agar with Tween 80 (bar = 5 μ m).

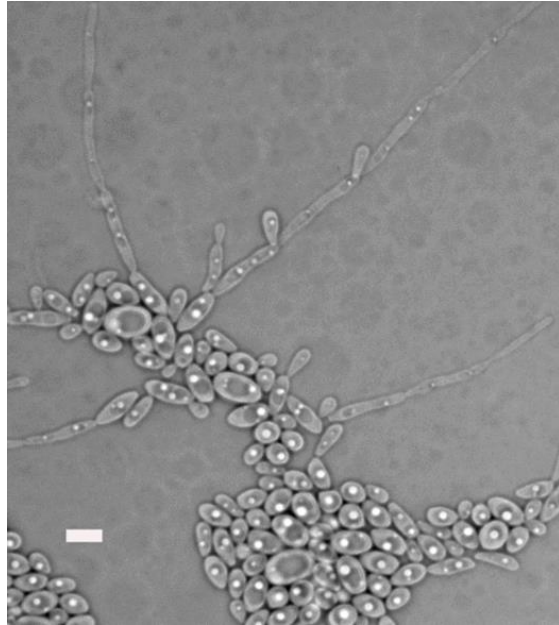
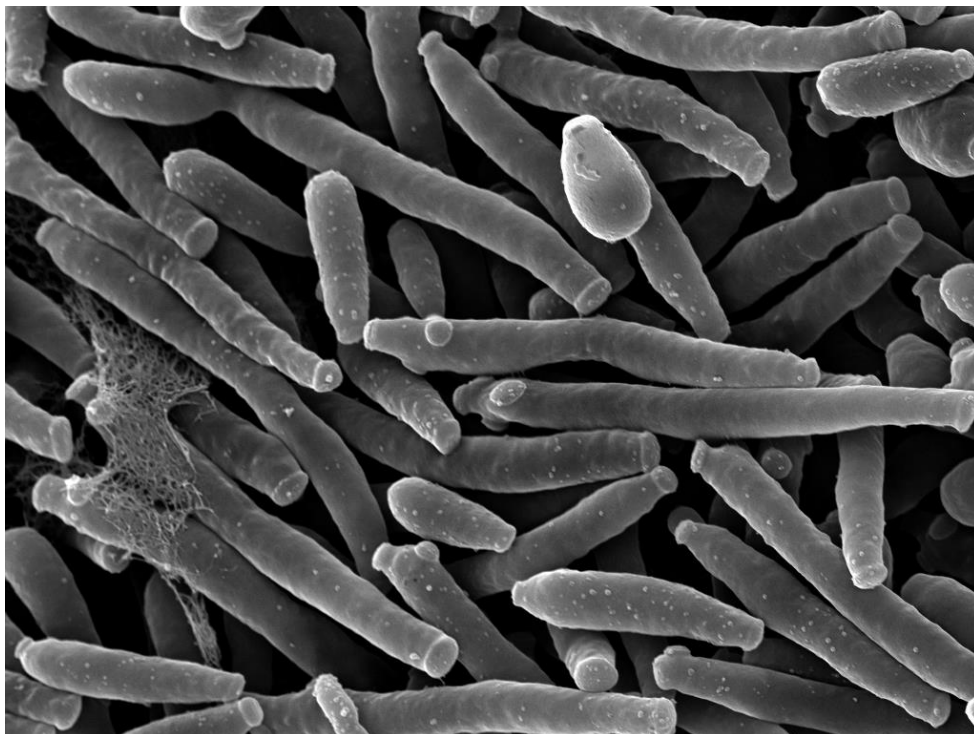


Figure 1A. Scanning electron micrograph with pseudohyphae and blastoconidia.



Further reading:

- Arendrup MC. 2010. Epidemiology of invasive candidiasis. *Curr Opin Crit Care*. 16: 445-452.
- Burton MJ, Shah P, Swiatlo E. 2011. Misidentification of *Candida parapsilosis* as *C. famata* in a clinical case of vertebral Osteomyelitis. *Am J Med Sci*. 341: 71-73.
- Deshpande K. 2003. *Candida parapsilosis* fungaemia treated unsuccessfully with amphotericin B and fluconazole but eliminated with caspofungin: a case report. *Crit Care Resusc*. 5: 20-23.
- Garzoni C, Nobre VA, Garbino J. 2007. *Candida parapsilosis* endocarditis: a comparative review of the literature. *Eur J Clin Microbiol Infect Dis*. 26: 915-926.
- Gilani AA, Barr CS. 2012. Recurrent *Candida parapsilosis* infective endocarditis aortic root replacement. *Br J Hosp Med (Lond)*. 73: 468-469.
- Hays C, Duhamel C, Cattoir V, Bonhomme J. 2011. Rapid and accurate identification of species belonging to the *Candida parapsilosis* complex by real-time PCR and melting curve analysis. *J Med Microbiol*. 60: 477-480.
- Kumar J, Fish D, Burger H, Weiser B, Ross J, Jones D, Robstad K, Li X, Chaturvedi V. 2011. Successful surgical intervention for the management of endocarditis due to multidrug resistant *Candida parapsilosis*: Case report and literature review. *Mycopathologia*. 172: 287-292.
- Moris DV, Melhem MS, Martins MA, Souza LR, Kacew S, Szeszs MW, Carvalho LR, Pimenta-Rodrigues MV, Berghs HA, Mendes RP. 2012. Prevalence and antifungal susceptibility of *Candida parapsilosis* complex isolates collected from oral cavities of HIV-infected individuals. *J Med Microbiol*. 61(Pt 12):1758-1765.
- Ruiz LD, Montelli AC, Sugizaki MD, Silva EG, Batista GC, Moreira D, Paula CR. 2013. Outbreak of fungaemia caused by *Candida parapsilosis* in a neonatal intensive care unit: Molecular investigation through microsatellite analysis. *Rev Iberoam Micol*. 30: 112-115.
- Schelenz S, Abdallah S, Gray G, Stubbings H, Gow I, Baker P, Hunter PR. 2011. Epidemiology of oral yeast colonization and infection in patients with hematological malignancies, head neck and solid tumors. *J Oral Pathol Med*. 40: 83-89.
- Singh R, Parija SC. 2012. *Candida parapsilosis*: an emerging fungal pathogen. *Indian J Med Res*. 136: 671-673.
- Romeo O, Delfino D, Costanzo B, Cascio A, Criseo G. 2011. Molecular characterization of Italian *Candida parapsilosis* isolates reveals the cryptic presence of the newly described species *Candida orthopsilosis* in blood cultures from newborns. *Diagn Microbiol Infect Dis*. 72: 234-238.
- Trfa D, Gácsér A, Nosanchuk JD. 2008. *Candida parapsilosis*, an emerging fungal pathogen. *Clin Microbiol Rev*. 21: 606-625.
- Yalaz M, Akisu M, Hilmioglu S, Calkavur S, Cakmak B, Kultursay N. 2006. Successful caspofungin treatment of multidrug resistant *Candida parapsilosis* septicaemia in an extremely low birth weight neonate. *Mycoses*. 49: 242-245.

Y-2 *Candida glabrata*

Source: Vaginal swab / Tissue

Clinical significance: *Candida glabrata* commonly causes urinary tract infections and vaginitis. Incidence of candidiasis caused by *C. glabrata* has increased in immunosuppressed patients due to more intensive anticancer chemotherapy, bone marrow, and organ transplantation.

Colony: *C. glabrata* colony is white to cream, smooth and shiny on Sabouraud's dextrose agar after 5 days at 25°C (Figure 2).

Microscopy: *C. glabrata* shows tiny, round or elliptical shape blastoconidia on corn meal agar with Tween 80 (Figure 2).

Differentiation: *C. glabrata* grows at 42°C but does not grow on media containing cycloheximide. It ferments glucose and trehalose. *C. glabrata* forms only blastoconidia and no pseudohyphae or true hyphae.

Molecular test: PCR amplification of a mitochondrial rRNA gene fragment, which is species specific, was developed to identify *C. glabrata*. Diversity of karyotype by pulse-field gel electrophoresis was used to confirm *C. glabrata* infection. Comparative sequence analysis of cytochrome oxidase gene has been reported for typing of *C. glabrata*.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with a reference strain of *Candida glabrata* CBS 138 (Genbank accession no: AY198398).

Antifungal susceptibility: *C. glabrata* is susceptible to amphotericin B, caspofungin, and 5-FC but resistant to azoles like fluconazole and itraconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	114
Laboratories with incorrect ID:	1
(<i>Saccharomyces cerevisiae</i>)	(1)

Illustrations:

Figure 2. *Candida glabrata* white and shiny colony on Sabouraud's dextrose agar, 25°C. Microscopic morphology of *Candida glabrata* with small elliptical shaped blastoconidia on corn meal agar with Tween 80 (bar = 25 µm).

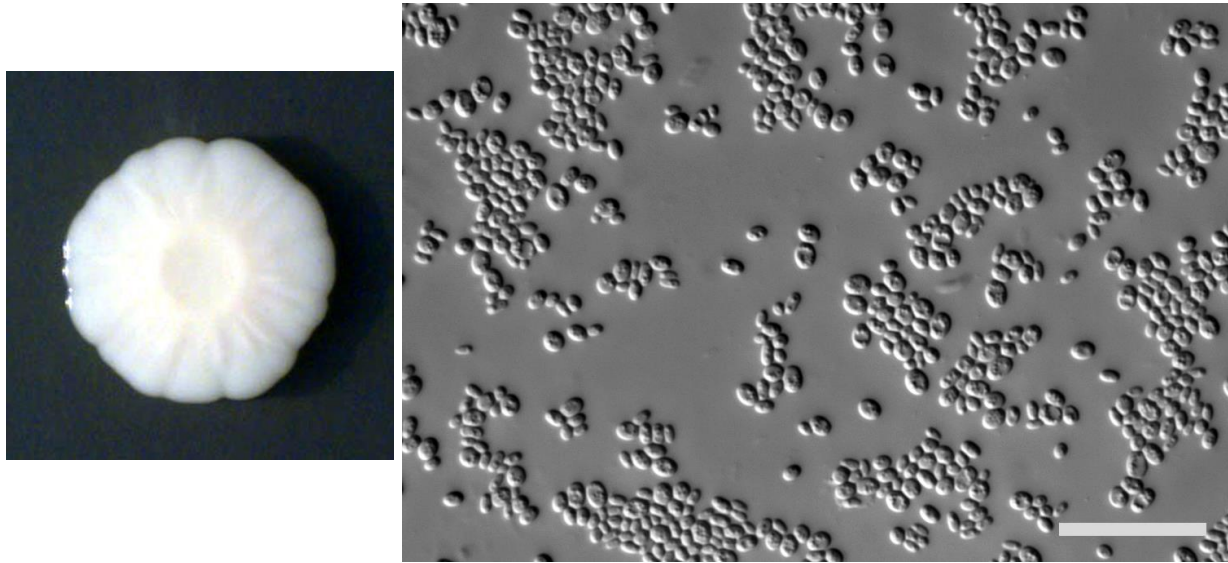
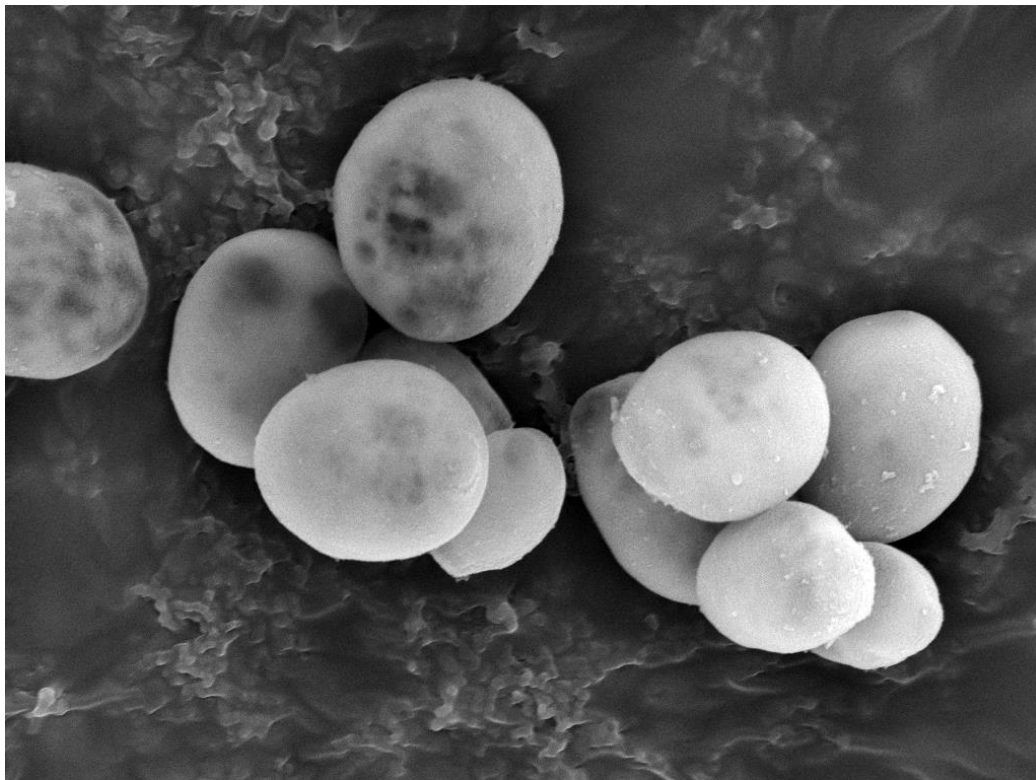


Figure 2A. Scanning electron micrograph with blastoconidia.



Further reading:

Becker K, Badehorn D, Keller B, Schulte M, Bohm KH, Peters G, Fegeler W. 2001. Isolation and characterization of a species specific DNA fragment for identification of *Candida (Torulopsis) glabrata* by PCR. *J.Clin. Microbiol.* 39: 3356-3359.

Coco BJ, Bagg J, Cross LJ, Jose A, Cross J, Ramage G. 2008. Mixed *Candida albicans* and *Candida glabrata* populations associated with the pathogenesis of denture stomatitis. *Oral Microbiol Immunol.* 23: 377-383.

Ghera M, Merz WG. 2009. Identification of *Candida albicans* and *Candida glabrata* within 1.5 Hours Directly from Positive Blood Culture Bottles with a Shortened PNA FISH Protocol. *J Clin Microbiol.* 47: 247-248.

Gugic D, Cleary T, Vincek V. 2008. *Candida glabrata* infection in gastric carcinoma patient mimicking cutaneous histoplasmosis. *Dermatol Online J.* 14: 15.

Khan ZU, Ahmad S, Al-Obaid I, Al-Sweih NA, Joseph L, Farhat D. 2008. Emergence of resistance to amphotericin B and triazoles in *Candida glabrata* vaginal isolates in a case of recurrent vaginitis. *J Chemother.* 20: 488-91.

Kiraz N, Dag I, Yamac M, Kiremitci A, Kasifoglu N, Akgun Y. 2009. Antifungal activity against *Candida glabrata* of caspofungin in combination with amphotericin B: Comparison of Disk diffusion, Etest and Time-kill methods. *Antimicrob Agents Chemother.* 53: 788-790.

Pasqualotto AC, Zimmerman RA, Alves SH, Aquino VR, Branco D, Wiltgen D, do Amaral A, Cechinel R, Colares SM, da Rocha IG, Severo LC, Sukiennik TC. 2008. Take control over your fluconazole prescriptions: the growing importance of *Candida glabrata* as an agent of candidemia in Brazil. *Infect Control Hosp Epidemiol.* 29: 898-899.

Pyrgos V, Ratanavanich K, Donegan N, Veis J, Walsh TJ, Shoham S. 2008. *Candida* bloodstream infections in hemodialysis recipients. *Med Mycol.* 16:1-5.

Sutherland A, Ellis D. 2008. Treatment of a critically ill child with disseminated *Candida glabrata* with a recombinant human antibody specific for fungal heat shock protein 90 and liposomal amphotericin B, caspofungin, and voriconazole. *Pediatr Crit Care Med.* 9: e23-25.

Thompson GR 3rd, Wiederhold NP, Vallor AC, Villareal NC, Lewis JS 2nd, Patterson TF. 2008. Development of caspofungin resistance following prolonged therapy for invasive candidiasis secondary to *Candida glabrata* infection. *Antimicrob Agents Chemother.* 52: 3783-3785.

Y-3 *Rhodotorula minuta*

Source: Sputum / Urine / Catheter

Clinical significance: *Rhodotorula minuta* is reported as a rare/unusual causative agent of systemic infections in patients with AIDS and leukemia. It is isolated from blood, sputum, throat swabs, and feces.

Colony: *R. minuta* colony is pink, smooth, and soft on Sabouraud's dextrose agar at 25°C (Figure 3).

Microscopy: *R. minuta* does not produce pseudohyphae, round blastoconidia are seen on corn meal agar with Tween 80 (Figure 3).

Differentiation: *R. minuta* does not assimilate maltose, which differentiate it from *R. glutinis* and *R. mucilaginosa*.

Molecular test: ITS sequence information is available to be used for molecular identification.

The ribosomal ITS1 region of the test isolate showed 100% nucleotide identity with *Rhodotorula minuta* (synonyms: *Rhodotorula slooffiae*) CBS 5706 (Genebank accession number: AF444627).

Antifungal susceptibility: *R. minuta* was susceptible to amphotericin B, 5-flucytosine, and itraconazole, but resistant to fluconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	102
Laboratories with incorrect ID:	0

Illustrations:

Figure 3. *Rhodotorula minuta*, colony smooth, soft, pink on Sabouraud's dextrose agar, 25°C (left panel). Microscopic morphology on corn meal agar with Tween 80, showing oval to round blastoconidia (right panel).

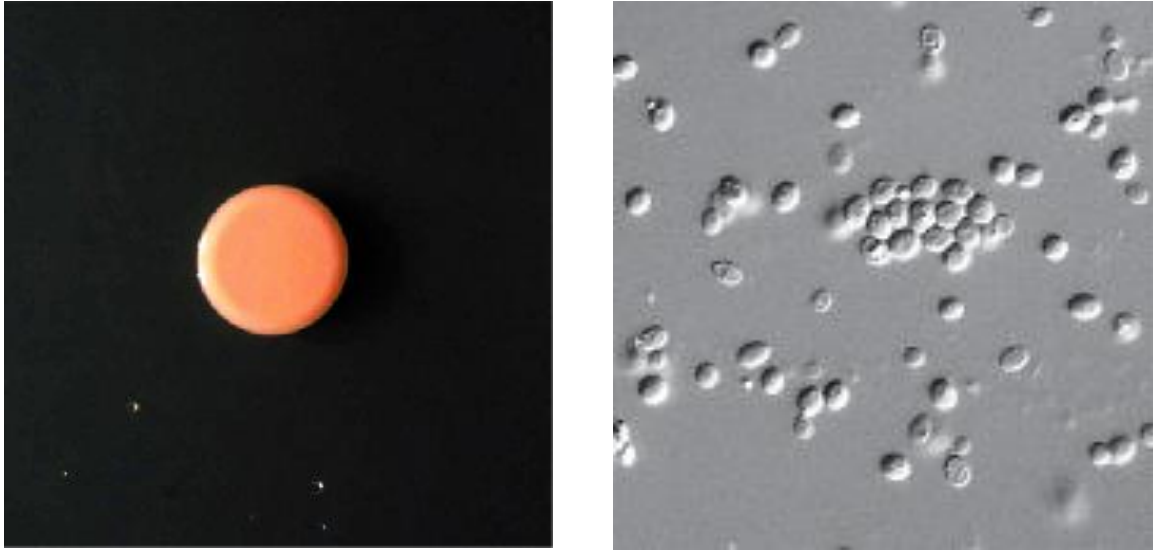
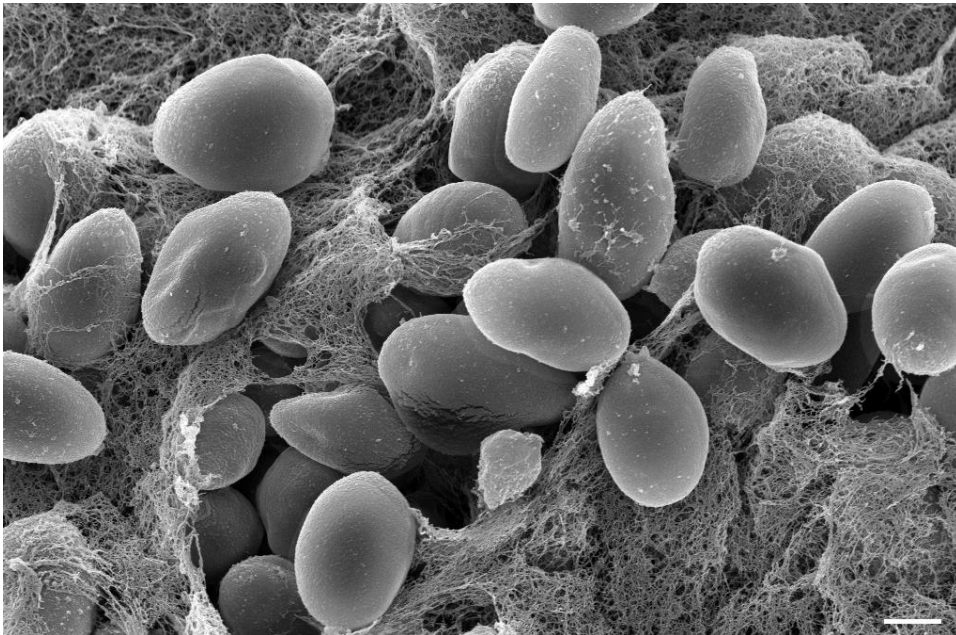


Figure 3A. Scanning electron micrograph of *Rhodotorula minuta* illustrates blastoconidia (bar = 1 μ m).



Further reading:

Cutrona AF, Shah M, Himes MS, Miladore MA. 2002. *Rhodotorula minuta*: an unusual fungal infection in hip-joint prosthesis. *Am. J. Orthop.* 31: 137-140.

Garcia-Martos P, Dominguez I, Marin P, Garcia-Agudo R, Aoufi S, Mira J. 2001. Antifungal susceptibility of emerging yeast pathogens. *Enferm. Infecc. Microbiol. Clin.* 19: 249-256.

Goldani LZ, Craven DE, Sugar AM. 1995. Central venous catheter infection with *Rhodotorula minuta* in a patient with AIDS taking suppressive doses of fluconazole. *J. Med. Vet. Mycol.* 33: 267-270.

Krzyściak P, Macura AB. 2010. Drug susceptibility of 64 strains of *Rhodotorula* sp. *Wiad Parazytol.* 56: 167-170.

Pinna A, Carta F, Zanetti S, Sanna S, Sechi LA. 2001. Endogenous *Rhodotorula minuta* and *Candida albicans* endophthalmitis in an injecting drug user. *Br. J. Ophthalmol.* 85: 759.

Thanos L, Mylona S, Kokkinaki A, Pomoni M, Tsiouris S, Batakis N. 2006. Multifocal skeletal tuberculosis with *Rhodotorula minuta* co-infection. *Scand J Infect Dis.* 38: 309-11.

Wirth F, Goldani LZ. 2012. Epidemiology of *Rhodotorula*: an emerging pathogen. *Interdiscip Perspect Infect Dis.* 2012:465717.

Zhou J, Chen M, Chen H, Pan W, Liao W. 2014. *Rhodotorula minuta* as onychomycosis agent in a Chinese patient: first report and literature review. *Mycoses.* 57:191-195.

Y-4 *Trichosporon asahii*

Source: Tissue / Nail / Urine

Clinical significance: *Trichosporon asahii* infections are not common, but have been associated with a wide spectrum of clinical manifestations. They range from superficial involvement in immunocompetent individuals to severe systemic disease in immunocompromised patients.

Colony: *T. asahii* colony is white to yellowish. The surface is wrinkled, velvety on Sabouraud's dextrose agar at 25°C (Figure 4).

Microscopy: – On corn meal agar with Tween 80, *T. asahii* produces true and pseudohyphae with blastoconidia singly or in short chains. Rectangular-to-oval arthroconidia are prominent; they originate by fragmentation of hyphae and hyphal branches (Figure 4).

Differentiation: *T. asahii* is non-fermentative, urease-positive, nitrate-negative, cycloheximide resistant, and metabolically active for assimilation of a wide range of carbohydrates. It can be distinguished from *Geotrichum candidum* by its wooly colony and production of urease. It is different from *T. mucoides* by no growth with melibiose.

Molecular test: Sequence analysis of the ribosomal DNA intergenic spacer regions allows distinction among closely related species and clinical isolates.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Trichosporon asahii* strain CBS 7137 (GenBank accession no. AF444466).

Antifungal susceptibility: *T. asahii* is susceptible to amphotericin B, flucytosine and azoles. Reduced-susceptibility to caspofungin is seen in some isolates.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	105
Laboratories with incorrect ID:	2
(<i>Trichosporon mucoides</i>)	(2)

Illustrations:

Figure 4. *Trichosporon asahii*, white to yellowish colony with wrinkled surface on Sabouraud's dextrose agar, 25°C. Microscopic morphology on corn meal agar showing arthroconidia (bar = 25 μ m).

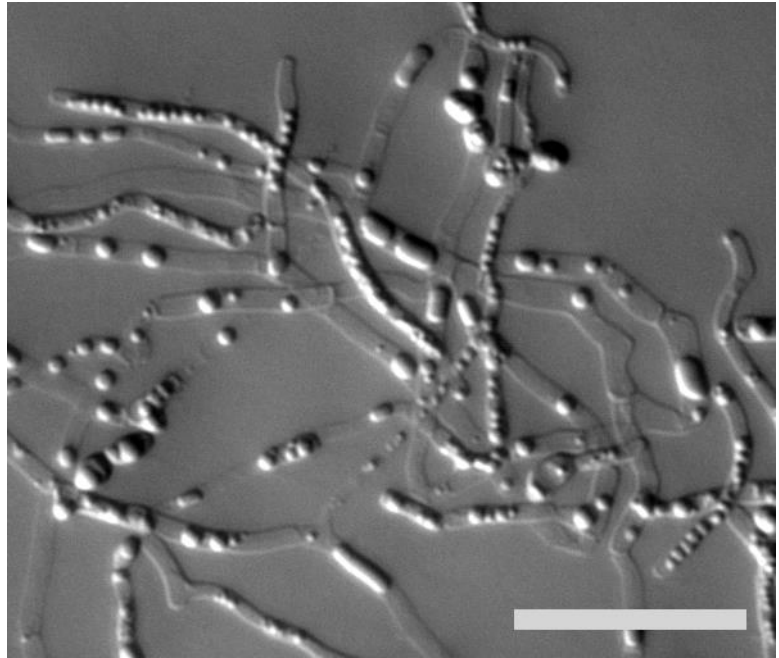
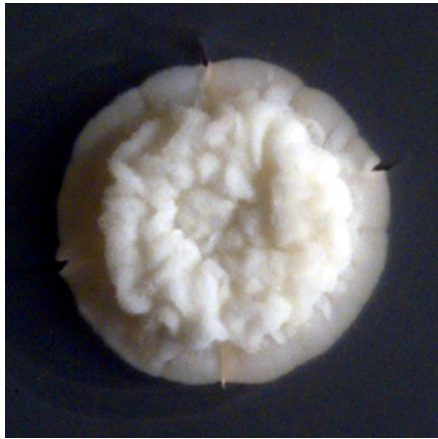
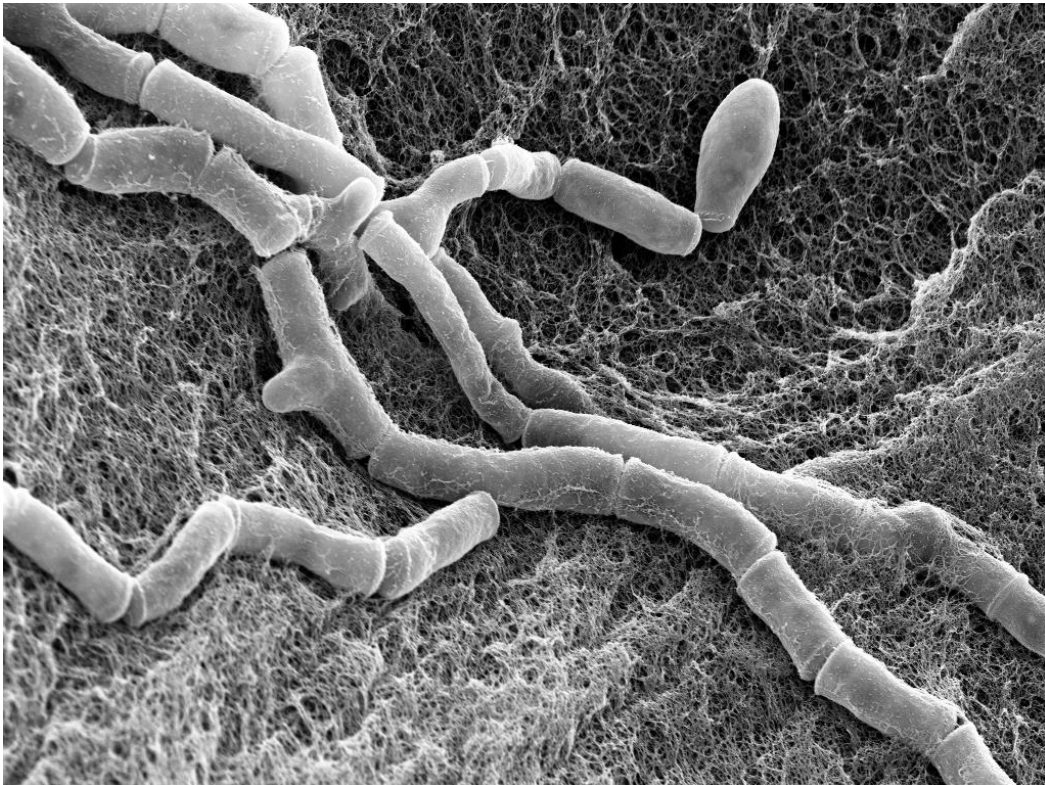


Figure 4A. Scanning electron micrograph illustrates arthroconidia.



Further reading:

- Basiri K, Meidani M, Rezaie F, Soheilnader S, Fatehi F. 2012. A rare case of *Trichosporon* brain abscess, successfully treated with surgical excision and antifungal agents. *Neurol Neurochir Pol.* 46: 92-95.
- Bayramoglu, G., Sonmez, M., Tosun, I., Aydin, K., and Aydin, F. 2007. Breakthrough *Trichosporon asahii* Fungemia in Neutropenic Patient with Acute Leukemia while Receiving Caspofungin. *Infection.* 36: 68-70.
- Chakrabarti, A., Marhawa, R.K., Mondal, R., Trehan, A., Gupta, S., Rao Raman, D.S., Sethi, S., and Padhyet, A.A. 2002. Generalized lymphadenopathy caused by *Trichosporon asahii* in a patient with Job's syndrome. *Med. Mycol.* 40: 83-86.
- Guého E, de Hoog GS, Smith MT. 1992. Neotypification of the genus *Trichosporon*. *Antonie van Leeuwenhoek.* 61: 285-288.
- Kudo K, Terui K, Sasaki S, Kamio T, Sato T, Ito E. 2011. Voriconazole for both successful treatment of disseminated *Trichosporon asahii* infection and subsequent cord blood transplantation in an infant with acute myelogenous leukemia. *Bone Marrow Transplant.* 46: 310-311.
- Li H, Lu Q, Wan Z, Zhang J. 2010. *In vitro* combined activity of amphotericin B, caspofungin and voriconazole against clinical isolates of *Trichosporon asahii*. *Int J Antimicrob Agents.* 35: 550-552.
- Mekha, N., Sugita, T., Ikeda, R., Nishikawa, A., and Poonwan, N. 2007. Real-time PCR assay to detect DNA in sera for the diagnosis of deep-seated trichosporonosis. *Microbiol Immunol.* 51(6): 633-635.
- Meyer, M.H., Letscher-Bru, V., Waller, J., Lutz, P., Marcellin, L., and Herbrecht, R. 2002. Chronic disseminated *Trichosporon asahii* infection in a leukemic child. *Clin. Infect. Dis.* 35: e22-25.
- Panagopoulou, P., Evdoridou, J., Bibashi, E., Filioti, J., Sofianou, D., Kremenopoulos, G., and Roilides, E. 2002. *Trichosporon asahii*: an unusual cause of invasive infection in neonates. *Pediatr. Infect. Dis. J.* 21: 169-170.
- Rastogi, V.L. and Nirwan, P.S. 2007. Invasive trichosporonosis due to *Trichosporon asahii* in a non-immunocompromised host: a rare case report. *Indian J Med Microbiol.* 25: 59-61.
- Rieger, C., Geiger, S., Herold, T., Nickenig, C., and Ostermann, H. 2007. Breakthrough infection of *Trichosporon asahii* during posaconazole treatment in a patient with acute myeloid leukaemia. *Eur J Clin Microbiol Infect Dis.* 26: 843-845.
- Sabharwal ER. 2010. Successful management of *Trichosporon asahii* urinary tract infection with fluconazole in a diabetic patient. *Indian J Pathol Microbiol.* 53: 387-388.
- Shang ST, Yang YS, Peng MY. 2010. Nosocomial *Trichosporon asahii* fungemia in a patient with secondary hemochromatosis: a rare case report. *J Microbiol Immunol Infect.* 43: 77-80.
- Sun W, Su J, Xu S, Yan D. 2012. *Trichosporon asahii* causing nosocomial urinary tract infections in intensive care unit patients: genotypes, virulence factors and antifungal susceptibility testing. *J Med Microbiol.* 61(Pt 12):1750-1757.

Y-5 *Prototheca wickerhamii*

Source: Bronchial wash / Vagina / Urine

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

Colony: *Prototheca wickerhamii* colony is moist, cream-colored on Sabouraud's dextrose agar 7 days at 25°C (Figure 5).

Microscopy: *Prototheca wickerhamii* shows sporangia of various sizes, some filled with sporangiospores (endospores) on corn meal agar with Tween 80. There is no budding, no hyphae (Figure 5).

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

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

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

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

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	106
Laboratories with incorrect ID:	0

Illustrations:

Figure 5. *Prototheca wickerhamii* colony moist, cream-colored on Sabouraud's dextrose agar, 7 days, 25°C. Microscopic morphology of *Prototheca wickerhamii* showing sporangia of various sizes, some filled with sporangiospores (endospores) on Corn meal agar with Tween 80 (bar = 10 μm).

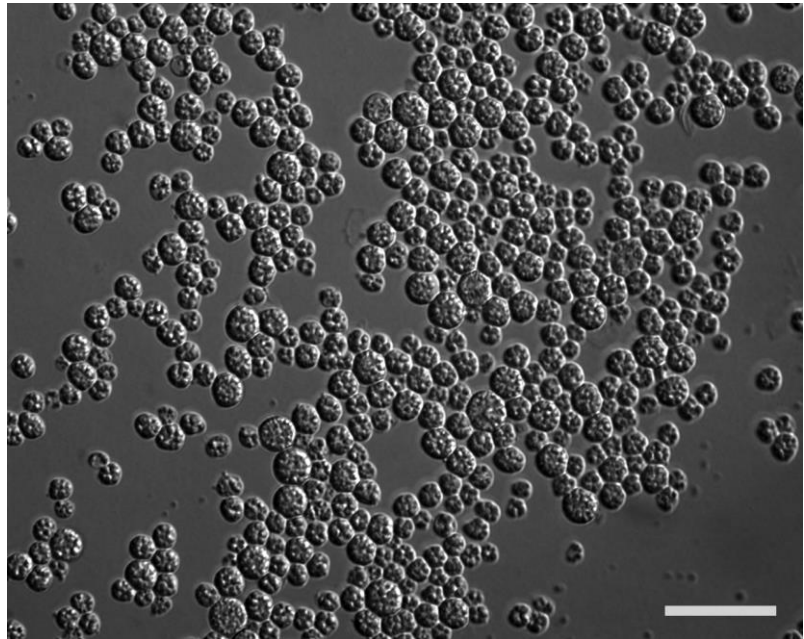
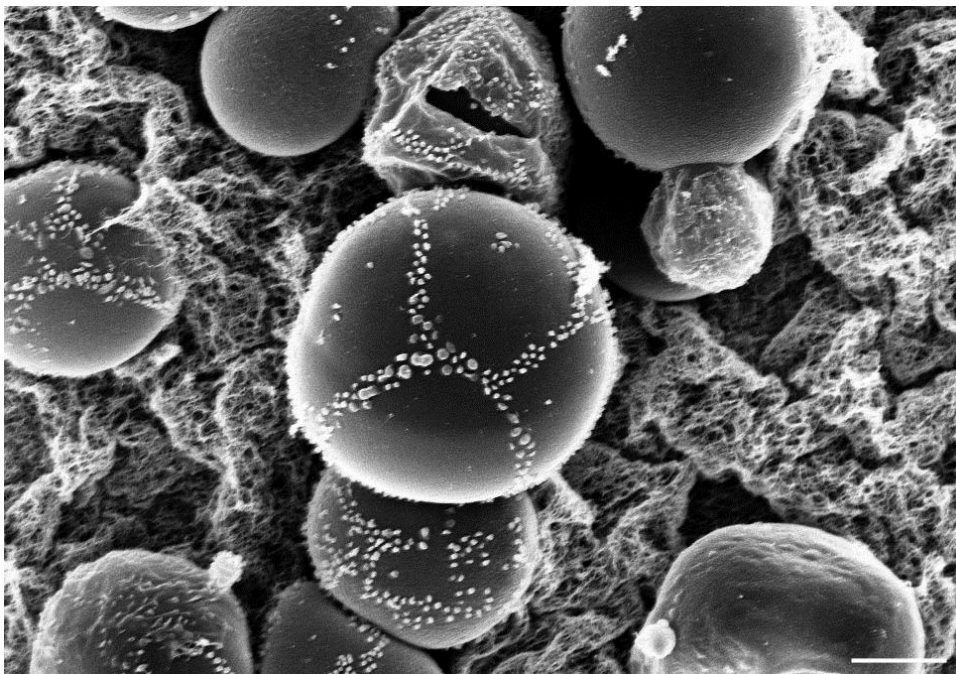


Figure 5A. Scanning electron micrograph with sporangiospores (bar = 2 μm).



Further reading:

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

Kano R, Sobukawa H, Suzuki M, Hiruma M, Shibuya K, Hasegawa A, Kamata H. 2014. Immunohistopathology of *Prototheca wickerhamii* in cutaneous lesions of protothecosis. *Med Mycol J*. 55: E29-32.

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

Min Z, Moser SA, Pappas PG. 2012. *Prototheca wickerhamii* algaemia presenting as cholestatic hepatitis in a patient with systemic lupus erythematosus: A case report and literature review. *Med Mycol Case Rep*. 2:19-22.

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

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

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

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

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

Zak I, Jagielski T, Kwiatkowski S, Bielecki J. 2012. *Prototheca wickerhamii* as a cause of neuroinfection in a child with congenital hydrocephalus. First case of human protothecosis in Poland. *Diagn Microbiol Infect Dis*. 74: 186-189.

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 albicans* (S-1) was the analyte in the May 28, 2014 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 3 of the 32 laboratories participating in this test event tested all 10 antifungal drugs. The reported results were as follows: itraconazole (27 laboratories), voriconazole (25 laboratories), flucytosine (23 laboratories), caspofungin (21 laboratories), amphotericin B (21 laboratories), anidulafungin (16 laboratories), micafungin (16 laboratories), posaconazole (15 laboratories), and ketoconazole (5 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 albicans* (M955)

Drug	No. labs	MIC (µg/ml)															
		0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
Amphotericin B	21							12	9								
Anidulafungin	16					12	4										
Caspofungin	21							2	14	4	1						
Flucytosine (5-FC)	23				2	5	15				1						
Fluconazole	31*											1			9	5	16
Itraconazole	26*					1	15	9		1							
Ketoconazole	4*								4								
Micafungin	16				1	12	3										
Posaconazole	15				3	8	3	1									
Voriconazole	25								8	15	2						

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

Colors represent the testing method used:

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

Table 4. Antifungal Susceptibility Interpretations Reported by the Participating Laboratories

S-1: *Candida albicans* (M955)

Drug	No. laboratories	Susceptible	Susceptible-dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	21	5					16
Anidulafungin	16	16					
Caspofungin	21	7		1	13		
Flucytosine	23	17					6
Fluconazole	32				32		
Itraconazole	27	1	18		2		6
Ketoconazole	5	1			1		3
Micafungin	16	16					
Posaconazole	15	5			7		10
Voriconazole	25	4	6	1	14		

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* M2039 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. Two laboratories used CLSI broth microdilution method while the remaining two laboratories used TREK YeastOne Colorimetric method.

Comments: Five 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 ($\mu\text{g/ml}$) Values of Mold Antifungal Susceptibility: *Aspergillus fumigatus* M2039

Drugs ($\mu\text{g/ml}$)	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	5						1	1	2	1						
Anidulafungin	4		2		2											
Caspofungin	3	1		1	1	1										
Fluconazole	4														2	2
Itraconazole	5											1	4			
Ketoconazole	2												1	1		
Micafungin	4	2		1	1											
Posaconazole	4					1		2	1							
Voriconazole	3						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:

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.