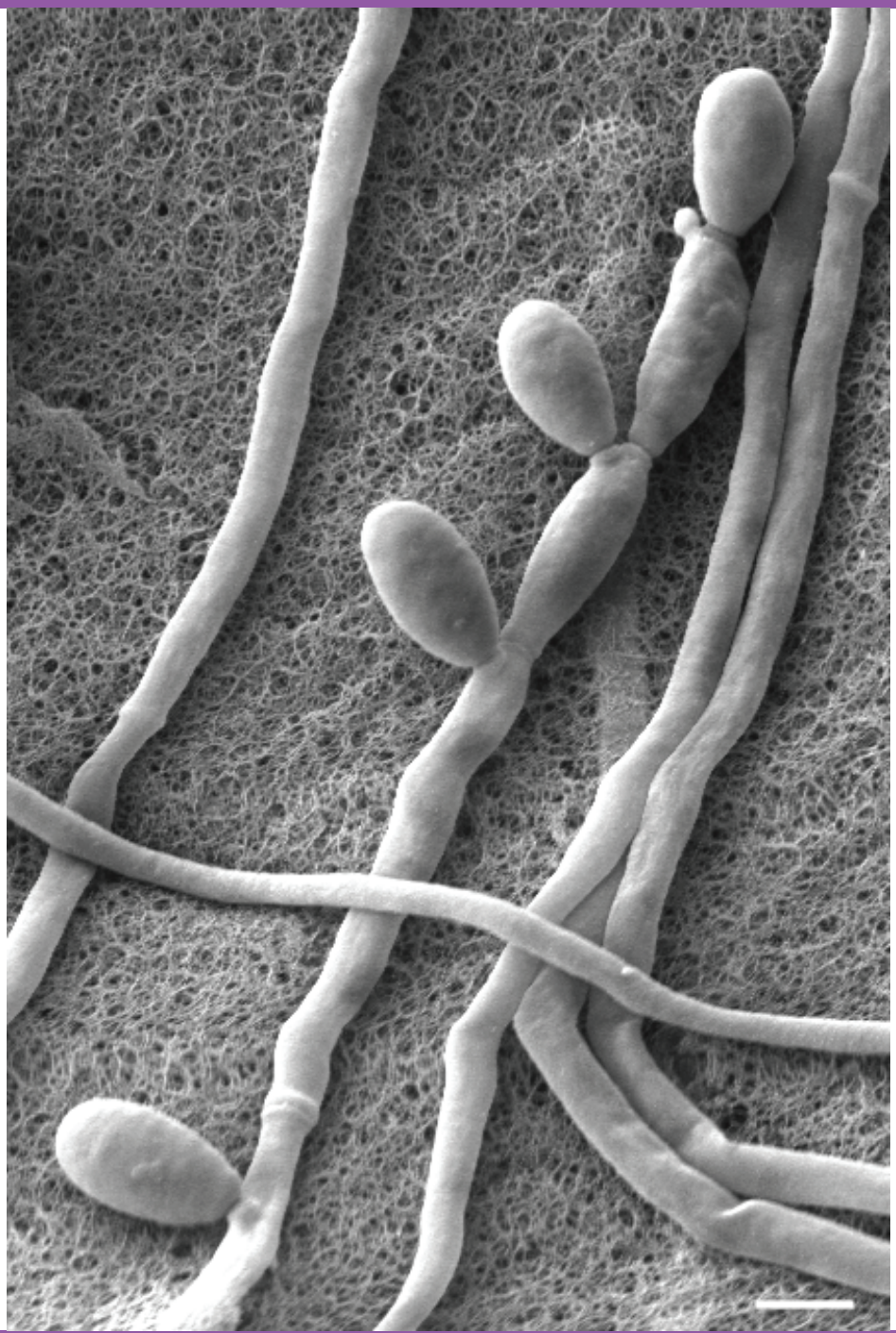
Mycology Proficiency Testing Program



Test Event Critique
May 2013

Wadsworth Center
NEW YORK STATE DEPARTMENT OF HEALTH
Mycology Laboratory

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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.
>
> **Molecular methods:** This category is for laboratories that use FDA-approved or lab-developed molecular methods for detection, identification, typing, characterization or determination of drug resistance against fungal pathogens. Laboratories using molecular methods under another Restricted permit category (e.g. Restricted: Antigen detection) or those holding a Comprehensive category permit are exempt from this-category.

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

PROFICIENCY TESTING ANALYTES OFFERED
(CMS regulated analytes or tests are indicated with an asterisk)

Comprehensive

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

Restricted

Identification Yeast Only

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

Antigen Detection

- Antigen detection of *Cryptococcus neoformans**

Molecular Methods

- No proficiency testing is offered at this time.

TEST SPECIMENS & GRADING POLICY

Test Specimens

At least two strains of each mold or yeast 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 it 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.

An acceptable answer will include names of the genus and species, when both genus and species are shown in this list e.g. *Candida albicans.* Any answer that only contains *Candida* sp. is not acceptable.

In some instances, a genus includes both specific species names and the epithet 'species', e.g. *Saccharomyces cerevisiae,* and *Saccharomyces* species. It is expected that most common pathogenic/non-pathogenic yeasts likely to be encountered in the clinical laboratory will be completely identified in such instances i.e. *Saccharomyces cerevisiae* while other members of the genus will be identified as '*Saccharomyces* species'. 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
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

Candida rugosa
Candida species
Candida tropicalis
Candida viswanathii
Candida zeylanoides
Cryptococcus albidus
Cryptococcus gattii
Cryptococcus laurentii
Cryptococcus neoformans
Cryptococcus neoformans-
Cryptococcus gattii species complex
Rhodotorula glutinis
Rhodotorula minuta
Rhodotorula mucilaginosa (*rubra*)
Rhodotorula species
Saccharomyces cerevisiae
Saccharomyces species
Sporobolomyces salmonicolor
Trichosporon asahii
Trichosporon inkin
Trichosporon mucoides
Trichosporon species

Summary of Laboratory Performance:

Mycology – Yeast Only

	Specimen key	Validated specimen	Laboratories with correct responses / Total laboratories (% correct responses)
Y-1	*Candida kefyr*	*Candida kefyr*	115/116 (99%)
Y-2	*Candida tropicalis*	*Candida tropicalis*	116/116 (100%)
Y-3	*Candida guilliermondii*	*Candida guilliermondii*	112/116 (97%)
Y-4	*Candida krusei*	*Candida krusei*	113/116 (97%)
Y-5	*Candida lusitaniae*	*Candida lusitaniae*	114/116 (98%)

Antifungal Susceptibility Testing for Yeast (S-1: *Candida glabrata* M1409)

Drugs	Acceptable MIC (μg/ml) Range	Acceptable interpretation	Laboratories with acceptable responses/ Total laboratories (% correct responses)
Amphotericin B	0.5 – 1	Susceptible / No interpretation	20/20 (100%)
Anidulafungin	0.03 – 0.125	Susceptible	16/16 (100%)
Caspofungin	0.06 – 0.25	Susceptible	22/22 (100%)
Flucytosine (5-FC)	0.03 – 0.06	Susceptible / No interpretation	24/24 (100%)
Fluconazole	> 64	Resistant	31/31 (100%)
Itraconazole	2 – 16	Resistant / No interpretation	28/28 (100%)
Ketoconazole	1 – 32	Resistant / No interpretation	4/4 (100%)
Micafungin	0.008 – 0.016	Susceptible	16/16 (100%)
Posaconazole	8 – 16	Resistant / No interpretation	15/15 (100%)
Voriconazole	1 – 4	No interpretation	24/24 (100%)

Commercial Device Usage Statistics:

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

Device	**No. laboratories**
Yeast Identification*	
AMS Vitek	6
API 20C AUX	70
Dade Behring MicroScan Rapid Yeast Identification Panel	5
MALDI-TOF	3
Remel RapID Yeast Plus System	8
Sequencing	3
Vitek2	62
Antifungal Susceptibility*	
Disk diffusion	1
Etest	2
Vitek II	1
YeastOne – Mold	2
YeastOne – Yeast	26
CLSI Microbroth dilution method – Yeast	3
CLSI Microbroth dilution method – Mold	2

*Include multiple systems used by some laboratories

YEAST DESCRIPTIONS

Y-1 *Candida kefyr*

Source: Urine / Vaginal / Wound

Clinical significance: *Candida kefyr* is rarely isolated in the clinical laboratory. *Candida kefyr* infections are reported from the reproductive and digestive tracts and the mucous linings.

Colony: *Candida kefyr* colonies appear smooth, creamy, and soft on Sabouraud's dextrose agar after 3 to 5 days, 25°C (Figure 1).

Microscopy: *Candida kefyr* shows abundant long pseudohyphae, and oval to elongated blastoconidia on cornmeal agar with Tween 80 (Figure 1). Ascospores within asci are observed in cultures on V-8 or malt extract agar (details not shown). The sexual or teleomorphic state is termed *Kluyveromyces marxianus*.

Differentiation: *C. kefyr* grows at 45°C and on the media containing cycloheximide. *C. kefyr* ferments glucose, sucrose, lactose, galactose, but not maltose, trehalose, and cellobiose, which differentiates it from other medically important *Candida* species.

Molecular test: Randomly amplified polymorphic DNA-polymorase chain reaction (RADP-PCR) was applied for the identification of *C. kefyr*. The ribosomal *ITS1* and *ITS2* regions of the test isolate showed 100 % nucleotide identity with a reference strain of *Candida kefyr* UWFP-208 (GenBank accession no. AF336841)

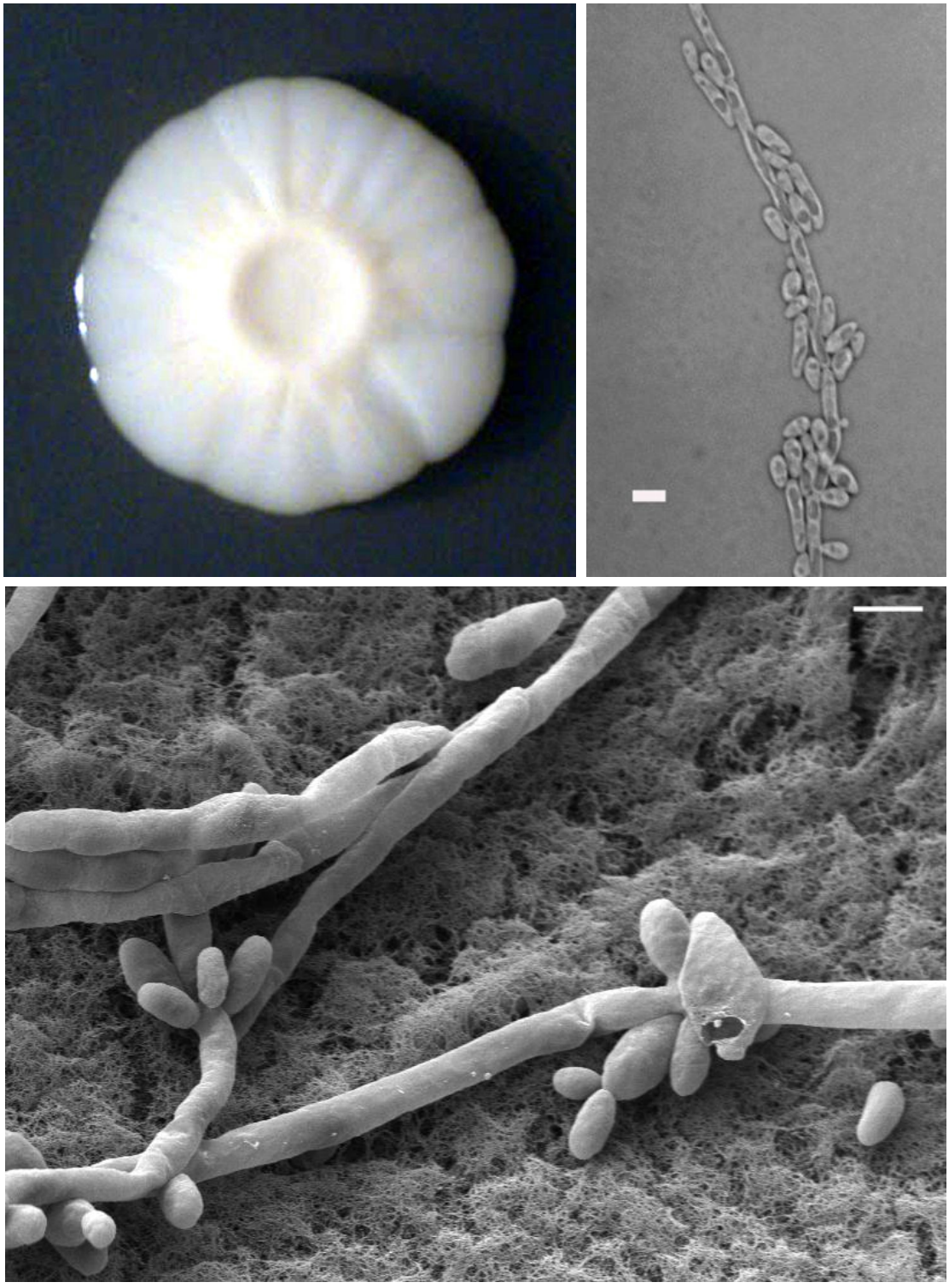
Antifungal susceptibility: *C. kefyr* is susceptible to amphotericin B, caspofungin, different azoles, and 5-fluorocytosine.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	115
Laboratories with incorrect ID:	01
(*Candida* species)	(1)

Illustrations:

Figure 1. *Candida kefyr* colony appearance is creamy to smooth on Sabouraud's dextrose agar 5-day, 25°C. Microscopic morphology of *Candida kefyr* showing long, pseudohyphae with oval to elongated blastoconidia on Corn meal agar with Tween 80 (bar = 5 μm). Scanning electron micrograph illustrating pseudohyphae and blastoconidia (bar = 2 μm).



Further reading:

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Corpus K, Hegeman-Dingle R, Bajjoka I. 2004. *Candida kefyr*, an uncommon but emerging fungal pathogen: report of two cases. *Pharmacotherapy*. 24: 1084-1088.

Gil-Lamaignere C, Muller FM. 2004. Differential effects of the combination of caspofungin and terbinafine against *Candida albicans*, *Candida dubliniensis* and *Candida kefyr*. *Int J Antimicrob Agents*. 23: 520-523.

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Sendid B, Lacroix C, Bougnoux ME. 2006. Is *Candida kefyr* an emerging pathogen in patients with oncohematological diseases? *Clin Infect Dis*. 43: 666-667.

Chopra T, Bhargava A, Kumar S, Chopra A, Dhar S, Afonso L, Sobel JD. 2010. *Candida kefyr* endocarditis in a patient with hypertrophic obstructive cardiomyopathy. *Am J Med Sci*. 339: 188-189.

Gomez-Lopez A, Pan D, Cuesta I, Alastruey-Izquierdo A, Rodriguez-Tudela JL, Cuenca-Estrella M. 2010. Molecular identification and susceptibility profile in vitro of the emerging pathogen *Candida kefyr*. *Diagn Microbiol Infect Dis.* 66: 116-119.

Y-2 *Candida tropicalis*

Source: Body fluid / Urine / 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 2).

Microscopy: *C. tropicalis* shows long true hyphae and pseudohyphae, with either single or small clusters of blastoconidia on Corn meal agar with Tween 80 (Figure 2).

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 corn meal 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 (Genebank accession no. EU924133)

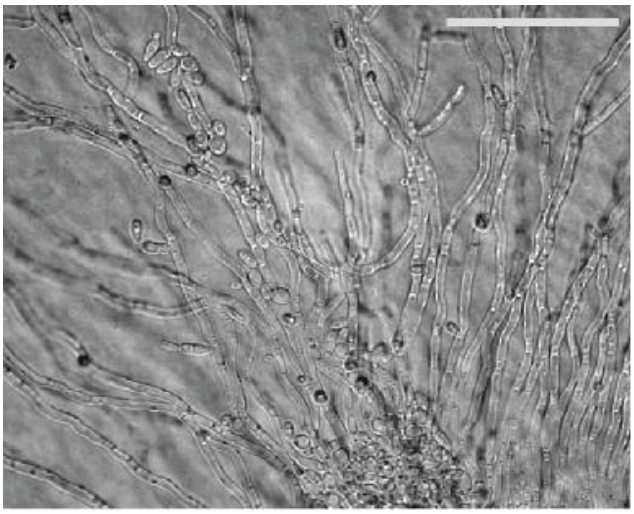
Antifungal susceptibility: *C. tropicalis* is generally susceptible to azoles and echinocandins, but variably susceptible to flucytosine. Few strains of *C. tropicalis* have been reported with high amphotericin B MIC.

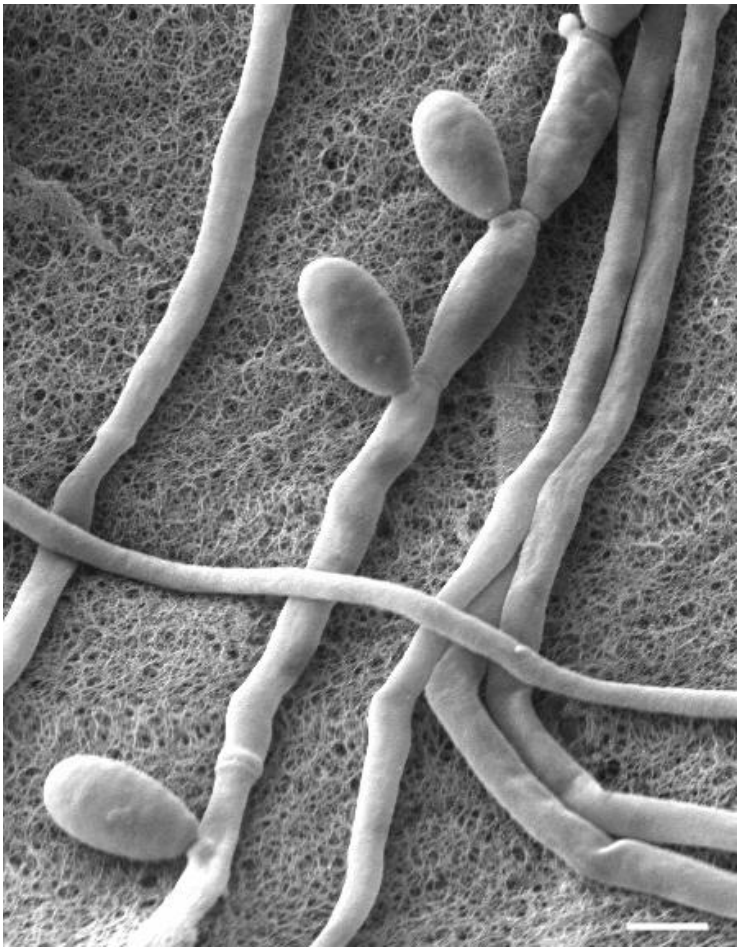
Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	116
Laboratories with incorrect ID:	0

Illustrations:

Figure 2. *Candida tropicalis,* smooth-to-wrinkled, creamish colony, Sabouraud's dextrose agar 7-days, 25°C**.** Microscopic morphology on corn meal 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:

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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.

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.

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de Carvalho Parahym AM, da Silva CM, Leao 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.

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.

Y-3 *Candida guilliermondii*

Source: Urine / Blood / Nail

Clinical significance: *Candida guilliermondii* is a frequent cause of nosocomial fungemia in immunosuppressed patients. It rarely causes infection of urinary tract, brain and eye.

Colony: *C. guilliermondii* colony is flat, smooth, cream-yellow on Sabouraud's dextrose agar after 7 days of incubation at 25°C (Figure 3).

Microscopy: *C. guilliermondii* shows few short pseudohyphae with clusters of blastoconidia on Corn meal agar with Tween 80 (Figure 3). Please check corn meal how it is written in book and change accordingly?

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

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

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

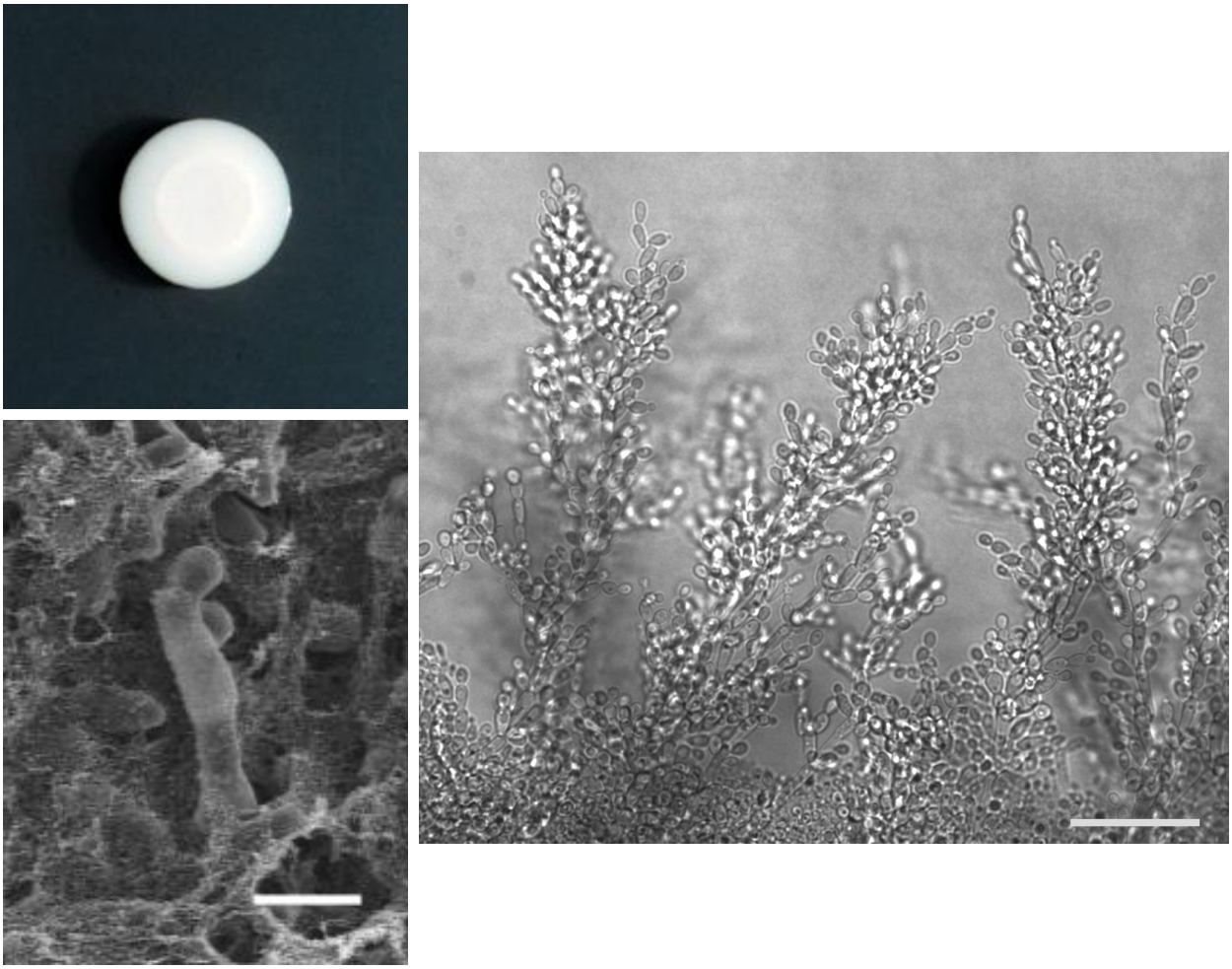
Antifungal susceptibility: Most clinical isolates are susceptible to amphotericin B, 5-flucytosine, echinocandins and azoles such as fluconazole, ketocoanzole, itraconazole. A few isolates are reported to have high MIC to azoles.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	112
Laboratories with incorrect ID:	06
(*Candida famata*)	(1)
(*Candida* sp.)	(2)
(Candida *viswanathii*)	(1)

Illustrations:

Figure 3. *Candida guilliermondii,* flat, smooth, creamish colony on Sabouraud's dextrose agar, 5 days, 25°C. Microscopic morphology on corn meal agar with Tween 80, showing short pseudohyphae with clusters of blastoconidia (bar = 10 μm). Scanning electron micrograph of *Candida guilliermondii* (*Pichia guilliermondii*) illustrates pseudohyphae and blastoconidia (bar = 2 μm)



Further reading:

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

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

Y-4 *Candida krusei*

Source: Tissue / Sputum / Urine / Stool

Clinical significance: *Candida krusei* causes nosocomial fungemia in immunosuppressed patients. It also causes disseminated disease including endocarditis, peritonitis, vaginitis, urinary tract infections, and sinusitis.

Colony: *C. krusei* colony is soft, cream to buff, glassy and wrinkled on Sabouraud's dextrose agar, after7 days of incubation at 25°C (Figure 4).

Microscopy: *C. krusei* shows branched pseudohyphae with elongated blastoconidia on Corn meal agar with Tween 80 (Figure 4).

Differentiation: *C. krusei* ferments glucose, but not sucrose or cellobiose, and does not grow on the media containing cycloheximide. *C. krusei* does not assimilate sucrose, which differentiates it from *C. parapsilosis* and *C. lusitaniae*. *C. krusei* grows well at 42°C, differentiating it from *C. lambica*. *C. krusei* does not produce arthroconidia, thus differentiating it from *Blastoschizomyces capitatus*.

Molecular test: DNA probes from the *ITS* regions are incorporated in a reverse hybridization line probe assay for the detection of ITS PCR products for identification of fungal pathogens. Panfungal PCR and multiplex liquid hybridization are developed for the detection of clinically important yeasts in tissue specimens. PFGE, RFLP, and RAPD procedures are used for DNA fingerprinting and electrophoretic karyotyping of oral *C. krusei* isolates. The ribosomal *ITS1* and *ITS2* regions of the test isolate showed 100% nucleotide identity with a reference strain of *C. krusei* (*Pichia kudriavzevii*) GenBank accession no. AF411417.

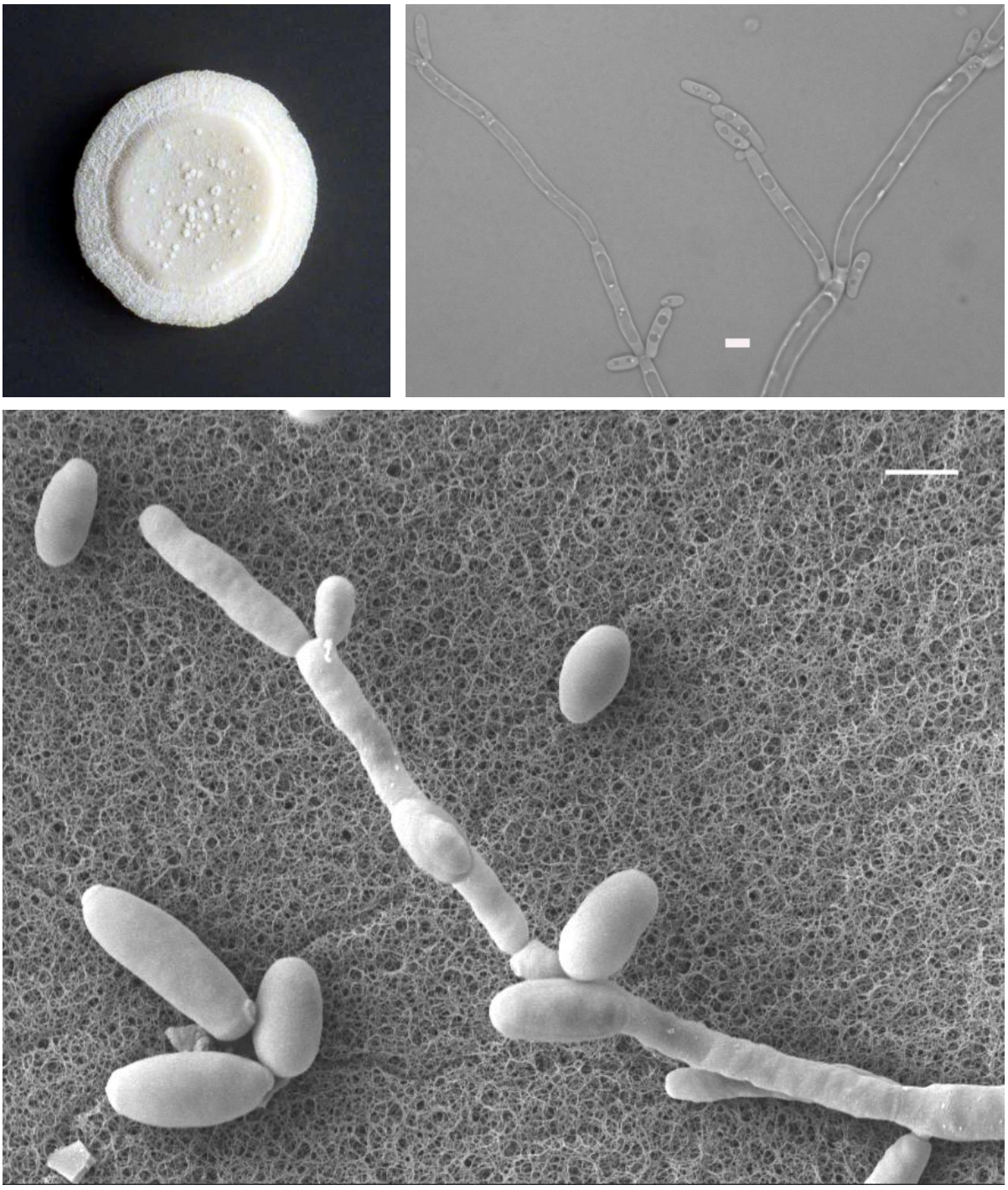
Antifungal susceptibility: *C. krusei* is susceptible to amphotericin B and flucytosine. *C. krusei* is innately resistant to fluconazole and variably resistant to other azoles such as itraconazole and ketoconazole, but not voriconazole. *C. krusei* is also susceptible to echinocandins.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	113
Laboratories with incorrect ID:	05
(*Candida norvegensis*)	(2)
(*Blastoschizomyces capitatus*)	(1)

Illustrations:

Figure 4. *Candida krusei* soft wrinkled colony on Sabouraud's dextrose agar, 7 days, 25°C; Microscopic morphology on corn meal agar showing long, branched pseudohyphae with oval blastoconidia (bar = 5 μm). Scanning electron micrograph illustrates pseudohyphae and blastoconidia (bar = 2 μm).



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Y-5 *Candida lusitaniae*

Source: Stool / Blood / Eye / Urine

Clinical significance: *Candida lusitaniae* causes fungemia and sepsis in immunocompromised and debilitated patients with cancer, diabetes, or asthma, and also neonates in intensive care units. The 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 5).

Microscopy: *C. lusitaniae* produced many short, branched ("bushy") pseudohyphae. Along the length of the pseudohyphae, elongated blastoconidia formed in short chains on Corn Meal Agar with Tween 80 (Figure 5).

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

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

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

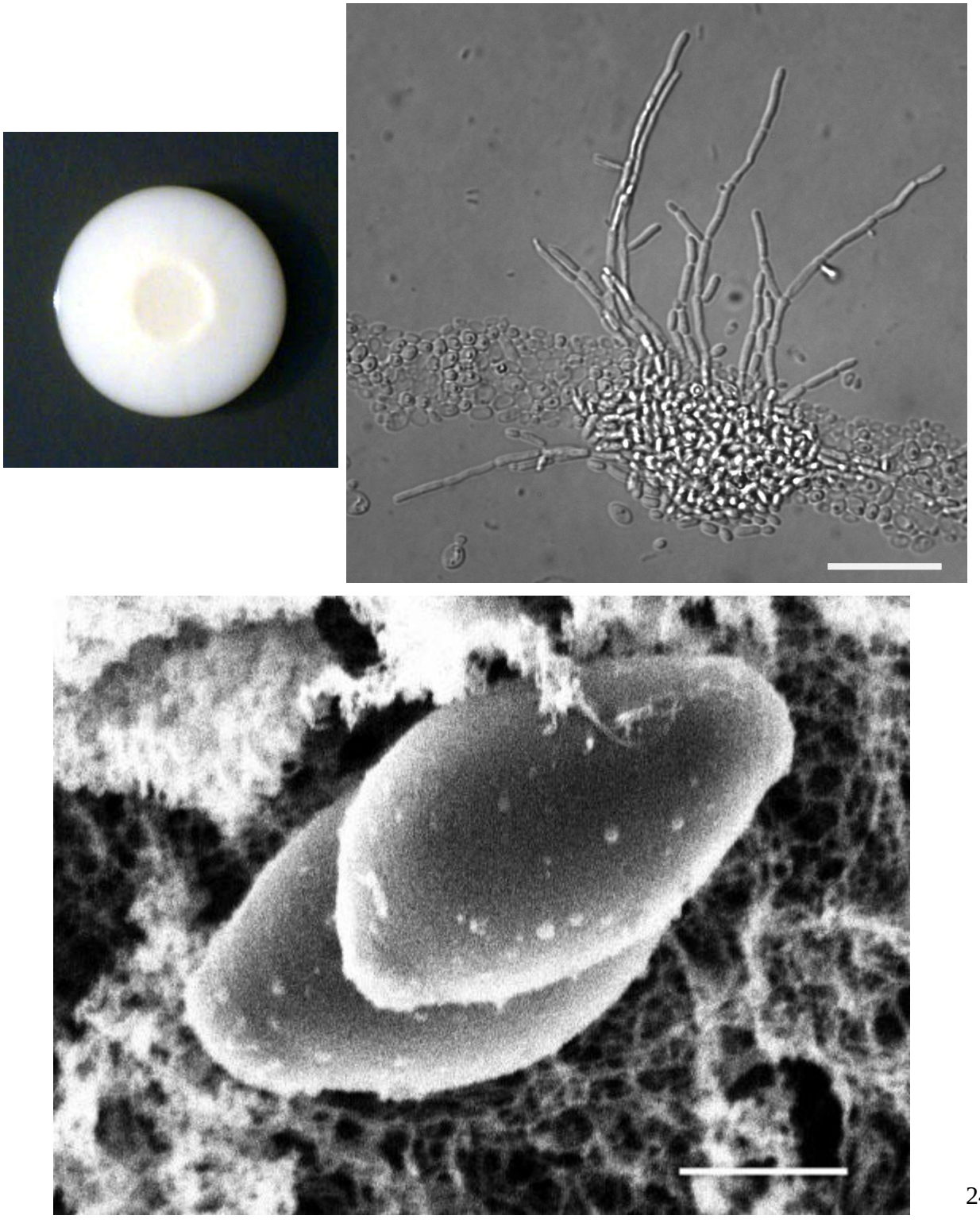
Antifungal susceptibility: Some *C. lusitaniae* strains are reported to be inherently resistant to amphotericin B. Amphotericin B susceptible strains are also known to develop resistance during the course of treatment with this drug. *C. lusitaniae* is reported as more susceptible to voriconazole than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

Participant performance:

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

Illustrations:

Figure 5. *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).



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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 glabrata* (S-1) was the analyte in the May 29, 2013 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 31 laboratories participating in this test event tested all 10 antifungal drugs. The reported results were as follows: itraconazole (28 laboratories), flucytosine (24 laboratories), voriconasole (24 laboratories), caspofungin (22 laboratories), amphotericin B (20 laboratories), anidulafungin (16 laboratories), micafungin (16 laboratories), posacoanazole (15 laboratories), and ketocoanzole (4 laboratories). Fluconazole was the only drug tested by all 31 laboratories.

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

S-1: *Candida glabrata* (M1409)

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	20							10	10								
Anidulafungin	16			7	6	3											
Caspofungin	22				1	16	5										
Flucytosine (5-FC)	24			6	18												
Fluconazole	30*														8	14	8
Itraconazole	27*									7	1		19				
Ketoconazole	4*									1	1			1			
Micafungin	16	1	15														
Posaconazole	15											14	1				
Voriconazole	24								1	10	13						

* 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 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 glabrata* (M1409)

Drug	No. laboratories	Susceptible	Susceptible-dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	20	9					11
Anidulafungin	16	16					
Caspofungin	22	22					
Flucytosine	24	22					2
Fluconazole	31				31		
Itraconazole	28	28			26		2
Ketoconazole	4				2		2
Micafungin	16	16					
Posaconazole	15				7		8
Voriconazole	24	1	6	1	12		4

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 used TREK YeastOne Colorimetric method.

Comments: Four out of thirty-one 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* M2039

Drugs (µg/ml)	Total # of labs	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1.0	2.0	4.0	8.0	16	32	64	256
Amphotericin B	4						1	2	1							
Anidulafungin	3		2		1											
Caspofungin	3	1		1	1											
Fluconazole	3														1	2
Itraconazole	4						1		1			1	1			
Ketoconazole	1													1		
Micafungin	3	2			1											
Posaconazole	3				1			2								
Voriconazole	3						1	1		1						

Colors represent the testing method used:
CLSI microdilution method
YeastOne Colorimetric method
Both CLSI microdilution and YeastOne Colorimetric methods

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