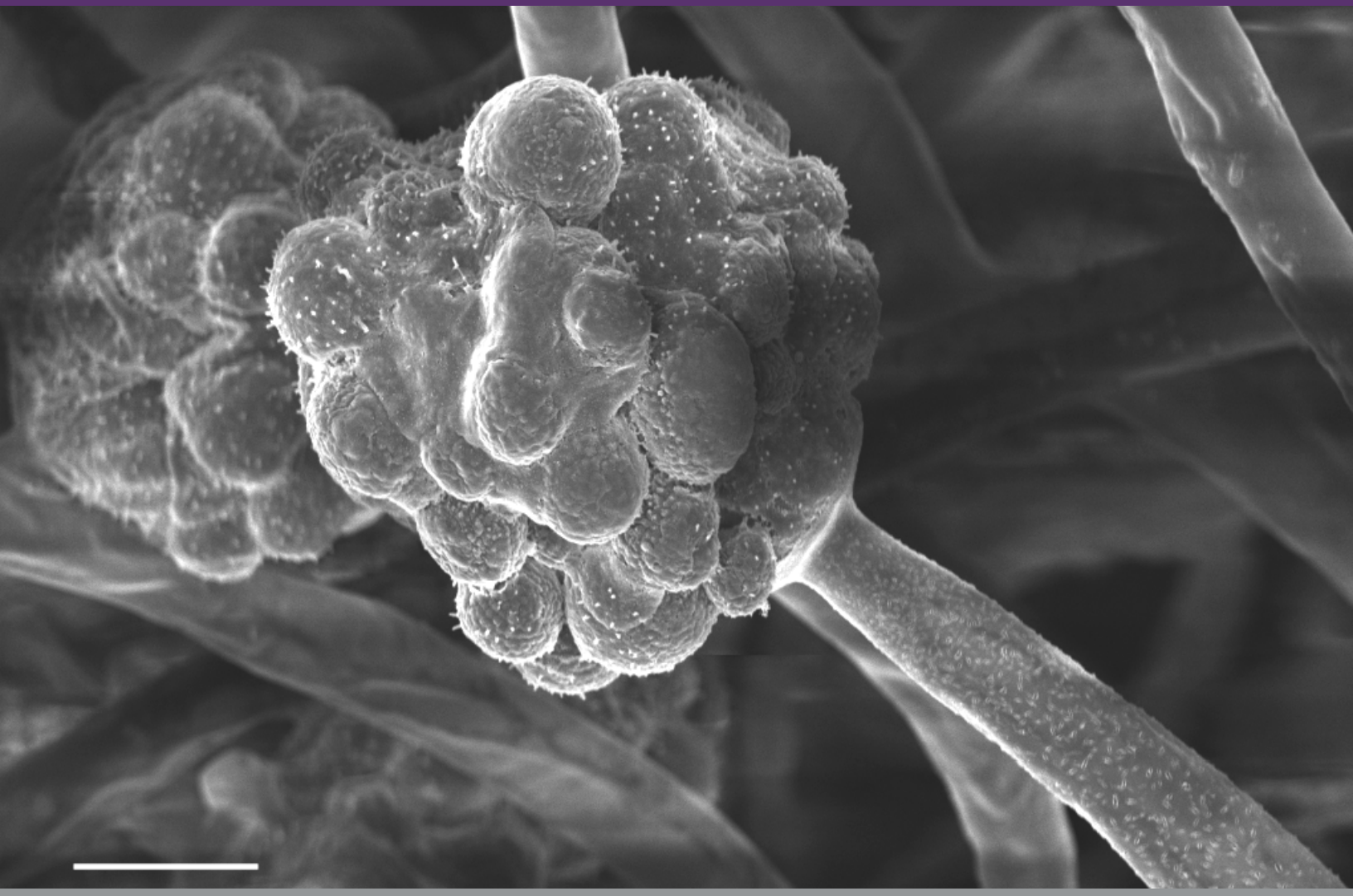
Mycology Proficiency Testing Program



Test Event Critique
January 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 below:

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

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/all participating laboratories' MIC values within +/- 2 dilutions and then the interpretation per CLSI guidelines or related, peer-reviewed publications. Especially, when there is no interpretation, MIC values are the key judge points. 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

Mold Master List

The mold master list is intended to provide guidance to the participating laboratories about the scope of the Mycology (Comprehensive) Proficiency Testing Program. The list includes most common pathogenic and non-pathogenic fungi likely to be encountered in the clinical laboratory. The list is compiled from published peer-reviewed reports as well as current practices in other proficiency testing programs. This list is meant to illustrate acceptable identifications used in grading of responses received after each test event. This list neither includes all molds 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. *Phaeoannellomyces werneckii* (*Hortea werneckii*). These guidelines supersede any previous instructions for identification of molds. The list is subject to change in response to significant changes in nomenclature, human disease incidence or other factors.

It is expected that major pathogenic fungi listed in the Master List will be completely identified to genus and species levels while those fungi either not listed (*Aspergillus lentulus*) or listed with genus name only (*Acremonium*) will be identified as *Aspergillus* species or *Acremonium* 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 "group" or "species complex" where appropriate e.g. *Aspergillus glaucus* group or *Fusarium solani* species complex if it is consistent with current reporting format used by the laboratory.

Absidia corymbifera
Absidia species
Acremonium species
Alternaria species
Arthrographis species
Aspergillus clavatus
Aspergillus flavus
Aspergillus fumigatus species complex
Aspergillus glaucus group
Aspergillus nidulans
Aspergillus niger
Aspergillus species
Aspergillus terreus
Aspergillus versicolor
Aureobasidium pullulans
Aureobasidium species
Basidiobolus ranarum
Beauveria species
Bipolaris species
Blastomyces dermatitidis
Chaetomium globosum
Chaetomium species
Chrysosporium species
Cladophialophora bantiana
Cladophialophora boppii
Cladophialophora carrionii species complex
Cladophialophora species
Cladosporium species
Coccidioides immitis
Coccidioides species
Cokeromyces recurvatus
Conidiobolus coronatus
Cunninghamella bertholletiae
Cunninghamella species
Curvularia species
Drechslera species
Emmonsia parva
Epicoccum species
Epidermophyton floccosum
Exophiala (*Wangiella*) *dermatitidis*
Exophiala jeanselmei species complex
Exophiala species
Exserohilum species
Fonsecaea species
Fusarium oxysporum species complex
Fusarium solani species complex
Fusarium species
Gliocladium species
Helminthosporium species
Histoplasma capsulatum
Hormonema dematioides
Malbranchea species
Microsporum audouinii
Microsporum canis
Microsporum cookei
Microsporum gypseum species complex
Microsporum nanum
Microsporum persicolor
Microsporum species
Mucor circinelloides
Mucor plumbeus
Mucor racemosus
Mucor species
Nigrospora species
Paecilomyces lilacinus
Paecilomyces species
Paecilomyces variotii
Penicillium marneffei
Penicillium species
Phaeoannellomyces werneckii (*Hortaea werneckii*)
Phialophora richardsiae
Phialophora species
Phialophora verrucosa species complex
Phoma species
Pithomyces species
Pseudallescheria boydii species complex
Pseudallescheria species
Rhizomucor pusillus
Rhizomucor species
Rhizopus oryzae
Rhizopus species
Scedosporium apiospermum (*Pseudallescheria apiospermum*)
Scedosporium prolificans (*inflatum*)
Scedosporium species
Scopulariopsis brevicaulis
Scopulariopsis brumptii
Scopulariopsis species
Scytalidium hyalinum
Scytalidium species
Sepedonium species
Sporothrix schenckii species complex
Sporothrix species
Stachybotrys atra (*chartarum / alternans*)
Stachybotrys species
Syncephalastrum racemosum
Syncephalastrum species
Trichoderma species
Trichophyton ajelloi
Trichophyton interdigitale
Trichophyton mentagrophytes species complex
Trichophyton rubrum
Trichophyton schoenleinii
Trichophyton species
Trichophyton terrestre
Trichophyton tonsurans
Trichophyton verrucosum
Trichophyton violaceum
Trichothecium species
Ulocladium species
Ustilago species
Verticillium species

Yeast Master List

The yeast master list is intended to provide guidance to the participating laboratories about the scope of the Mycology - Restricted to Yeasts Only Proficiency Testing Program. This list includes most common pathogenic and non-pathogenic yeasts likely to be encountered in the clinical laboratory. The list is compiled from published peer-reviewed reports as well as current practices in other proficiency testing programs. The list is meant to illustrate acceptable identifications used in grading of responses received after each test event. 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 – Mold

	Specimen key	Validated specimen	Other acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
M-1	*Mucor* species	*Mucor* species	*Mucor circinelloides*	55/57 (96%)
M-2	*Trichoderma* species	*Trichoderma* species	*Trichoderma asperellum*	55/57 (96%)
M-3	*Aspergillus glaucus* group	*Aspergillus glaucus* group	*Aspergillus glaucus*	56/56 (100%)
M-4	*Chaetomium* species	(Not validated)	*Chaetomium globosum*	16/57 (28%)
M-5	*Epidermophyton floccosum*	(Not validated)		40/57 (70%)

Mycology – Yeast Only

	Specimen key	Validated specimen	Other acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
Y-1	*Candida krusei*	*Candida krusei*		52/52 (100%)
Y-2	*Cryptococcus uniguttulatus*	*Cryptococcus uniguttulatus*		50/51 (98%)
Y-3	*Candida glabrata*	*Candida glabrata*		54/54 (100%)
Y-4	*Candida lipolytica*	*Candida lipolytica*		51/51 (100%)
Y-5	*Cryptococcus neoformans*		*Cryptococcus neoformans* - *Cryptococcus gattii* species complex	49/49 (100%)

Mycology – Direct detection (*Cryptococcus* Antigen Test)

	Specimen key (Titer)	Validated specimen	Correct responses / Total laboratories (% correct responses)	
			Qualitative	Quantitative
Cn-Ag-1	Negative	Negative	64/65 (98%)	NA
Cn-Ag-2	Negative	Negative	65/65 (100%)	NA
Cn-Ag-3	Negative	Negative	64/65 (98%)	NA
Cn-Ag-4	Negative	Negative	65/65 (100%)	NA
Cn-Ag-5	Negative	Negative	65/65 (100%)	NA

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

Drugs	Acceptable MIC (μg/ml) range	Interpretation	Laboratories with acceptable responses/ Total laboratories (% correct responses)
Amphotericin B	0. 5 – 1.0	Susceptible / No interpretation	21/21 (100%)
Anidulafungin	0.03 – 0.125	Susceptible	18/18 (100%)
Caspofungin	0.03 – 0.25	Susceptible	22/22 (100%)
Flucytosine (5-FC)	<0.06 – 0.25	Susceptible / No interpretation	22/22 (100%)
Fluconazole	<1 – 2	Susceptible	32/32 (100%)
Itraconazole	<0.06 – 0.5	Resistant / No interpretation	26/26 (100%)
Ketoconazole	0.125 – 2.0	No interpretation	4/4 (100%)
Micafungin	0.015 – 0.03	Susceptible	18/18 (100%)
Posaconazole	0.06 – 0.25	No interpretation	17/17 (100%)
Voriconazole	0.06 – 0.25	Susceptible / Susceptible-Dose Dependent	28/28 (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	1
API 20C AUX	21
Dade Behring MicroScan Rapid Yeast Identification Panel	3
MALDI-TOF	2
Molecular Sequencing	2
Remel RapID Yeast Plus System	4
Vitek2	28
Antifungal Susceptibility*	
Disk diffusion	1
Etest	1
Vitek II	2
YeastOne– Mold	2
YeastOne –Yeast	23
CLSI Microbroth dilution method – Yeast	5
CLSI Microbroth dilution method – Mold	2
Cryptococcal antigen*	
Immuno-Mycologics Latex Cryptococcus Antigen Detection System	6
Immuno-Mycologics CrAg Lateral Flow Assay	12
Meridien BioScience Cryptococcal Antigen Latex Agglutination System (CALAS)	37
Immuno-Mycologics ALPHA Cryptococcal Antigen enzyme immunoassay(CrAg EIA)	1
Remel Cryptococcal Antigen Latex Test	9

*Include multiple systems used by some laboratories

MOLD DESCRIPTIONS

M-1 *Mucor* species

Source: Sinus / Sputum / Nail

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

Colony: *Mucor* sp. grow rapidly on Sabouraud's dextrose agar. At 25°C, the colony is grayish on surface, very wooly, covering the entire Petri dish (Figure 1).

Microscopy: Lactophenol cotton blue mount shows hyaline hyphae, which are broad and predominantly aseptate. The long and straight sporangiophores arise irregularly from the hyphae - branched or unbranched. Sporangia with columellas lacks apophyses. Rhizoids and stolons are absent (Figure 1).

Differentiation: *Mucor* differs from *Rhizopus* and *Rhizomucor* by absence of rhizoids, and from *Absidia* by absence of an apophysis beneath the sporangium. *Mucor* spp. can be differentiated from *Rhizomucor* by their preference for growth at certain temperatures. In general *Mucor* spp. grow below 37°C except for few but *Rhizomucor* spp. can grow all the way up to 54°C.

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

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

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

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	55
Laboratories with incorrect ID:	02
(*Cunninghamella* spp.)	(1)
(*Rizomucor* spp.)	(1)

Illustrations:

Figure 1. Five-day-old, grayish and very wooly colony of *Mucor* sp. on Sabouraud's dextrose agar, 25°C; the reverse of the colony appears pale yellow (Upper panel). Microscopic morphology of *Mucor* sp. showing hyaline aseptate hyphae. Sporangia with columella that lack apophyses (bar = 10 μm; lower panel)

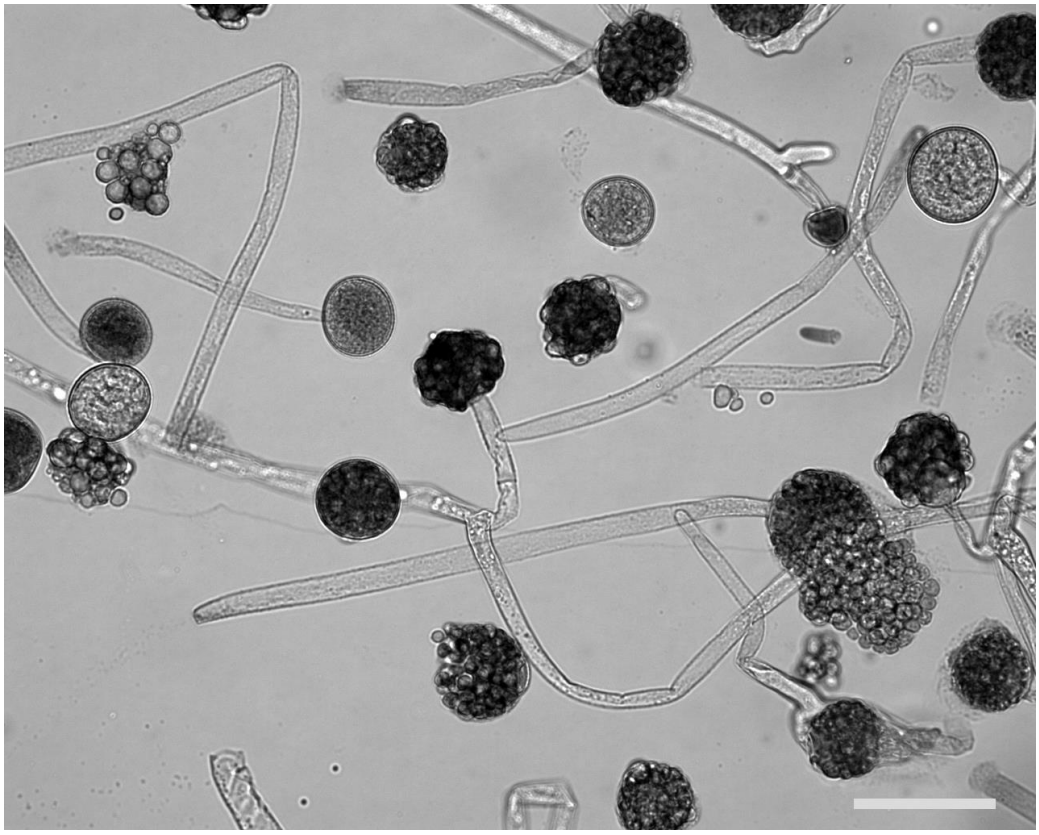
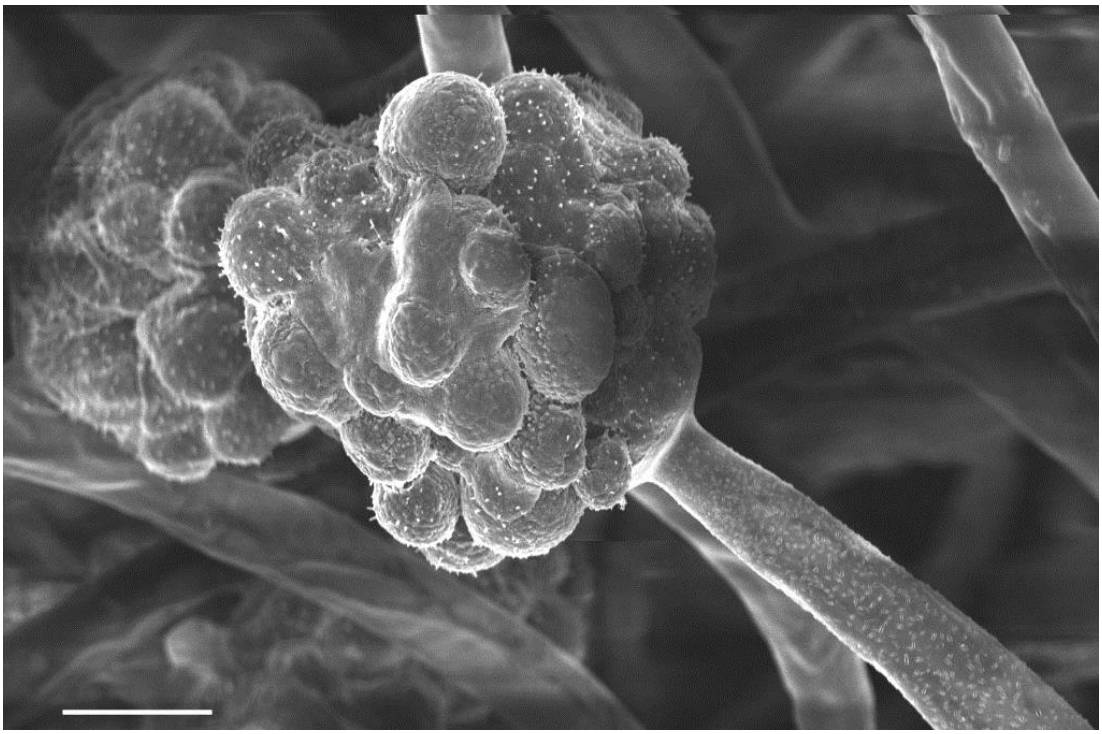


Figure 1A. Scanning electron micrograph of *Mucor* sp. highlighting characteristic sporangium and hypha (bar = 10 μm).



Further reading:

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

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

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

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

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

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

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

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

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

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

M-2 *Trichoderma* species

Source: Wound / Nail / Sputum

Clinical significance: *Trichoderma* spp. are distributed worldwide which rarely infect humans but can cause from localized infections to fatal disseminated disease particularly in patients with debilitating underlying conditions.

Colony: *Trichoderma* colony is fast growing, white turning into wooly texture with green tufts on Sabouraud's dextrose agar at 25°C. The reverse is pale to yellowish (Figure 2).

Microscopy: Lactophenol cotton blue mount shows branched conidiophores with pyramidal arrangement (Figure 2). Phialides are mostly single and flask-shaped bearing globose, semiglobose, or ellipsoidal, greenish conidia on the tip.

Differentiation: *Trichoderma* spp. have characteristic macroscopic and microscopic morphology for easy differentiation from other molds. The phialides are flask shaped as compared to *Gliocladium* spp., which has brush-like phialides similar to *Penicillium* spp.

Molecular test: A PCR diagnostic test targeting the ribosomal DNA internal transcribed spacer (ITS) regions of *Trichoderma* spp., has been described. The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Trichoderma asperellum* strain LT85 (Genebank accession no. HQ392486.1).

Antifungal susceptibility: Amphotericin MICs for *Trichoderma* spp. is variable (0.06 - 2.0 μg/ml), voriconazole MIC is in the susceptible range while the fungus is resistant to fluconazole and 5-fluorocytosine.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	54
Laboratories with incorrect ID:	02
(*Phialophora* sp.)	(1)
(*Trichothecium* sp.)	(1)

Illustrations:

Figure 2. Seven-day-old, colony of *Trichoderma* sp. with wooly texture and green tufts, Sabouraud's dextrose agar, 25°C; the reverse is pale to yellowish (Upper panels). Microscopic morphology of *Trichoderma* sp. showing branched conidiophore with pyramidal arrangement (400× magnification). Phialides are mostly single and flask-shaped bearing globose, semiglobose, or ellipsoidal, greenish conidia on the tip (Lower panel).

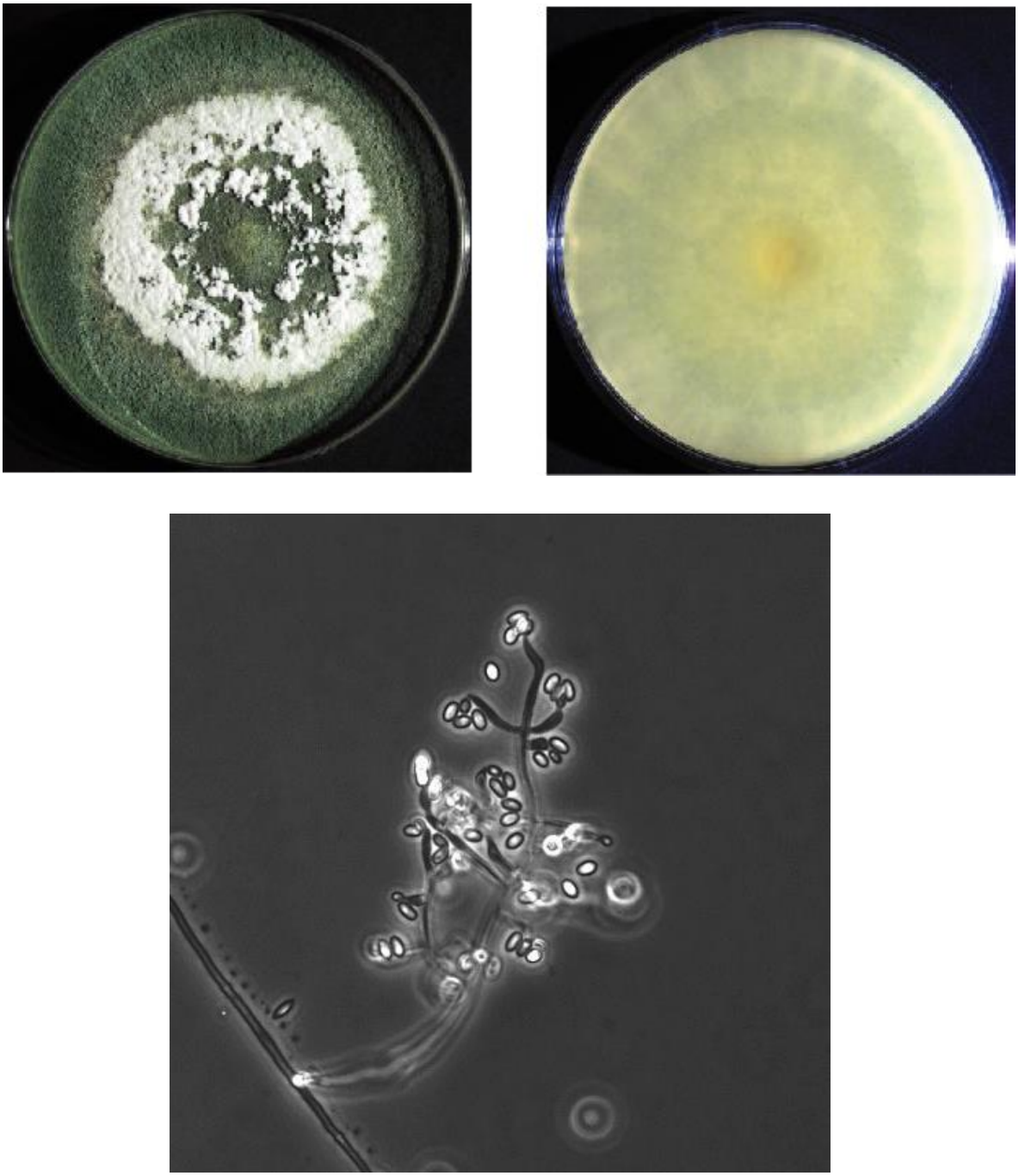
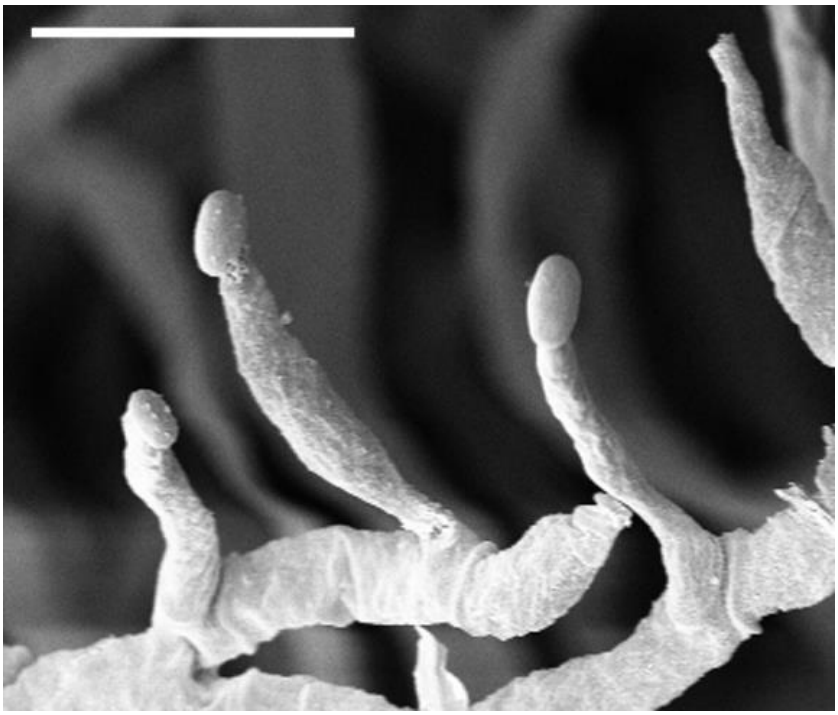
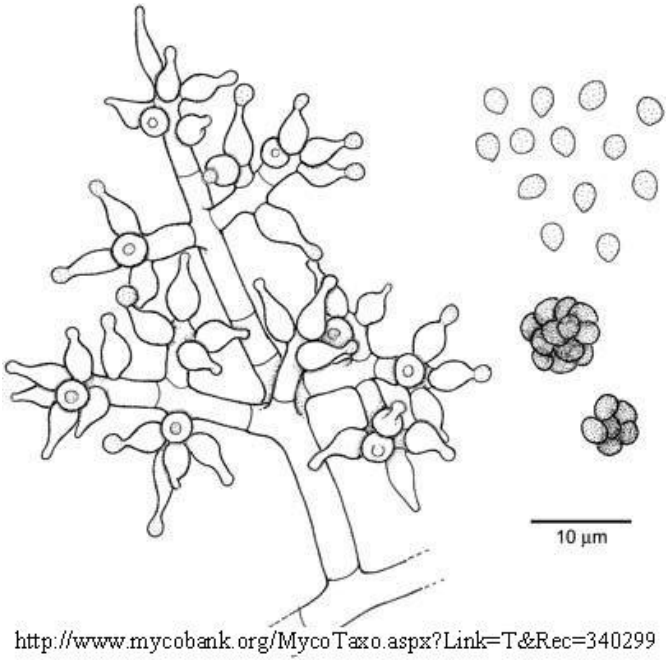


Figure 2A. Scanning electron micrograph of conidia and conidiophores of *Trichoderma* sp. on Sabouraud's dextrose agar (Bar = 10 μm; upper panel). Line drawings of phialides and conidia (lower panel).





Further reading:

Espinel-Ingroff A. 2001. *In vitro* fungicidal activities of voriconazole, itraconazole, and amphotericin B against opportunistic moniliaceous and dematiaceous fungi. *J Clin Microbiol*. 39: 954-958.

Chouaki T, Lavarde V, Lachaud L, Raccurt CP, Hennequin, C. 2002. Invasive infections due to *Trichoderma* species: report of 2 cases, findings of *in vitro* susceptibility testing, and review of the literature. *Clin Infec Dis*. 35: 1360-1367.

Kredics L, Antal Z, Doczi I, Manczinger L, Kevei F, Nagy, E. 2003. Clinical importance of the genus *Trichoderma*. A review. *Acta Microbiol Immunol Hung.* 50(2-3): 105-117.

De Miguel D, Gomez P, Gonzalez R, Garcia-Suarez J, Cuadros JA, Banas MH, Romanyk J, Burgaleta C. 2005. Nonfatal pulmonary *Trichoderma viride* infection in an adult patient with acute myeloid leukemia: report of one case and review of the literature. *Diagn Microbiol Infect Dis.* 53: 33-37.

Kratzer C, Tobudic S, Schmoll M, Graninger W, Georgopoulos A. 2006. *In vitro* activity and synergism of amphotericin B, azoles and cationic antimicrobials against the emerging pathogen *Trichoderma* spp. *J Antimicrob Chemother.* 58: 1058-1061.

Alanio A, Brethon B, Feuilhade de Chauvin M, de Kerviler E, Leblanc T, Lacroix C, Baruchel A, Menotti J. 2008. Invasive pulmonary infection due to *Trichoderma longibrachiatum* mimicking invasive aspergillosis in a neutropenic patient successfully treated with voriconazole combined with caspofungin. *Clin Infect Dis.* 46: e116-118.

Kantarcioğlu AS, Celkan T, Yücel A, Mikami Y, Kurugoglu S, Mitani H, Altas K. 2009. Fatal *Trichoderma harzianum* infection in a leukemic pediatric patient. *Med Mycol.* 47: 207-215.

Santillan Salas CF, Joshi AY, Dhiman N, Banerjee R, Huskins WC, Wengenack NL, Henry NK. 2011. Fatal post-operative *Trichoderma longibrachiatum* mediastinitis and peritonitis in a paediatric patient with complex congenital cardiac disease on peritoneal dialysis. *J Med Microbiol.* 60: 1869-1871.

M-3 *Aspergillus glaucus* group

Source: Scalp / Bronchial wash / Lung

Clinical significance: *Aspergillus glaucus* has been reported from cases of onychomycosis, otitis, and orofacial, cerebral, cardiovascular, and visceral infections. It is an infrequent agent of nail infections, subcutaneous and systemic infections in various organ systems.

Colony: *Aspergillus glaucus* grows moderately rapid on Sabouraud's dextrose agar at 25°C. Colony is green to yellow, downy to powdery on the surface and yellow to brown in color on the reverse (Figure 3).

Microscopy: Lactophenol cotton blue mount shows radiate conidial head with uniseriate phialides, and round to oval conidia in chain. Numerous large cleistothecia are easily oberseved (Figure 3).

Differentiation: *Aspergillus glaucus* is distinguished from other *Aspergillus* species by the green and yellow coloration of the colony with numerous cleistothecia.

Molecular test: PCR-RFLP and sequencing of the ITS regions of rDNA were reported to be used for molecular identification of *Aspergillus glaucus*.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Aspergillus chevalieri* strain KAUh4 (Genebank accession number: LN813026.1).

Antifungal susceptibility: Usually susceptible to itraconazole and terbinafine

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	57
Laboratories with incorrect ID:	0

Illustrations:

Figure 3. *Aspergillus glaucus* colony is yellow-green on Sabouraud's dextrose agar (upper panel). Microscopic morphology of *A. glaucus* showing a radiate conidial head with uniseriate phialides. Round to ellipsoidal conidia form in chain. Large cleistothecia present (lower panel).

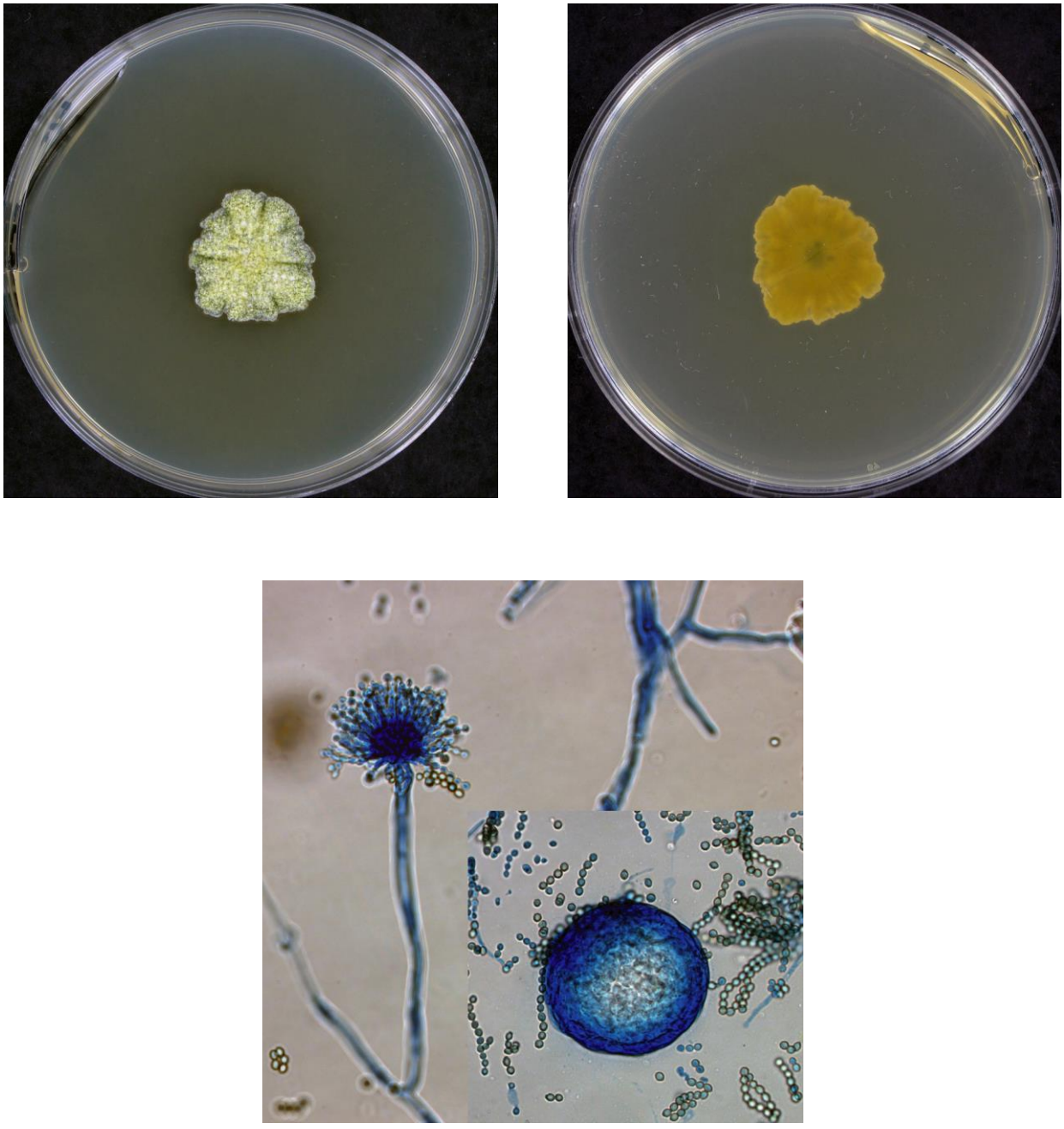
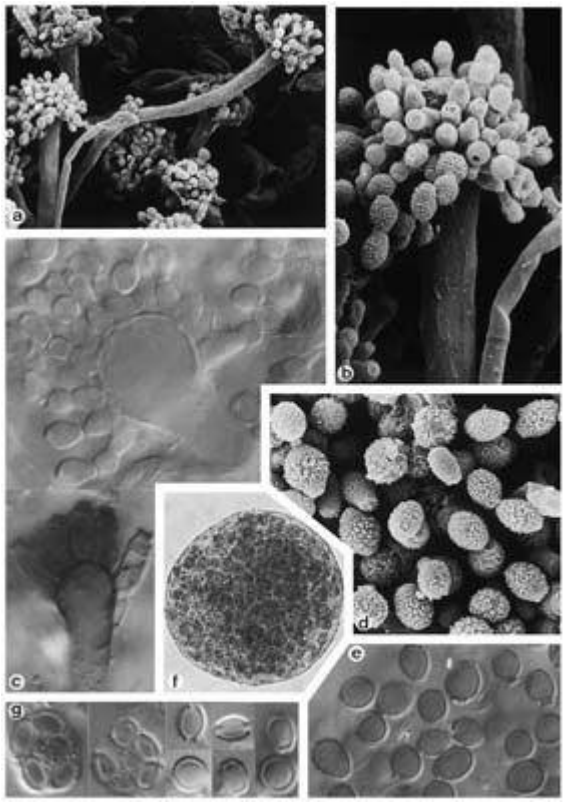


Figure 3A. Scanning electron micrograph of *A. glaucus* (upper panel), line drawing (lower panel).



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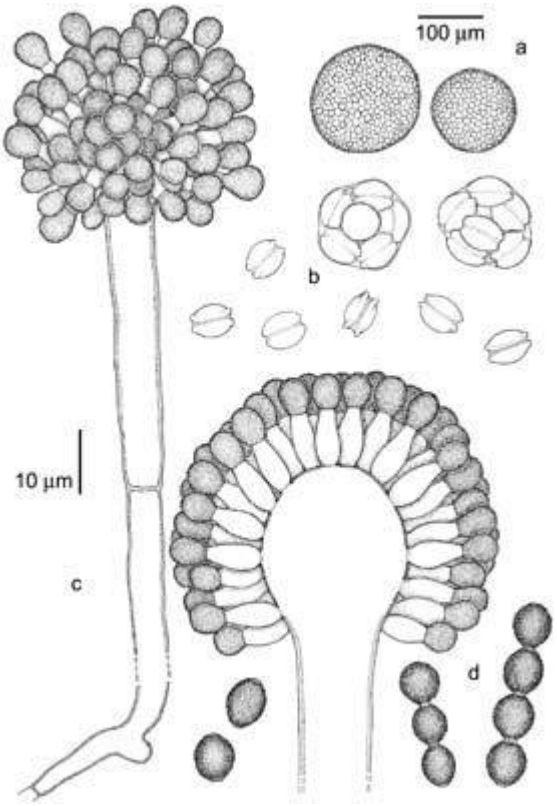

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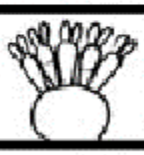
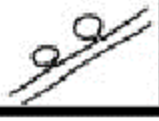
Table 1. Scheme for differentiation of *Aspergilli* most commonly involved in human diseases.

	A. flavus	*A. fumigatus*	*A. nidulans*	*A. niger*	*A. terreus*	*A. versicolor*
Colony	Yellow-green	Blue-green	Dark-green	Black	Tan - buff	Pale - green
Conidiophores						
Vesicle						
Sterigmata						
Conidia						
Other Structures						

Further reading:

Araujo R, Pina-Vaz C, Rodrigues AG. 2007. Susceptibility of environmental versus clinical strains of pathogenic *Aspergillus*. *Int J Antimicrob Agents.* 29:108-111.

Curtis L, Cali S, Conroy L, Baker K, Ou CH, Hershow R, Norlock-Cruz F, Scheff P. 2005. *Aspergillus* surveillance project at a large tertiary-care hospital. *J Hosp Infect*. 59: 188-196.

Hansen D, Healy M, Reece K, Smith C, Woods GL. 2008. Repetitive-sequence-based PCR using the DiversiLab system for identification of *Aspergillus* species. *J Clin Microbiol.* 46: 1835-1839.

Sridhar H, Jayshree RS, Bapsy PP, Appaji L, Navin Kumar M, Shafiulla M, VijayKumar BR. 2002. Invasive aspergillosis in cancer. *Mycoses.* 45: 358-363.

Traboulsi RS, Kattar MM, Dbouni O, Araj GF, Kanj SS. 2007. Fatal brain infection caused by *Aspergillus glaucus* in an immunocompetent patient identified by sequencing of the ribosomal 18S-28S internal transcribed spacer. *Eur J Clin Microbiol Infect Dis*. 26: 747-750.

Willinger B, Obradovic A, Selitsch B, Beck-Mannagetta J, Buzina W, Braun H, Apfalter P, Hirschl AM, Makristathis A, Rotter M. 2003. Detection and identification of fungi from fungus balls of the maxillary sinus by molecular techniques. *J Clin Microbiol.* 41: 581-585.

M-4 *Chaetomium* species

Source: Nail / Toe

Clinical significance: *Chaetomium* spp. is commonly encountered in clinical laboratories as a contaminant. It is occasionally reported as an agent of phaeohyphomycosis.

Colony: *Chaetomium* sp. grows rapidly on Sabouraud's dextrose agar at 25°C. The colony is white to gray, yellowish, wooly surface and pale yellow on reverse (Figure 4).

Microscopy: Lactophenol cotton blue mount shows round, oval, or flask-shaped perithecia (best seen on potato dextrose agar) with long, brown setae. Ascospores, oval to lemon shaped, emerge from the ostiole (opening) of the perithecium (Figure 4).

Differentiation: *Chaetomium* sp. is differentiated from other molds by its very typical perithecium, which is a large round or pear-shaped structure with a small rounded opening called ostiole (which differentiates it from cleistothecium) and containing asci and ascospores. The ascospores are unicellular and commonly lemon-shaped.

Molecular test: Oligonucleotide fingerprinting of rRNA genes (OFRG) was reported to identify *Alternaria, Ascobolus, Chaetomium, Cryptococcus,* and *Rhizoctonia* clades.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Chaetomium globosum* isolate wxm152 (Genebank accession number: HM061327).

Antifungal susceptibility: *Chaetomium* sp. is susceptible to amphotericin B, itraconazole, ketoconazole, ravuconazole, voriconazole, and albaconazole, but resistant to micafungin, fluconazole and flucytosine in general.

Participant performance:

Referee Laboratories with correct ID:	3
Laboratories with correct ID:	16
Laboratories with incorrect ID:	41
(*Acremonium* species)	(21)
(*Paecilomyces* species)	(5)
(*Scytalidium* species)	(5)

Illustrations:

Figure 4. White to gray, yellowish, wooly colony of *Chaetomium* sp. on Sabouraud's dextrose agar. (upper panels). Microscopic morphology of *Chaetomium* sp. showing round, oval, or flask-shaped perithecia, oval to lemon shaped ascospores (lower panel).

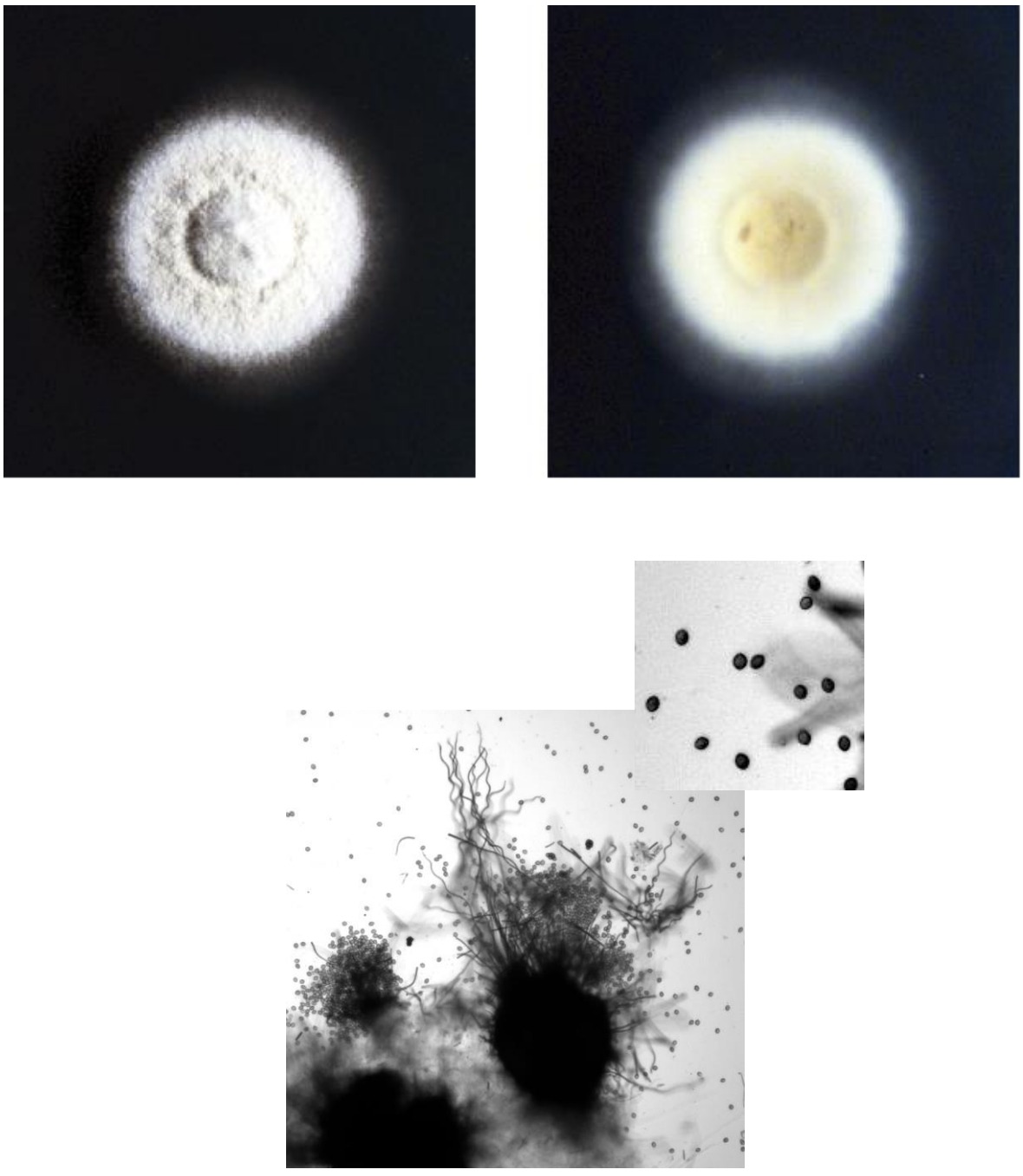
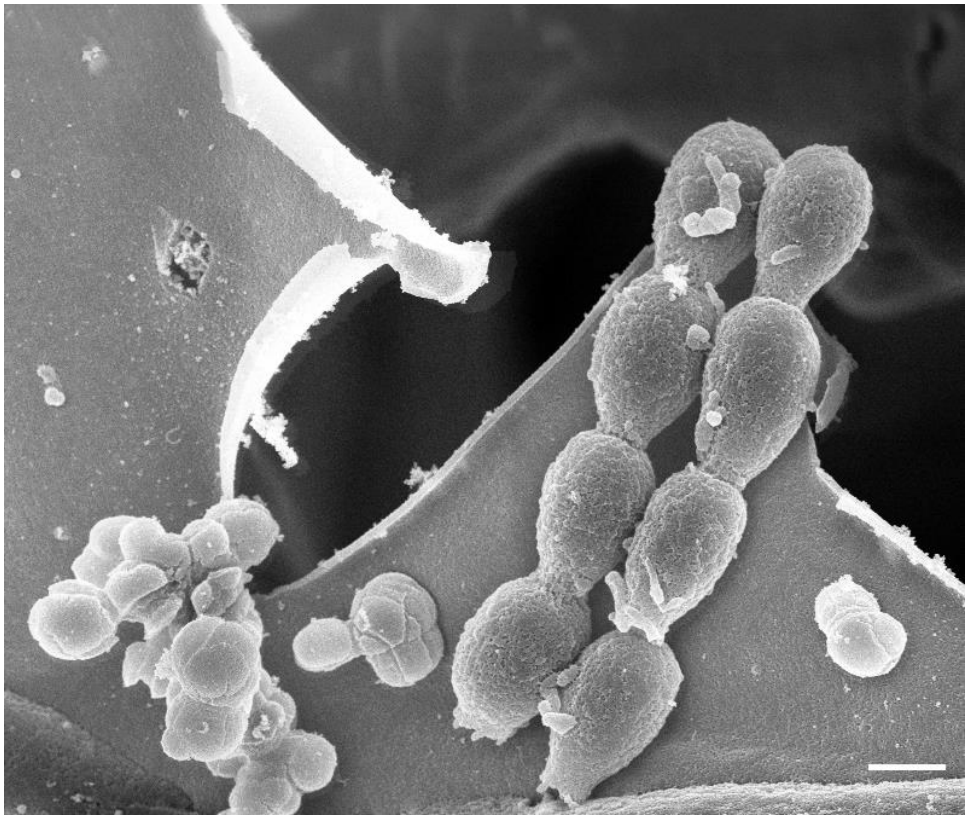
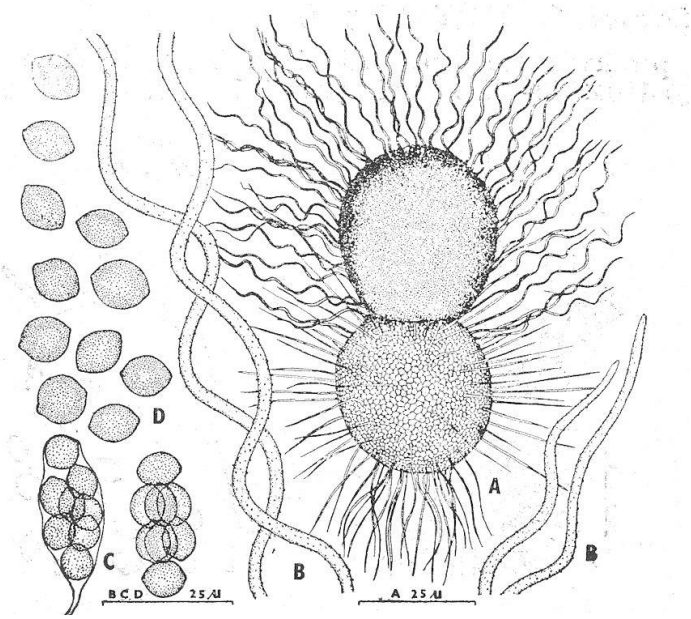


Figure 4A. Scanning electron micrograph of *Chaetomium* sp. (bar = 1 µm; upper panel). Line drawing depicting details of *Chaetomium* sp. (lower panel).





http://www.mycobank.org/TempFiles/20150402/gpal0hom5nvegulxu0grveft/TempF13276_Record__13276.jpg

Further reading:

Barron, M.A., Sutton, D.A., Veve, R., Guarro, J., Rinaldi, M., Thompson, E., Cagnoni, P.J., Moultney, K., and Madinger, N.E. 2003. Invasive mycotic infections caused by *Chaetomium perlucidum*, a new agent of cerebral phaeohyphomycosis. *J. Clin. Microbiol.* 41: 5302-5307.

Serena, C., Ortoneda, M., Capilla, J., Pastor, F.J., Sutton, D.A., Rinaldi, M.G., and Guarro, J. 2003. *In vitro* activities of new antifungal agents against *Chaetomium* spp. and inoculum standardization. *Antimicrob. Agents. Chemother.* 47: 3161-3164.

Teixeira, A.B., Trabasso, P., Moretti-Branchini, M.L., Aoki, F.H., Vigorito, A.C., Miyaji, M., Mikami, Y., Takada, M., and Schreiber, A.Z. 2003. Phaeohyphomycosis caused by *Chaetomium globosum* in an allogeneic bone marrow transplant recipient. *Mycopathologia*. 156: 309-312.

Tomsikova, A. 2002. Causative agents of nosocomial mycoses. *Folia Microbiol (Praha).* 47: 105-112.

Valinsky, L., Della Vedova, G., Jiang, T., and Borneman, J. 2002. Oligonucleotide fingerprinting of rRNA genes for analysis of fungal community composition. *Appl. Environ. Microbiol*. 68: 5999-6004.

M-5 *Epidermophyton floccosum*

Source: Foot / Hair / Skin

Clinical significance: *Epidermophyton floccosum* is a frequent causal agent of nail and skin infections in feet and groin. Unlike many other dermatophytes, *E. floccosum* does not infect hair. There is one case report on invasive disease in immunocompromised patient with Behcet's syndrome.

Colony: *Epidermophyton floccosum* is a slow-growing fungus. Colonies measure up to 3 cm in 2 weeks. At 25°C, on Sabouraud's dextrose agar, colonies are initially white to yellow, later becoming greenish-yellow, folded in center and radically grooved, reverse tan (Figure 5).

Microscopy: Lactophenol cotton blue mounts show septate hyphae; macroconidia single or in clusters, smooth, thin-walled, clubshaped, with 2-6 septations. No microconidia are seen. Chlamydoconidia are present in old cultures (Figure 5).

Differentiation: *Epidermophyton floccosum* is differentiated from other fungi by slow growth, absence of microconidia, and club-shaped macroconidia. The second species in this genus, *E. stockdaleae,* has been isolated from soil. It is differentiated from *E. floccosum* by its production of longer conidia with nine septations.

Molecular test: ITS1 sequences of clinical isolates are species-specific. Specific DNA bands in arbitrarily primed polymerase chain reaction (AP – PCR) also provides rapid identification of dermatophytes including *E. floccosum*.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *E. floccosum* ATCC 52066 (Genebank accession number: EF631604).

Antifungal susceptibility: Most clinical isolates are susceptible to terbinafine and variably to griseofulvin, itraconazole, ketoconazole, and clotrimazole.

Participant performance:

Referee Laboratories with correct ID:	8
Laboratories with correct ID:	40
Laboratories with incorrect ID:	17
(*Trichophyton* species)	(5)
(*Trichophyton* verrucosum)	(5)
(*Trichophyton tonsurans*)	(2)
(*Arthrographis* species)	(1)
(*Emmonsia parva*)	(1)
(*Microsporum* species)	(1)
(*Trichophyton interdigitale*)	(1)
(*Trichophyton mentagrophytes* species complex)	(1)

Illustrations:

Figure 5. White to pale yellow colony *E. floccosum* on Sabouraud's dextrose agar (upper panel). Microscopic morphology of *E. floccosum* showing hyphae and smooth, thin walled, club-shaped macroconidia (lower panel).

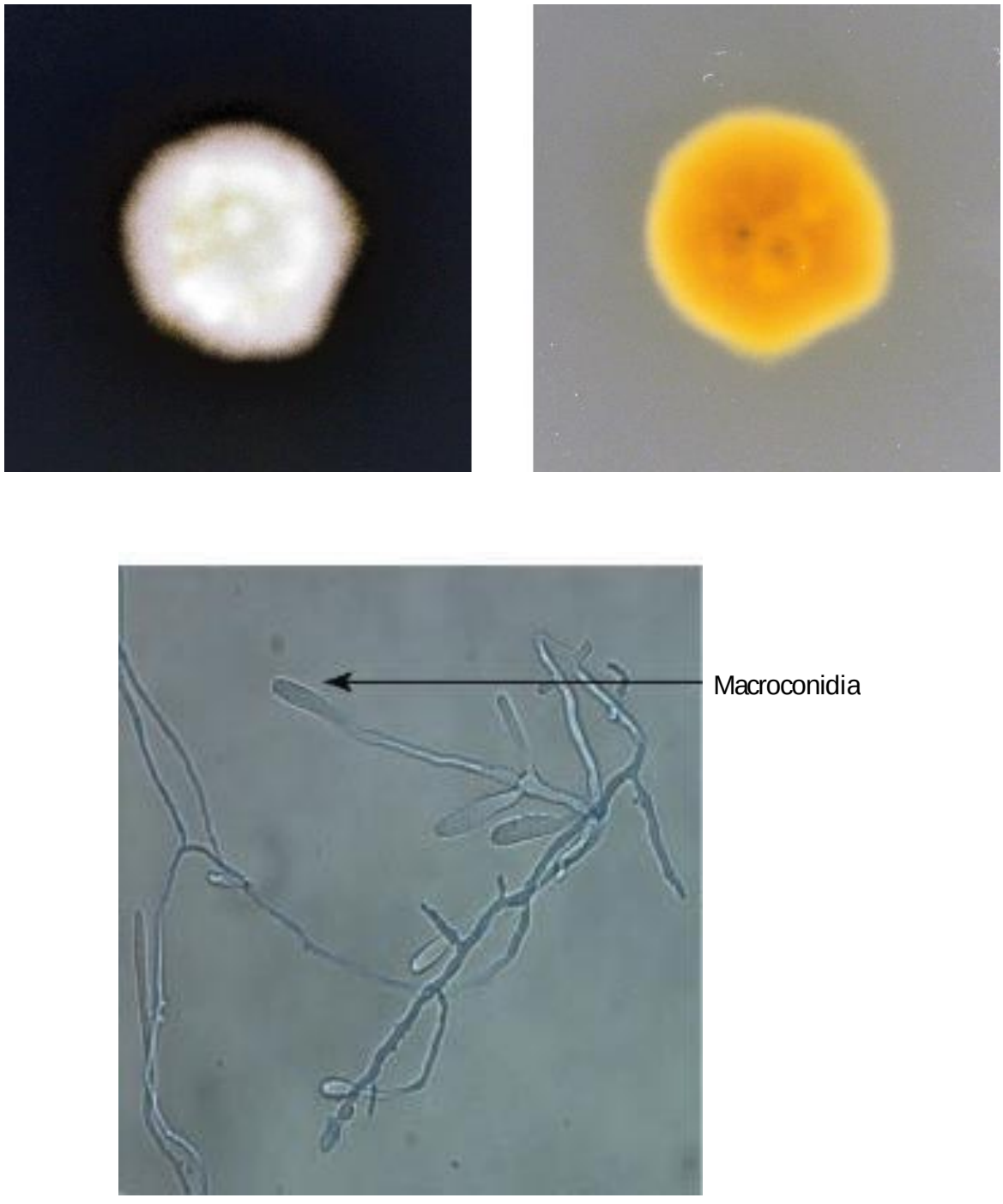
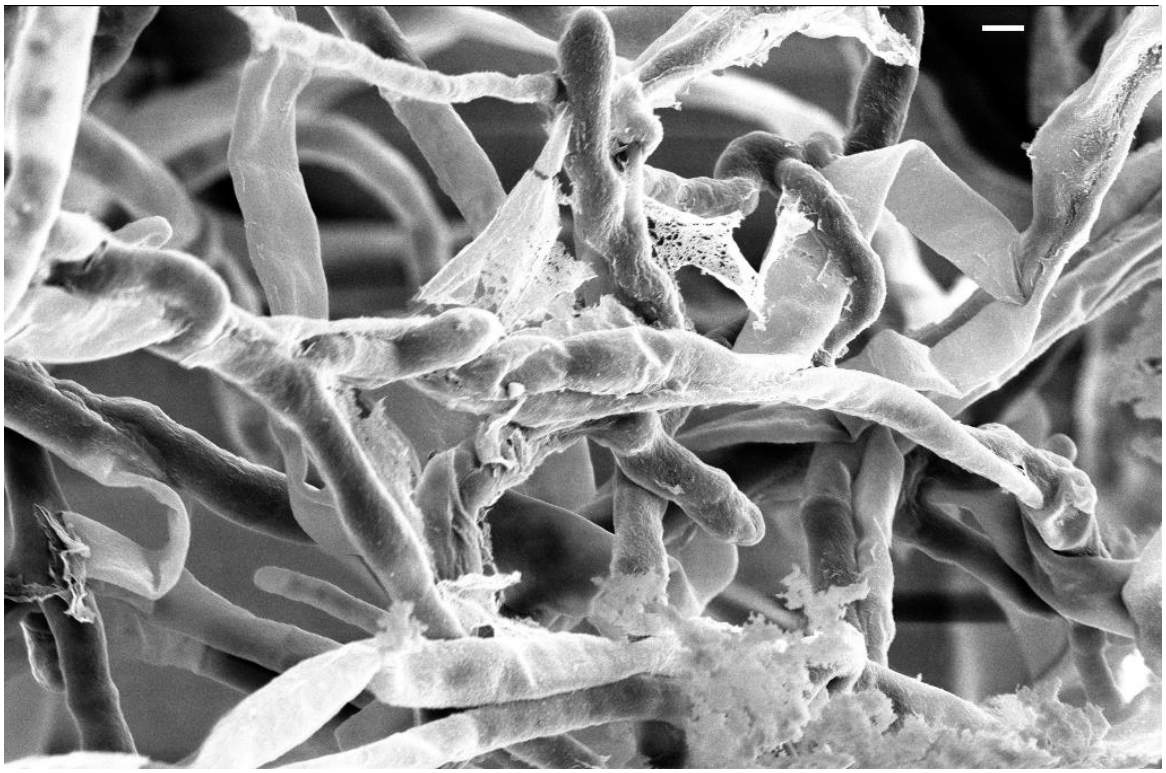
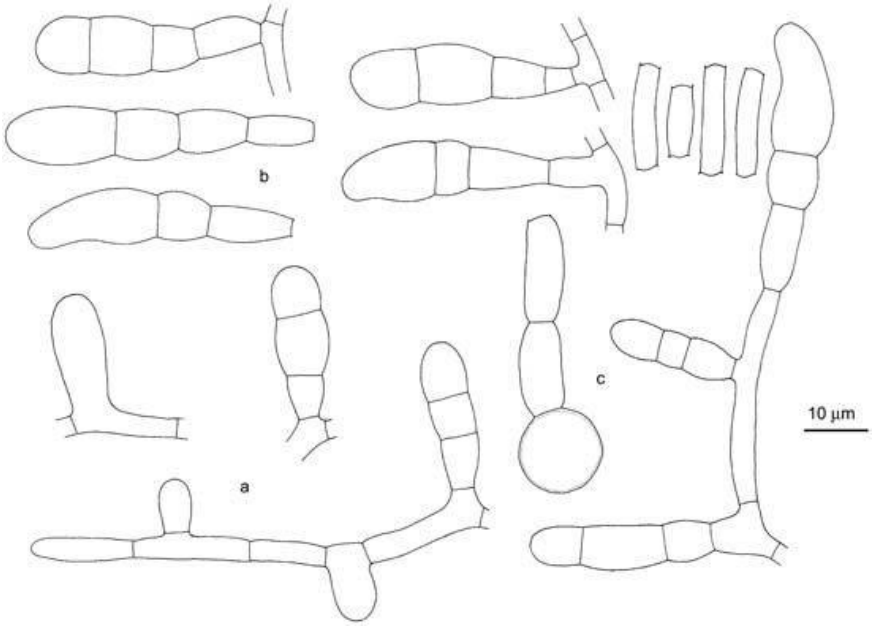


Figure 5A. Scanning electron micrograph of *E. floccosum* (bar = 2 µm, upper panel). Line drawing depicting details of *E. floccosum* (lower panel).





http://www.mycobank.org/TempFiles/20150402/gpal0hom5nvegulxu0grveft/TempF3791_Record__3791.jpg

Further reading:

Jessup, C. J., N. S. Ryder, and M. A. Ghannoum. 2000. An evaluation of the *in vitro* activity of terbinafine. *Med. Mycol*. 38: 155-159.

Kano, R., Y. Nakamura, S. Watanabe, H. Tsujimoto, and A. Hasegawa. 1999. Phylogenetic relation of *Epidermophyton floccosum* to the species of *Microsporum* and *Trichophyton* in chitin synthase 1 (CHS1) gene sequences. *Mycopathologia.* 146**:** 111-113.

Khosravi A, Behzad F, Sabokbar A, Shokri H, Haddadi S, Masoudi-Nejad A. 2010. Molecular typing of *Epidermophyton floccosum* isolated from patients with dermatophytosis by RAPD-PCR. *J Basic Microbiol*. 50 Suppl 1: S68-73.

Korting, H. C., and S. Rosenkranz. 1990. *In vitro* susceptibility of dermatophytes from Munich to griseofulvin, miconazole and ketoconazole. *Mycoses*. 33**:** 136-139.

Liu, D., S. Coloe, R. Baird, and J. Pedersen. 1997. Molecular determination of dermatophyte fungi using the arbitrarily primed polymerase chain reaction. *Br J Dermatol*. 137: 351-355.

Mochizuki, T., M. Kawasaki, H. Ishizaki, and K. Makimura. 1999. Identification of several clinical isolates of dermatophytes based on the nucleotide sequence of internal transcribed spacer 1 (ITS 1) in nuclear ribosomal DNA. *J Dermatol*. 26: 276-281.

Mochizuki, T., N. Sugie, and M. Uehara. 1997. Random amplification of polymorphic DNA is useful for the differentiation of several anthropophilic dermatophytes. *Mycoses*. 40: 405-409.

Pau M, Atzori L, Aste N, Tamponi R, Aste N. 2010. Epidemiology of Tinea pedis in Cagliari, Italy. *G Ital Dermatol Venereol*. 145: 1-5.

Seddon, M. E., and M. G. Thomas. 1997. Invasive disease due to *Epidermophyton floccosum* in an immunocompromised patient with Behcet's syndrome. *Clin Infect Dis*. 25: 153-154.

Weitzman, I., N. X. Chin, N. Kunjukunju, and P. Della-Latta. 1998. A survey of dermatophytes isolated from human patients in the United States from 1993 to 1995. *J Am Acad Dermatol.* 39(2 Pt 1): 255-261.

YEAST DESCRIPTIONS

Y-1 *Candida krusei*

Source: Urine / Stool / Sputum / Tissue

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: *Candida krusei* colony is soft, cream to buff, glassy and wrinkled on Sabouraud's dextrose agar, after7 days of incubation at 25°C (Figure 6).

Microscopy: *Candida krusei* shows branched pseudohyphae with elongated blastoconidia on corn meal agar with Tween 80 (Figure 6).

Differentiation: *Candida 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.

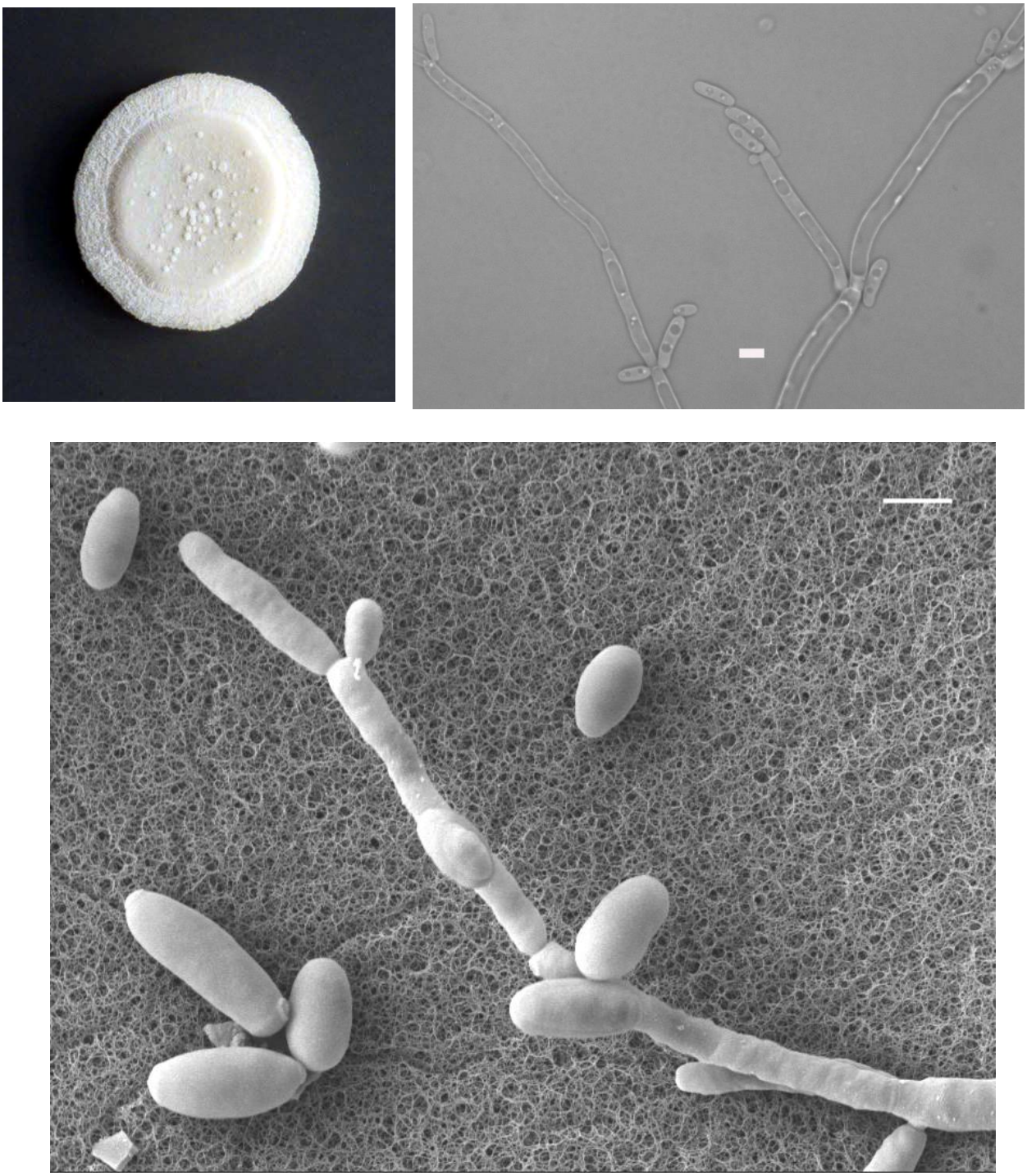
Antifungal susceptibility: *C. krusei* is susceptible to amphotericin B and flucytosine. *C. krusei* is innately resistant to fluconazole and variably resistant to other azoles such as itraconazole and ketoconazole, but not voriconazole. *C. krusei* is also susceptible to echinocandins.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	52
Laboratories with incorrect ID:	0

Illustrations:

Figure 6. *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).



Further reading:

Sili U, Yilmaz M, Ferhanoglu B, Mert A. 2007. *Candida krusei* arthritis in a patient with hematologic malignancy: successful treatment with voriconazole. *Clin Infect Dis.* 45: 897-898.

Jacobsen MD, Gow NA, Maiden MC, Shaw DJ, Odds FC. 2007. Strain typing and determination of population structure of *Candida krusei* by multilocus sequence typing. *J Clin Microbiol*. 45: 317-323.

Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Nagy E, Dobiasova S, Rinaldi M, Barton R, Veselov A; the Global Antifungal Surveillance Group. 2008. *Candida krusei*, a Multidrug-Resistant Opportunistic Fungal Pathogen: Geographic and Temporal Trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001-2005. *J Clin Microbiol.* 46: 515-521.

Natale F, Castronovo A, Regoli D, De Curtis M, Manzoni P. 2009. Successful treatment with caspofungin of refractory *Candida krusei* candidemia in a very low birth weight preterm infant. *Pediatr Infect Dis J*. 28: 452.

Cascio A, Barone M, Micali V, Iaria C, Delfino D, David A, Monaco M, Monaco F. 2010. On a fatal case of *Candida krusei* pleural empyema in a pregnant woman with spontaneous esophagus perforation. *Mycopathologia.* 169: 451-455.

Hager JL, Mir MR, Hsu S. 2010. *Candida krusei* fungemia in an immunocompromised patient. *Dermatol Online J.* 16: 5.

Schilling A, Seibold M, Mansmann V, Gleissner B. 2007. Successfully treated *Candida krusei* infection of the lumbar spine with combined caspofungin/posaconazole therapy. *Med Mycol.* 46: 79-83.

Shorr AF, Wu C, Kothari S. 2011. Outcomes with micafungin in patients with candidaemia or invasive candidiasis due to *Candida glabrata* and *Candida krusei*. *J Antimicrob Chemother*. 66: 375-380.

Y-2 *Cryptococcus uniguttulatus*

Source: Catheter / Urine / Skin

Clinical significance: *Cryptococcus uniguttulatus* is a rarely encountered yeast. Like other *Cryptococcus* species, it has been isolated from the gastrointestinal tract and droppings of birds. *Cryptococcus uniguttulatus* ventriculitis was documented in a case report in 2001.

Colony: *Cryptococcus uniguttulatus* colony is smooth, dull, cream colored on Sabouraud's dextrose agar, at 7 days post-incubation at 25°C (Figure 6).

Microscopy: *Cryptococcus uniguttulatus* produced round blastoconidia on corn meal agar with Tween-80 (Figure 6). No pseudo- or true hyphae is formed.

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

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

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

Antifungal susceptibility: Very limited information is available for this species. In general, it is susceptible to amphotericin B and itraconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	51
Laboratories with incorrect ID:	1
(*Sporobolomyces salmonicolor*)	(1)

Illustrations:

Figure 6. *Cryptococcus uniguttulatus*, smooth, creamy colored colony of on Sabouraud's dextrose agar, 7 days, 25°C. Microscopic morphology on corn meal agar showing round blastoconidia (bar = 10 μm).

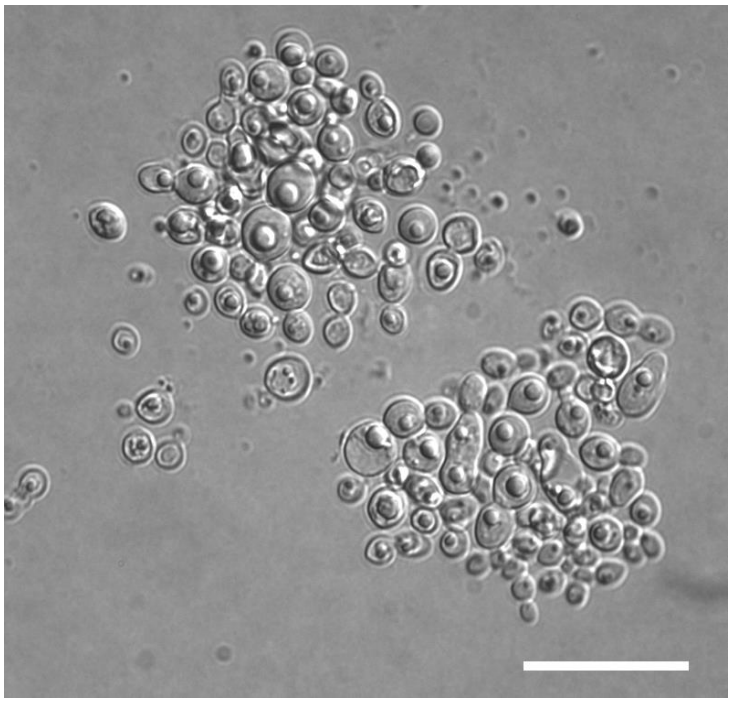
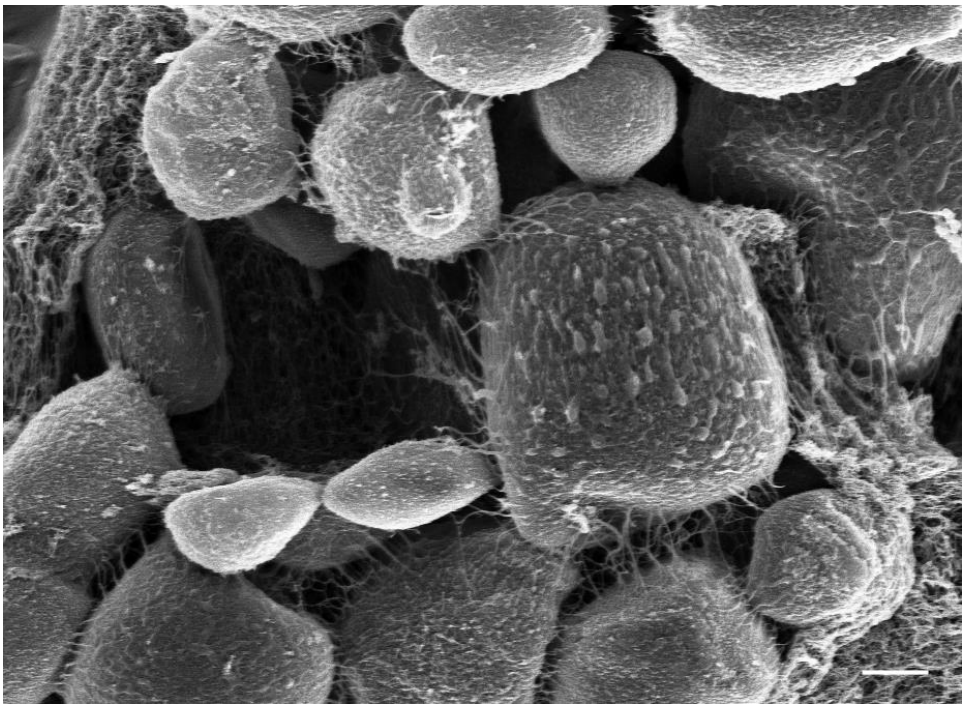


Figure 6A. Scanning electron micrograph illustrates blastoconidia (bar = 1 μm).



Further reading:

Arendrup MC, Boekhout T, Akova M, Meis JF, Cornely OA, Lortholary O; ESCMID EFISG study group and ECMM. 2014. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. *Clin Microbiol Infect*. Suppl 3:76-98.

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

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

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

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

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

Y-3 *Candida glabrata*

Source: Blood / Vaginal Swab / Urine

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

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

Differentiation: *Candida 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 *C. glabrata* CBS 138 (Genebank accession no: AY198398).

Antifungal susceptibility: *Candida 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:	54
Laboratories with incorrect ID:	0

Illustrations:

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

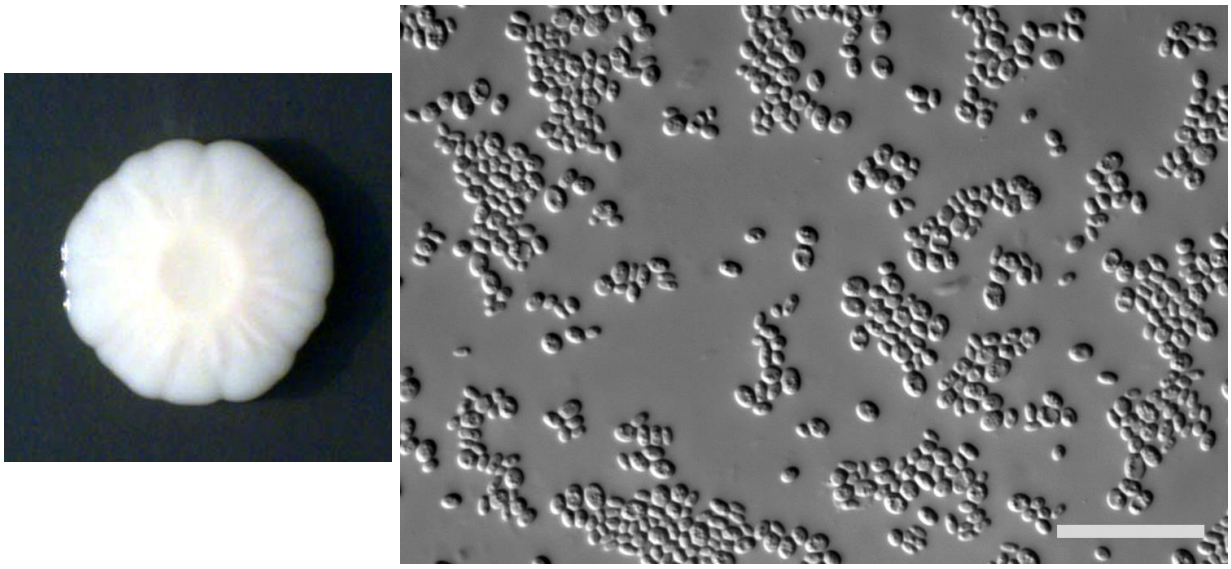
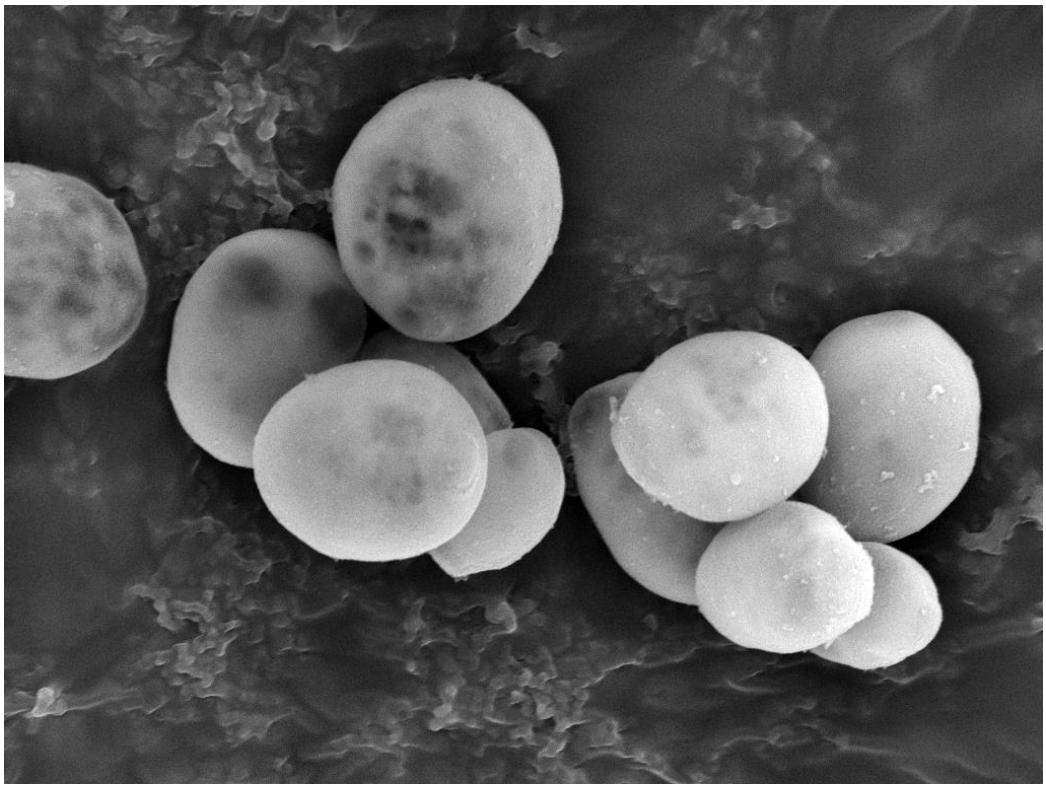


Figure 2A. Scanning electron micrograph with blastoconidia.



Further reading:

Becker K, Badehorn D, Keller B, Schulte M, Bohm KH, Peters G, FegelerW. 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.

Gherna 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, Zimerman 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-4 *Candida lipolytica*

Source: Wound / Tissue / Nail

Clinical significance: *Candida lipolytica* causes catheter-related fungemia and sinusitis in immunocompromised patients. It is also reported from traumatic ocular infections. It has been isolated as a colonizer from human vagina.

Colony: *Candida lypolytica* colony is white to cream, wrinkled on Sabouraud's dextrose agar at 25°C (Figure 9).

Microscopy: *Candida lypolytica* produces abundant, multibranched true hyphae and infrequent blastoconidia along the hyphae on Corn meal agar with Tween 80 (Figure 9). *Yarrowia lipolytica*, the teleomorph (sexual form) of *C. lypolytica*, can form ascospores on yeast malt agar in 3 to 7 days at 25°C.

Differentiation: *Candida lypolytica* grows on media containing cycloheximide, grows well at 25°C, is urease positive, and negative on nitrate reactions. Sugars are not fermented by *C. lypolytica*. No growth at 42°C and positive growth on media containing cycloheximide differentiates it from *C. krusei*. Positive urease reaction and growth on media containing cycloheximide differentiates it from *C. lambia*. *C. lypolytica* is differentiated from *Geotrichum* species by negative urease reaction by the later. On the API 20C AUX, a specific assimilation biocode differentiates this organism from the genus *Trichosporon*.

Molecular test: Comparisons of partial rRNA/rDNA sequences analysis demonstrated that *C. lypolytica* is distinctly related to selected members of the genus *Candida*. Randomly amplified polymorphic DNA (RAPD) PCR has been used for the identification of *C. lypolytica* isolates from dairy products.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Yarrowia lipolytica* (*Candida lipolytica*) strain ATCC 9773 (GenBank accession no. GQ458037.1).

Antifungal susceptibility: *Ccandida lypolytica* is less susceptible to amphotericin B, but more susceptible to caspofungin. Most isolates are susceptible to azoles like fluconazole and ketoconazole and 5FC, but resistant to itraconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	51
Laboratories with incorrect ID:	0

Illustrations:

Figure 9. *Candida lypolytica*, white to cream colony with wrinkled surface on Sabouraud's dextrose agar, 25°C. Microscopic morphology on corn meal agar showing bushy pseudohyphae (bar = 50 μm).

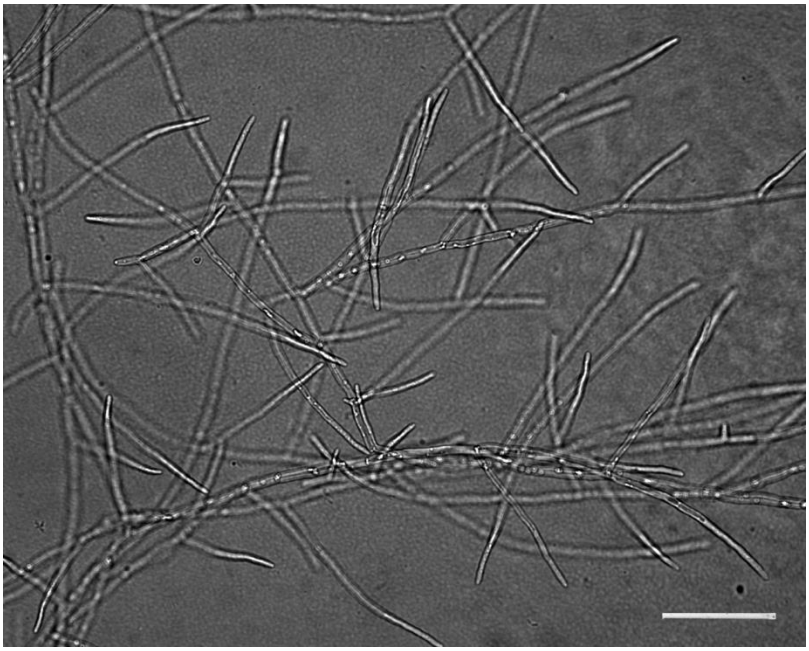
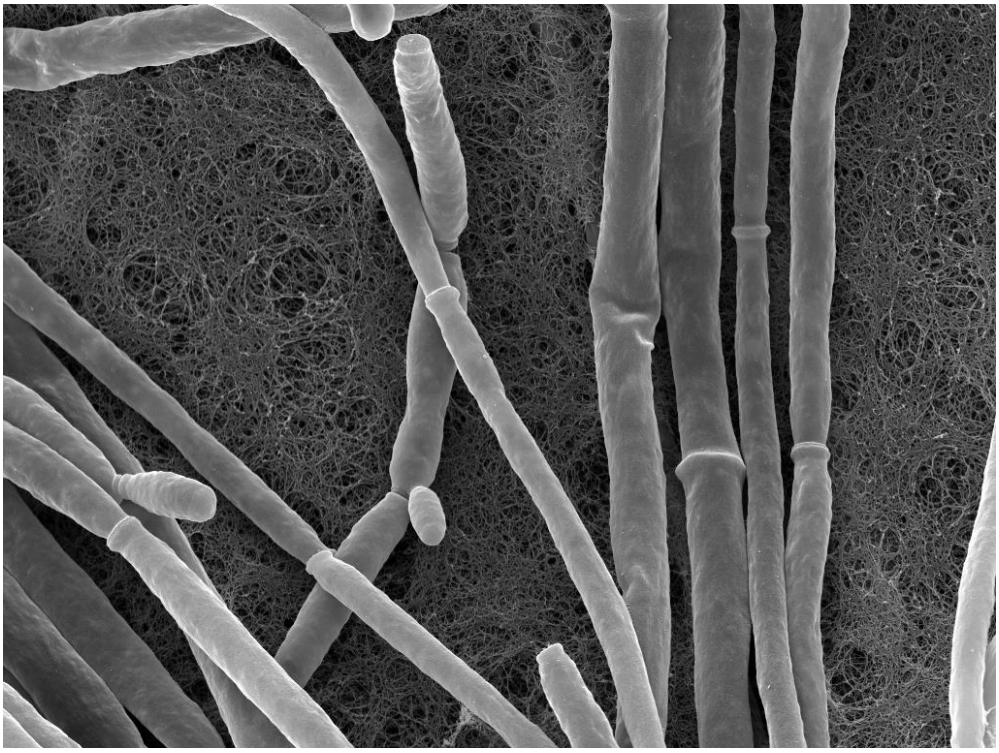


Figure 9A. Scanning electron micrograph illustrates pseudohyphae and blastoconidia.



Further reading:

Agarwal S, Thakur K, Kanga A, Singh G, Gupta P. 2008. Catheter-related candidemia caused by *Candida lipolytica* in a child with tubercular meningitis. *Indian J Pathol Microbiol*. 51: 298-300.

Alberth M, Majoros L, Kovalecz G, Borbas E, Szegedi I, J Marton I, Kiss C. 2006. Significance of oral *Candida* Andrighetto, C.E., Psomas, N., Tzanetakis, G., Suzzi, and Lombardi, A. 2000. Randomly amplified polymorphic DNA (RAPD) PCR for the identification of yeasts isolated from dairy products. *Lett Appl Microbiol.* 30: 5-9.

Barchiesi F, Tortorano AM, Di Francesco LF, Cogliati M, Scalise G, Viviani MA. 1999. In-vitro activity of five antifungal agents against uncommon clinical isolates of *Candida* spp. *J Antimicrob Chemother.* 43: 295-299.

Belet N, Ciftci E, Ince E, Dalgic N, Oncel S, Guriz H, Yagmurlu A, Dindar H, Dogru U. 2006. Caspofungin treatment in two infants with persistent fungaemia due to *Candida lipolytica. Scand J Infect Dis.* 38: 559-562.

D'Antonio D, Romano F, Pontieri E, Fioritoni G, Caracciolo C, Bianchini S, Olioso P, Staniscia T, Sferra R, Boccia S, Vetuschi A, Federico G, Gaudio E, Carruba G.2002. Catheter-related candidemia caused by *Candida lipolytica* in a patient receiving allogeneic bone marrow transplantation. *J Clin Microbiol*. 40: 1381-1386.

Lai CC, Lee MR, Hsiao CH, Tan CK, Lin SH, Liao CH, Huang YT, Hsueh PR. 2012. Infections caused by *Candida lipolytica*. *J Infect*. 65: 372-374.

Liu WC, Chan MC, Lin TY, Hsu CH, Chiu SK. 2013. *Candida lipolytica* candidemia as a rare infectious complication of acute pancreatitis: a case report and literature review. *J Microbiol Immunol Infect.* 46: 393-396.

López-Martínez R. 2010. Candidosis, a new challenge. *Clin Dermatol*. 28: 178-184.

Ozdemir H, Karbuz A, Ciftçi E, Dinçaslan HU, Ince E, Aysev D, Yavuz G, Doğru U. 2011. Successful treatment of central venous catheter infection due to *Candida lipolytica* by caspofungin-lock therapy. *Mycoses*. 54: e647-649.

Shin JH, Kook H, Shin DH, Hwang TJ, Kim M, Suh SP, Ryang DW. 2000. Nosocomial cluster of *Candida lipolytica* fungemia in pediatric patients. *Eur J Clin Microbiol Infect Dis*. 19: 344-349.

Y-5 *Cryptococcus neoformans*

Source: CSF / Bronchial lavage

Clinical significance: *Cryptococcus neoformans* (var. *grubii* and var. *neoformans*) is a major pathogen of humans and animals. It is differentiated from its sibling pathogenic species *C. gattii* by biochemical and genetic features and by host predilection. *C. neoformans* var. *neoformans* is most common in Europe while *C. neoformans* var. *grubii* is endemic in North America. *Cryptococcus gattii*, earlier thought to be restricted to tropical and sub-tropical countries, is now an emerging pathogen in North America. The incidence of cryptococcosis due to *C. neoformans* increased with the spread of AIDS and other immunosuppressive conditions. Unlike *C. neoformans, C. gattii* is not particularly associated with AIDS or other forms of immunosuppression. The fungus can cause disease in healthy people.

Colony: *Cryptococcus neoformans* colony is cream to tan in color, smooth, moist, and soft on Sabouraud's dextrose agar at 25°C (Figure 6).

Microscopy: *Cryptococcus neoformans* yeast cells are large and round, with no pseudohyphae or true hyphae on corn meal agar with Tween 80. In India-ink preparation, encapsulated yeasts are seen (Figure 6).

Differentiation: *Cryptococcus neoformans* does not ferment any carbohydrates and does not grow on media containing cycloheximide, but it grows at 37°C. *C. neoformans* produces dark brown colonies on Niger seed agar. It produces urease enzyme and it is negative on nitrate reaction. *C. neoformans* and *C. gattii* are distinguished by 1) differential media. *C. gattii* growth on canavanine-glycine-bromthymol blue (CGB) agar turn the medium blue-green after 2 – 5 days of incubation at 25°C; 2) PCR technique: *C. gattii* can be differentiated from the other two varieties using a number of primers; 3) serotyping: *C. neoformans* var. *grubii* is serotype A, *C. neoformans* var. *neoformans* is serotype D, *C. gattii* is serotype B and C.

Molecular test: *Cr. neoformans* is one of the most intensely studied pathogenic fungi. The molecular biology of this organism has revealed various virulence factors and unique genotypes among clinical strains.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Cryptococcus neoformans* var. *grubii* isolate H99 (GenBank accession no. CP003821.1).

Antifungal susceptibility: Most isolates are susceptible to amphotericin B, 5-flucytocine, and to azoles like fluconazole, itraconazole, and posaconazole. A few isolates with high MIC to fluconazole have been isolated from AIDS patients. *Cryptococus* spp. are intrinsically resistant to echinocandins.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	52
Laboratories with incorrect ID:	0

Illustrations:

Figure 6. *Cryptococcus neoformans* colony cream to tan colored, smooth, moist, and soft colony of on Sabouraud's dextrose agar, 25°C. Microscopic morphology of *C. neoformans* showing round, large blastoconidia on Corn meal agar with Tween 80 (bar = 25 μm).

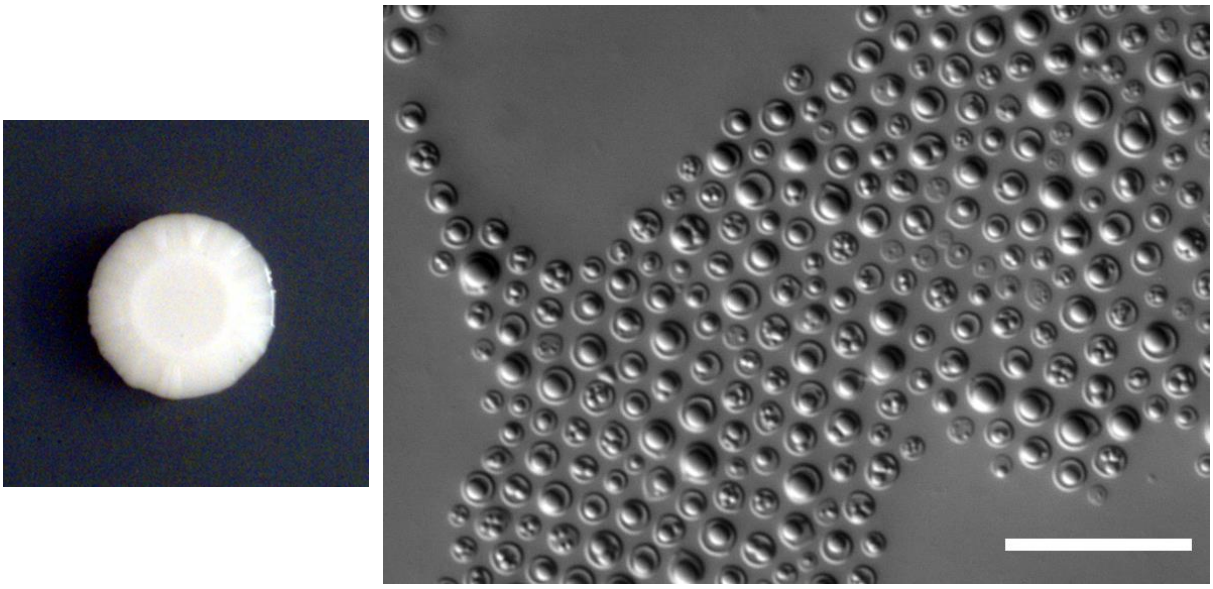
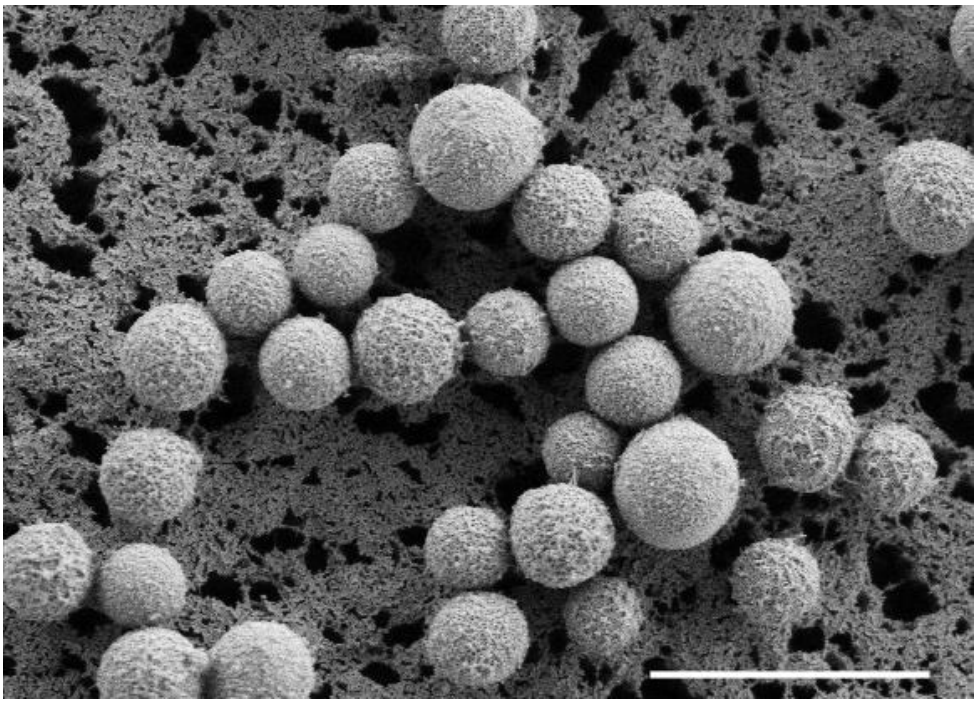


Figure 6A. Scanning electron micrograph with *C. neoformans* (bar = 10 μm).



Further reading:

De Baere, T., Claeys, G., Swinne, D., Verschraegen, G., Muylaert, A., Massonet C., and Vaneechoutte, M. 2002. Identification of cultured isolates of clinically important yeast species using fluorescent fragment length analysis of the amplified internally transcribed rRNA spacer 2 region (ITS2). *BMC Microbiol.* 2: 21.

Dromer F, Bernede-Bauduin C, Guillemot D, Lortholary O; French Cryptococcosis Study Group. 2008. Major role for amphotericin B-flucytosine combination in severe cryptococcosis. *PLoS ONE*. 3: e2870.

Espinel-Ingroff A, Aller AI, Canton E, Castañón-Olivares LR, Chowdhary A, Cordoba S, Cuenca-Estrella M, Fothergill A, Fuller J, Govender N, Hagen F, Illnait-Zaragozi MT, Johnson E, Kidd S, Lass-Flörl C, Lockhart SR, Martins MA, Meis JF, Melhem MS, Ostrosky-Zeichner L, Pelaez T, Pfaller MA, Schell WA, St-Germain G, Trilles L, Turnidge J. 2012. C*Cryptococcus neoformans-Cryptococcus gattii* species complex: an international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for fluconazole, itraconazole, posaconazole, and voriconazole. *Antimicrob Agents Chemother.* 56: 5898-5906.

Flores VG, Tovar RM, Zaldivar PG, Martinez EA. 2012. Meningitis due to *Cryptococcus neoformans*: treatment with posaconazole. *Curr HIV Res*. 10: 620-623.

Heitman, J., Kozel, T.R., Kwon-Chung, K.J., Perfect, J.R. and Casadevall A. 2010. Cryptococcus: From Human Pathogen to Model Yeast. ASM Press, Washington D.C.

Jarvis JN, Dromer F, Harrison TS, Lortholary O. 2008. Managing cryptococcosis in the immunocompromised host. *Curr Opin Infect Dis.* 21: 596-603.

Kwon-Chung, K.J., Polacheck, I., and Bennett, J.E. 1982. Improved diagnostic medium for separation of *Cryptococcus neoformans* var. *neoformans* (serotypeA and D) and *Cryptococcus neoformans* var. *gattii* (serotype B and C). *J Clin Microbiol.* 15: 535-537.

Larsen RA, Bauer M, Pitisuttithum P, Sanchez A, Tansuphaswadikul S, Wuthiekanun V, Peacock SJ, Simpson AJ, Fothergill AW, Rinaldi MG, Bustamante B, Thomas AM, Altomstone R, Day NP, White NJ. 2011. Correlation of susceptibility of *Cryptococcus neoformans* to amphotericin B with clinical outcome. *Antimicrob Agents Chemother*. 55: 5624-5630.

Lui, G., Lee, N., Ip, M., Choi, K.W., Tso, Y.K., Lam, E., Chau, S., Lai, R., Cockram, C.S. 2006. Cryptococcosis in apparently immunocompetent patients. *QJM*. 99:143-51.

McTaggart L, Richardson SE, Seah C, Hoang L, Fothergill A, Zhang SX. 2011. Rapid identification of *Cryptococcus neoformans* var. *grubii*, *C. neoformans* var. *neoformans*, and *C. gattii* by use of rapid biochemical tests, differential media, and DNA sequencing. *J Clin Microbiol*. 49: 2522-2527.

Singh N, Lortholary O, Dromer F, Alexander BD, Gupta KL, John GT, del Busto R, Klintmalm GB, Somani J, Lyon GM, Pursell K, Stosor V, Munoz P, Limaye AP, Kalil AC, Pruett TL, Garcia-Diaz J, Humar A, Houston S, House AA, Wray D, Orloff S, Dowdy LA, Fisher RA, Heitman J, Wagener MM, Husain S; Cryptococcal Collaborative Transplant Study Group. 2008. Central nervous system cryptococcosis in solid organ transplant recipients: clinical relevance of abnormal neuroimaging findings. *Transplantation.* 86: 647-651.

Springer DJ, Chaturvedi V. 2010. Projecting global occurrence of *Cryptococcus gattii*. *Emerg Infect Dis.* 16: 14-20.
Steenbergen, J.N., and Casadevall. 2000. Prevalence of *Cryptococcus neoformans* var. *neoformans* (serotype D) and *Cryptococcus neoformans* var. *grubii* (serotype A) isolates in New York City. *J Clin Microbiol*. 38:1974-1976.

Wiesner DL, Boulware DR. 2011. *Cryptococcus*-related immune reconstitution inflammatory syndrome (IRIS): pathogenesis and its clinical implications. *Curr Fungal Infect Rep.* 5: 252-261.

DIRECT DETECTION (*Cryptococcus neoformans* ANTIGEN TEST)

Introduction: In early 1960s, a simple, sensitive latex test, capable of detecting the capsular polysaccharide of *C. neoformans* in serum, was described. The test proved superior in sensitivity to the India ink mount of CSF from suspected patients. Further clinical studies established the prognostic value of the test, and showed it to be a valuable aid in establishing a diagnosis when culture was negative. Paired serum and CSF specimens allowed detection of antigen in confirmed cases. In early 1990s, an enzyme immunoassay based upon monoclonal antibody against capsular polysaccharide, was described. More recently, a lateral flow immunoassay was described as an immunochromatographic test system for the qualitative or semi-quantitative detection of the capsular polysaccharide antigens of *C. neoformans* and *C. gattii* complex in serum and CSF.

Materials: Sixty-five laboratories participated in the January 28, 2015 direct antigen detection test event. Five negative (Cn-Ag-1 to Cn-Ag-5) serum samples for cryptococcal antigen were included.

Results: The consensus results for all specimens were negative. One laboratory each reported Cn-Ag-1 and Cn-Ag-3 positive respectively with low titers.

Further Reading:

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

Binnicker MJ, Jespersen DJ, Bestrom JE, Rollins LO. 2012. Comparison of four assays for the detection of cryptococcal antigen. *Clin Vaccine Immunol*. 19: 1988-1990.

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

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

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

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

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

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

Hansen J, Slechta ES, Gates-Hollingsworth MA, Neary B, Barker A, Bauman S, Kozel TR, Hanson KE. 2013. Large scale evaluation of the Immuno-Mycologics Inc. (IMMY) Lateral Flow and Enzyme-linked Immunoassays for the detection of Cryptococcal antigen in serum and cerebrospinal fluid. *Clin Vaccine Immunol.*20: 52-55.

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

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

McMullan BJ, Halliday C, Sorrell TC, Judd D, Sleiman S, Marriott D, Olma T, Chen SC. 2012. Clinicalutility of the cryptococcalantigenlateralflowassay in a diagnosticmycologylaboratory. *PLoS One.*7: e49541.

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

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 tropicalis* (S-1) was the analyte in the January 28, 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 the consensus/all participating laboratories' MIC values within +/- 2 dilutions and then the interpretation per latest CLSI and EUCAST documents to score proficiency testing results. Especially, when there is no interpretation, MIC values are the key judge points. 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: fluconazole (31 laboratories), itraconazole (25 laboratories), voriconasole (23 laboratories), caspofungin (22 laboratories), flucytosine (21 laboratories), amphotericin B (20 laboratories), anidulafungin (17 laboratories), micafungin (17 laboratories), posacoanazole (16 laboratories), and ketocoanzole (4 laboratories). CLSI document M27-S4 specifically stated that the current data are insufficient to demonstrate a correlation between *in vitro* susceptibility testing and clinical outcome for *C. glabrata* and voriconazole. So we strongly suggest laboratories follow the M27-S4 guidelines.

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

S-1: *Candida tropicalis* (M2698)

Drug	No. labs	MIC (µg/ml)															
		0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
Amphotericin B	21						2	15	4								
Anidulafungin	17			1	10	6											
Caspofungin	24			2	10	6	6										
Flucytosine (5-FC)	22			2	17	2	1										
Fluconazole	31*			1					20	9							
Itraconazole	26*				1	10	12	2									
Ketoconazole	4*		1				2										
Micafungin	18		5	13													
Posaconazole	17				2	7	8										
Voriconazole	28				5	17	6										

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

Colors represent the testing method used:

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

Table 4. Antifungal Susceptibility Interpretations Reported by the Participating Laboratories

S-1: *Candida glabrata* (M956)

Drug	No. laboratories	Susceptible	Susceptible-dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	21	4					17
Anidulafungin	18	18					
Caspofungin	24	24		1			
Flucytosine	22	15					7
Fluconazole	32	32					
Itraconazole	26	8	10				8
Ketoconazole	4	1					3
Micafungin	18	18					
Posaconazole	17	6	1		5		10
Voriconazole	28	24	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 molds. The following nine drugs are included in the antifungal susceptibility panel - amphotericin B, anidulafungin, caspofungin, fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole.

Materials: *Aspergillus fumigatus* M2036 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* M2036

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	64	256
Amphotericin B	4						1	1	1	1					
Anidulafungin	4		1	1	1				1						
Caspofungin	4			1	1	1		1							
Fluconazole	3													2	1
Itraconazole	4									1		1	2		
Ketoconazole	1												1		
Micafungin	4	1		1	1				1						
Posaconazole	4						1	2		1					
Voriconazole	4							2		1	1				

CLSI microbroth dilution 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.