Mycology Proficiency Testing Program

Test Event Critique
September 2011

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 fungal diseases. The services include testing for dimorphic pathogenic fungi, unusual molds and yeasts pathogens, antifungal susceptibility testing including tests with research protocols, molecular tests including rapid identification and strain typing, outbreak and pseudo-outbreak investigations, laboratory contamination and accident investigations and environmental samples related to fungal diseases. The laboratory maintains proficiency and certification for handling Select Agents and to assist clinical laboratories in compliance with the latest regulations. Fungal Culture Collection of mycology laboratory is an important resource for high quality cultures used for proficiency testing program and for in house development of new diagnostic tests.

Mycology Proficiency Testing Program provides technical expertise to NYSDOH Clinical Laboratory Evaluation Program (CLEP). The program is responsible for conducting the CLIA-compliant proficiency testing (Mycology) for clinical laboratories in New York. All analytes for these test events are prepared and standardized internally. The program also provides continuing educational activities in form of detailed critiques of test events, workshops and occasional one-on-one training of laboratory professionals.

Mycology Laboratory Staff and Contact Details

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Mycology Proficiency Testing Program (PTP)

CATEGORY DESCRIPTION

COMPREHENSIVE: This category is for laboratories that examine clinical specimens for pathogenic molds and yeasts routinely encountered in a clinical microbiology laboratory. These laboratories are expected to identify fungi to the genus and species level as appropriate. Laboratories holding this category may also perform antifungal susceptibility testing, antigen detection, molecular identification or other tests described under any of the categories listed below.

RESTRICTED: This category is for laboratories that restrict their testing to one or more of the following:

IDENTIFICATION YEAST ONLY: This category is for laboratories that isolate and identify to genus and species, as appropriate, yeast-like fungi routinely encountered in a clinical microbiology laboratory. Laboratories holding this category may also perform susceptibility testing on yeast. These laboratories are expected to refer mold specimens to another laboratory holding Mycology – Comprehensive permit.

ANTIGEN DETECTION: This category is for laboratories that perform direct antigen detection methods.

MOLECULAR METHODS: This category is for laboratories that use FDA-approved or lab-developed molecular methods for detecting, identifying, typing, characterizing or determining drug resistance of infectious agents. Laboratories using molecular methods under another Restricted permit category (e.g. Restricted: Antigen detection) or those holding a Comprehensive category permit, do not need to request this molecular method category.

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

PROFICIENCY TESTING ANALYTES OFFERED

(CMS regulated analytes or tests are indicated with an asterisk)

COMPREHENSIVE

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

RESTRICTED

Identification Yeast Only

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

Antigen Detection

- Cryptococcus neoformans* Antigen detection

Molecular Methods

- No proficiency testing is offered at this time.

TEST SPECIMENS & GRADING POLICY

Test Specimens

At least two strains of each mold or yeast specimens are examined for inclusion in the proficiency test event. The colony morphology of molds is studied on Sabouraud dextrose agar. The microscopic morphologic features are examined by potato dextrose agar slide cultures. The physiological characteristics such as cycloheximide sensitivity and growth at higher temperatures are investigated with appropriate test media. The strain that best demonstrates the morphologic and physiologic characteristics typical of the species is included as a test analyte. Similarly, two or more strains of yeast species are examined for inclusion in the proficiency test. The colony morphology of all yeast strains is studied on corn meal agar with Tween 80 plates inoculated by Dalmau or streak-cut method. Carbohydrate assimilation is studied with the API 20C AUX identification kit (The use of brand and/or trade names in this report does not constitute an endorsement of the products on the part of the Wadsworth Center or the New York State Department of Health). The fermentations of carbohydrates, i.e., glucose, maltose, sucrose, lactose, trehalose, and cellobiose, are also documented using classical approaches. Additional physiologic characteristics such as nitrate assimilation, urease activity, and cycloheximide sensitivity are investigated with the appropriate test media. The 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 percent 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 consensus/references laboratories MIC values within +/- 2 dilutions and interpretation per CLSI (NCCLS) guidelines or related, peer-reviewed publications. One yeast and/or mold is to be tested against following drugs: amphotericin B, anidulafungin, caspofungin, flucytosine (not for molds), fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. The participating laboratories are free to select any number of antifungal drugs from the test panel based upon 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 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 identification used in grading of responses received after each test event. However, the laboratory can elect to provide only genus level identification if it reflects the standard operating procedures (SOP) for patient testing. This list does not include all molds that might be encountered in a clinical laboratory nor is it intended to be used for competency assessment of laboratory personnel in diagnostic mycology.

The nomenclature used in the mold master list is based upon currently recognized species in published literature, monographs and in 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.

Absidia corymbifera

Absidia species

Acremonium species

Alternaria species

Arthrographis species

Aspergillus clavatus

Aspergillus flavus

Aspergillus fumigatus species complex

Aspergillus glaucus

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

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. However, the laboratory can elect to provide only genus level identification if it reflects the standard operating procedures (SOP) for patient testing. This list does not include all yeasts that might be encountered in a clinical laboratory nor is it intended to be used for competency assessment of the laboratory personnel in diagnostic mycology.

The nomenclature used in this list is based upon currently recognized species in published literature, monographs, and catalogues of recognized culture collections. No attempt has been made to include teleomorphic states of fungi if they are not routinely encountered in the clinical specimens. Where appropriate, current nomenclature has been included under parentheses to indicate that commonly accepted genus and/or species name is no longer valid, e.g. *Blastoschizomyces capitatus* (*Geotrichum capitatum*). These guidelines supersede any previous instructions for identification of yeasts. The list is subject to change in response to significant changes in nomenclature, human disease incidence or other factors.

Blastoschizomyces capitatus (*Geotrichum capitatum*)
Blastoschizomyces species
Candida albicans
Candida dubliniensis
Candida famata
Candida glabrata
Candida guilliermondii species complex
Candida kefyr
Candida krusei
Candida lipolytica (*Yarrowia lipolytica*)
Candida lusitaniae
Candida norvegensis
Candida parapsilosis species complex
Candida rugosa
Candida species
Candida tropicalis
Candida viswanathii
Candida zeylanoides
Cryptococcus albidus
Cryptococcus gattii
Cryptococcus laurentii
Cryptococcus neoformans
Cryptococcus neoformans-Cryptococcus gattii species complex
Cryptococcus species
Cryptococcus terreus
Cryptococcus uniguttulatus
Geotrichum candidum
Geotrichum species
Hansenula anomala (*Candida pelliculosa*)
Malassezia furfur
Malassezia pachydermatis
Malassezia species
Pichia ohmeri (*Kodamaea ohmeri*)
Prototheca species
Prototheca wickerhamii
Prototheca zopfii
Rhodotorula glutinis
Rhodotorula minuta
Rhodotorula mucilaginosa (*rubra*)
Rhodotorula species
Saccharomyces cerevisiae
Saccharomyces species
Sporobolomyces salmonicolor
Trichosporon asahii
Trichosporon inkin
Trichosporon mucoides
Trichosporon species

Summary of Laboratory Performance:

Mycology – Mold

	Specimen key	Validated specimen	Acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
M-1	*Pithomyces* species	*Pithomyces* species		57/66 (86%)
M-2	*Trichoderma* species	*Trichoderma* species		65/66 (98%)
M-3	*Fonsecaea* species	*Fonsecaea* species		63/66 (95%)
M-4	*Trichophyton tonsurans*	*Trichophyton tonsurans*		54/66 (82%)
M-5	*Rhizomucor* species	*Rhizomucor* species	*Rhizomucor pusillus*	57/66 (86%)

Mycology – Yeast Only

	Specimen key	Validated specimen	Laboratories with correct responses / Total laboratories (% correct responses)
Y-1	*Candida tropicalis*	*Candida tropicalis*	56/56 (100%)
Y-2	*Candida krusei*	*Candida krusei*	51/56 (91%)
Y-3	*Candida kefyr*	*Candida kefyr*	55/56 (98%)
Y-4	*Candida glabrata*	*Candida glabrata*	56/56 (100%)
Y-5	*Candida parapsilosis*	*Candida parapsilosis*	56/56 (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	67/68 (99%)	NA
Cn-Ag-2	Negative	Negative	68/68 (100%)	NA
Cn-Ag-3	Negative	Negative	68/68 (100%)	NA
Cn-Ag-4	Positive (1:256)	Positive (1:256)	68/68 (100%)	62/63 (98%)
Cn-Ag-5	Negative	Negative	67/68 (99%)	NA

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

Drugs	Acceptable MIC (µg/ml) Range	Acceptable interpretation	Laboratories with acceptable responses/ Total laboratories (% correct responses)
Amphotericin B	0.12 – 2.0	Susceptible / No interpretation	23/23 (100%)
Anidulafungin	0.5 – 2.0	Susceptible	17/17 (100%)
Caspofungin	0.12 – 2.0	Susceptible	22/22 (100%)
Flucytosine (5-FC)	0.015 – 0.25	Susceptible	26/26 (100%)
Fluconazole	0.25 – 4.0	Susceptible	30/31 (97%)
Itraconazole	0.03 – 0.5	Susceptible	30/30 (100%)
Ketoconzole	0.03 – 0.25	Susceptible / No interpretation	5/5 (100%)
Micafungin	0.5 – 8.0	Susceptible / Nonsusceptible	17/17 (100%)
Posaconazole	0.015 – 0.25	Susceptible / No interpretation	18/18 (100%)
Voriconazole	0.004 – 0.06	Susceptible	24/24 (100%)

Antifungal SusceptibilityTesting for Mold (MS-1: *Aspergillus fumigatus* M2036)

Drugs	Acceptable MIC (µg/ml) Range	Laboratories with acceptable responses / Total laboratories (%)
Amphotericin B	0.12 – 2.0	6/6 (100%)
Anidulafungin	0.004 – 0.06	4/4 (100%)
Caspofungin	0.015 – 2.0	4/5 (80%)
Fluconazole	≥ 64	5/5 (100%)
Itraconazole	0.12 – 2.0	6/6 (100%)
Ketoconzole	Not validated	NA
Micafungin	0.004 – 0.06	4/4 (100%)
Posaconazole	0.06 – 1.0	5/5 (100%)
Voriconazole	0.12 – 2.0	5/5 (100%)

Commercial Device Usage Statistics:

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

Process/ Device	No. laboratories
Yeast Identification*	
API 20C AUX	46
AMS Vitek	4
Vitek2	23
Remel Uni-Yeast-Tek	6
Microscan	1
API 20C AUX	46
Antifungal Susceptibility*	
YeastOne -Yeast	26
YeastOne- Mold	3
Etest	3
Disk diffusion	1
Vitek2	1
Others‡ - Yeast	3
Others - Mold	3
LA Cryptococcal antigen	
Immuno-Mycologics	10
Meridien Diagnostics	45
Remel	13

* Include multiple systems used by some laboratories

‡ Include laboratories using CLSI Microbroth dilution method

MOLD DESCRIPTIONS

M-1. *Pithomyces* species

Source: Toe

CLINICAL SIGNIFICANCE: *Pithomyces* spp. may rarely cause human disease. Two strains deposited in the CBS-KNAW Fungal Biodiversity Centre Culture Collection (CBS 243.96 and 244.96) originated from chronic nasal polyposis and skin scrapings of patients in California. *Pithomyces* species might be encountered as a contaminant in the clinical specimens. *Pithomyces chartarum* causes pithomycotoxicosis (facial eczema) in ruminants.

COLONY: *Pithomyces* spp. grew at moderate rate in the laboratory (Figure 1). The colonies were grey to black, downy ('featherlike projections') on Sabouraud's dextrose agar, 25°C.

MICROSCOPY: Lactophenol cotton blue mount showed muriform (brick wall pattern) conidia on conidiophores; conidiophore was indistinguishable from hyphae (Figure 1). Conidia were dark brown, broadly ellipsoidal, and echinulate ('spikes') or verrucose (wart-like projections). An annular frill at the base of the conidium was visible after detachment.

DIFFERENTIATION: *Pithomyces* spp. differ from *Alternaria* spp. and *Ulocladium* spp. by the lack of geniculate (bent) conidiophores, and solitary conidia. *Pithomyces* spp. differ from *Bipolaris*, *Curvularia*, and *Drechslera* by muriform (brickwall pattern) conidia and from *Stemphylium* by lacking percurrent (extending through the length) proliferation.

MOLECULAR TEST: No specific diagnostic test has been described in the published literature.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Pithomyces chartarum* (*Leptosphaerulina chartarum*) isolate ATT044 (GenBank accession no. HQ607815.1).

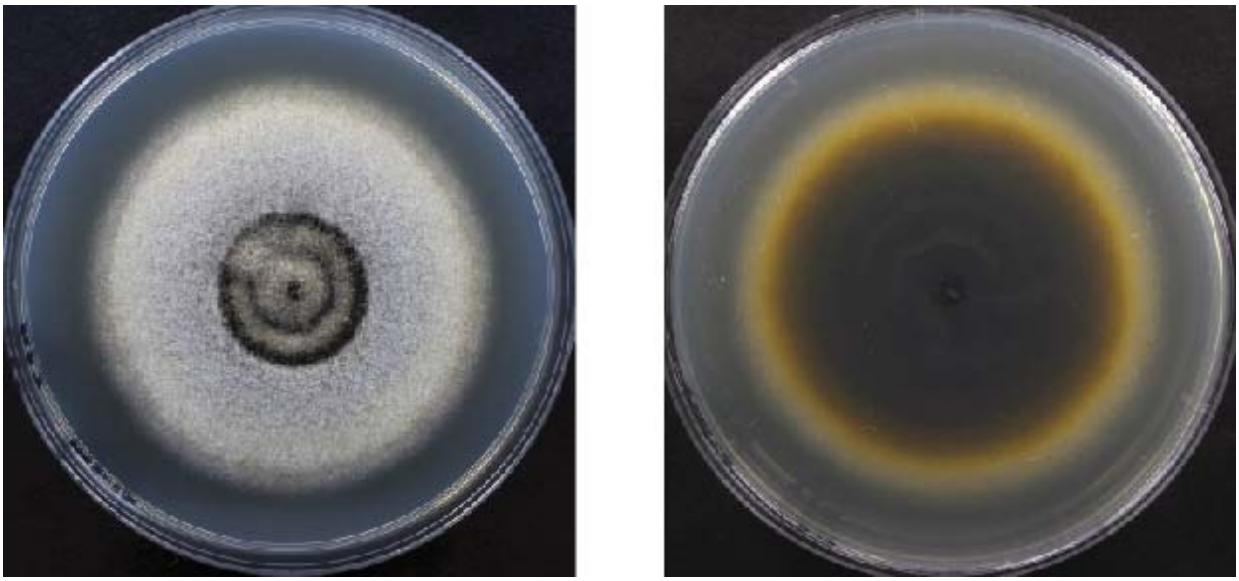
ANTIFUNGAL SUSCEPTIBILITY: No information is available.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	09
Laboratories with correct ID:	57
Laboratories with incorrect ID:	09
(*Ulacladium* spp.)	(5)
(*Acremonium* spp.)	(1)
(*Arthrographis* spp.)	(1)
(*Aureobasidium pullulans*)	(1)
(*Ustilago* spp.)	(1)

Illustrations:

FIGURE 1. (Upper panel) Five-day-old, grey,'downy'colony of *Pithomyces* sp. on Sabouraud's dextrose agar; the reverse of the colony appears olive brown to black. (Lower panel) Microscopic morphology of *Pithomyces* spp. showing septate brown hyphae, muriform conidia (400× magnification).



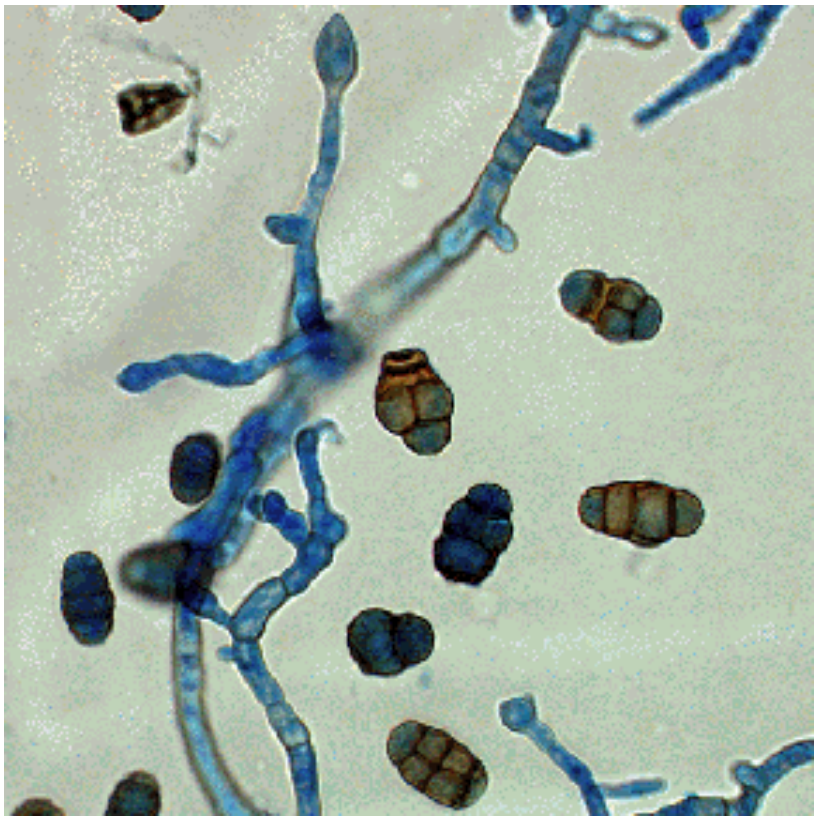
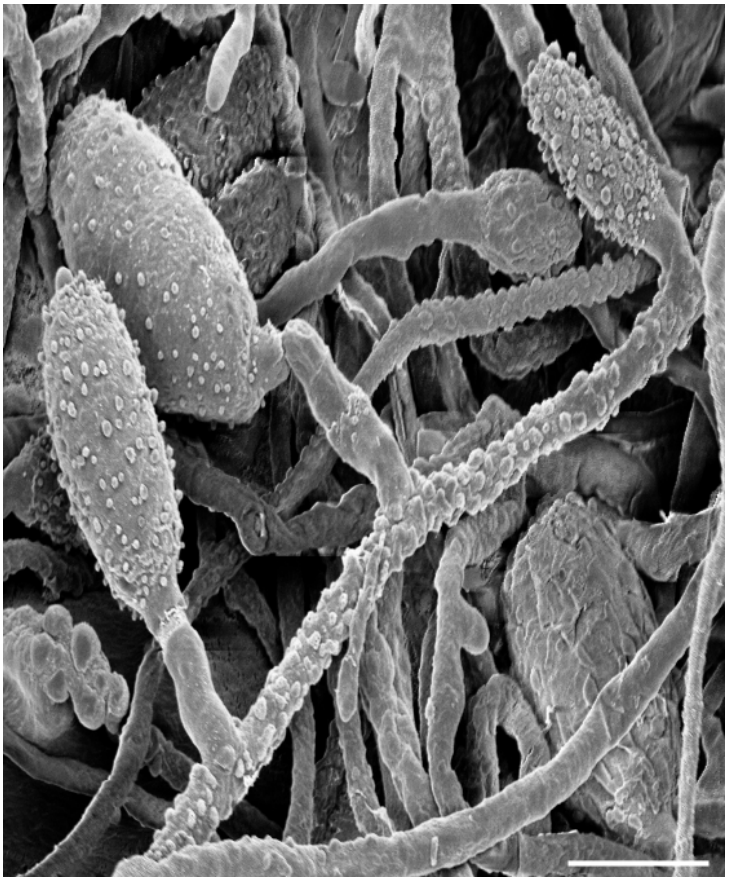
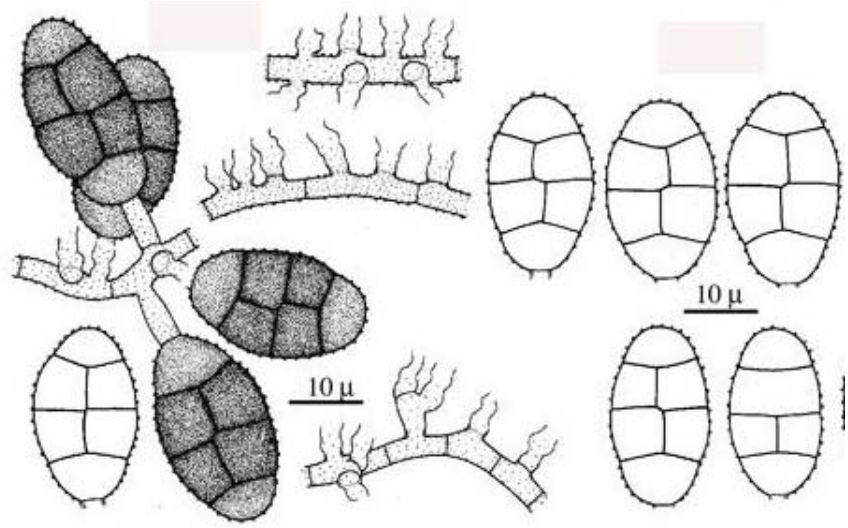


FIGURE 1A. (Upper panel) Scanning electron micrograph of *Pithomyces* spp. with characteristic ellipsoidal, echinulate or verrucose conidia and inconspicuous conidiophores (Bar = 10 μm; Lower panel) Line drawings to highlight microscopic features of *Pithomyces* spp.





http://www.mycobank.org/MycoTaxo.aspx?Link=T&Rec=337066

Further reading:

Roux C. 1986. *Leptosphaerulina chartarum* sp. nov, the teleomorph of *Pithomyces chartarum*. *Trans Brit Mycol Soc*. 86: 319-323.

Baxter DM, Perkins JL, McGhee CR, Seltzer JM. 2005. A regional comparison of mold spore concentrations outdoors and inside "clean" and "mold contaminated" Southern California buildings. *J Occup Environ Hyg*. 2: 8-18.

Pinto C, Santos VM, Dinis J, Peleteiro MC, Fitzgerald JM, Hawkes AD, Smith BL. 2005. Pitomycotoxicosis (facial eczema) ruminants in the Azores, Portugal. *Vet Record* 157: 805-810.

Green BJ, Tovey ER, Beezhold DH, Perzanowski MS, Acosta LM, Divjan AI, Chew GL. 2009. Surveillance of fungal allergic sensitization using the fluorescent halogen immunoassay. *J Mycol Med*. 19:253-261.

M-2 *Trichoderma* species

Source: Hair

CLINICAL SIGNIFICANCE: *Trichoderma* spp. rarely cause systemic infection in humans. However, many systemic infections have been reported from patients with debilitating underlying conditions.

COLONY: *Trichoderma* colony was fast growing, white turning into wooly texture with green tufts on Sabouraud's dextrose agar, after 7 days at 25°C. The reverse was pale to yellowish (figure 2).

MICROSCOPY: Lactophenol cotton blue mount showed branched conidiophores with pyramidal arrangement (Figure 2). Phialides were mostly single and flask-shaped. Greenish condia were globose, semiglobose, or like an ellipsoid.

DIFFERENTIATION: *Trichoderma* spp. have characteristic macroscopic and microscopic morphology for easy differentiation from other molds. This fungus has flask shaped phialides while *Gliocladium* spp. reported by one laboratory has *Penicillium*-like phialides and conidiophores.

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).

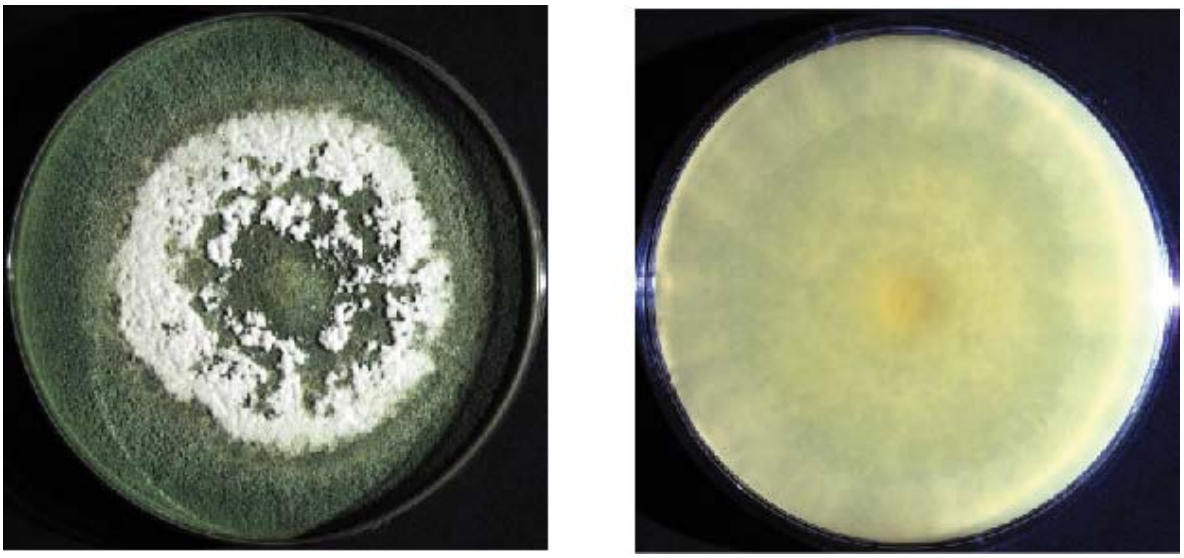
ANTIFUNGAL SUSCEPTIBILITY: Amphotericin MICs for *Trichoderma* spp. is variable (0.06 - 2.0 µg/ml), voriconazole MIC is in the susceptible range while the fungus is resistant to fluconazole and 5-fluorocytosine.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	65
Laboratories with incorrect ID:	01
(*Gliocladium* sp.)	(1)

Illustrations:

FIGURE 2. Seven-day-old, colony of *Trichoderma* spp. with wooly texture and green tufts, Sabouraud's dextrose agar, 25°C; the reverse is pale to yellowish (Upper panels). (Lower panel) Microscopic morphology of *Trichoderma* spp. 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.



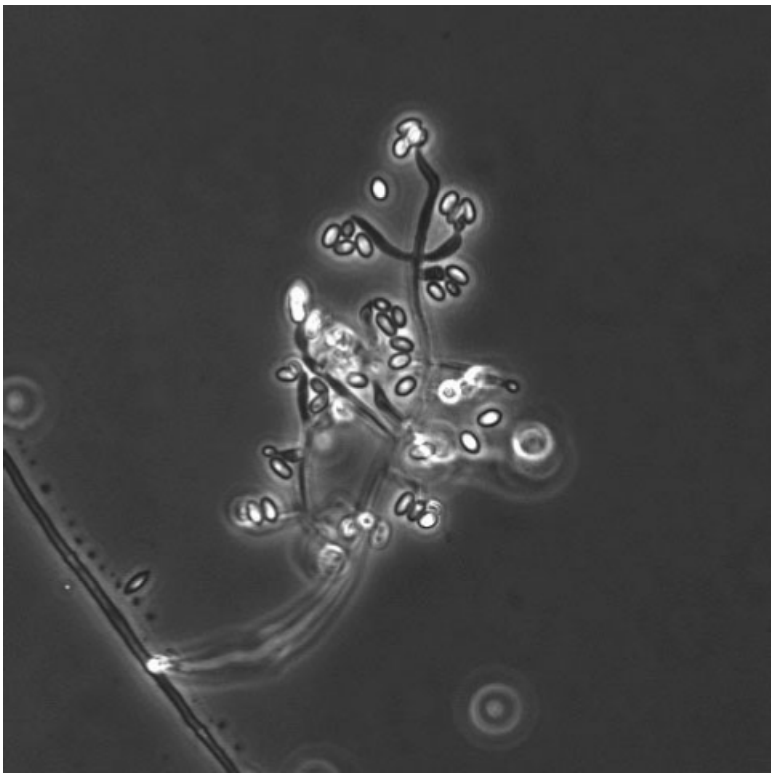
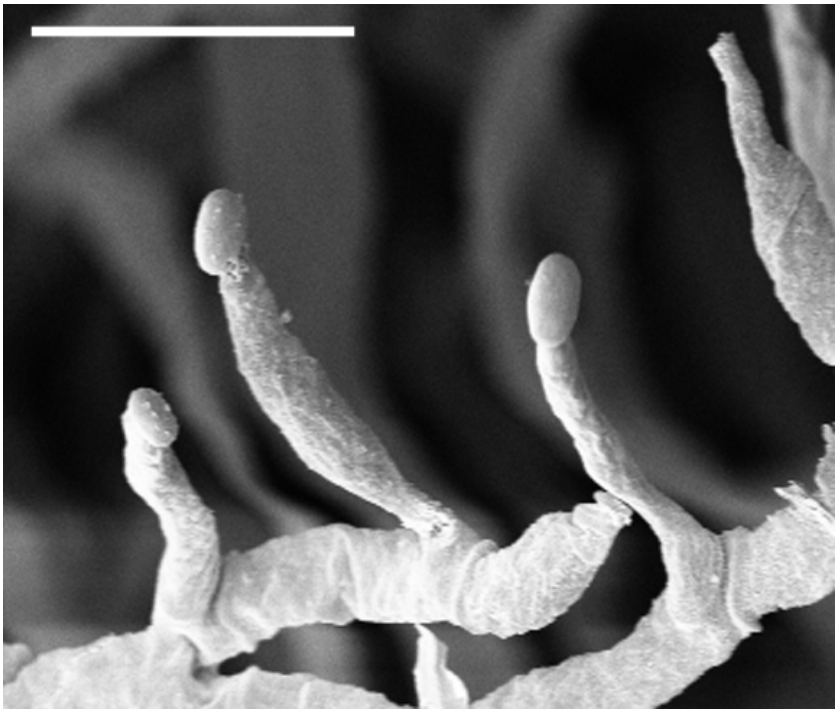
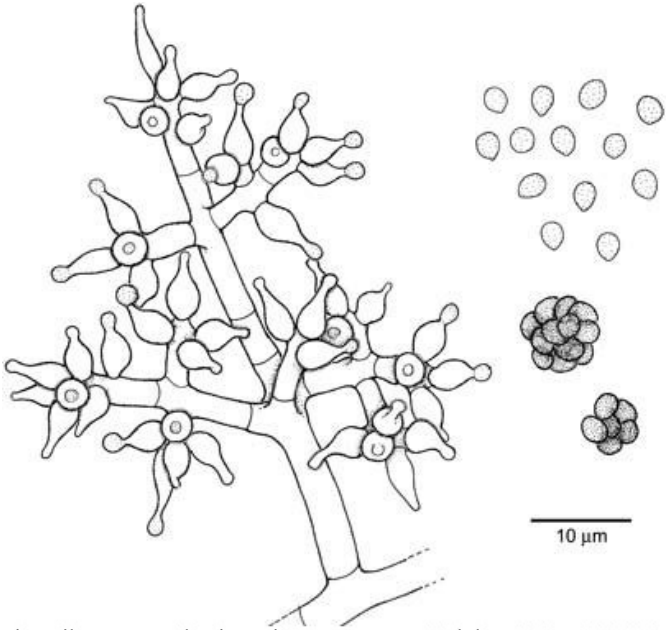


FIGURE 2A. Scanning electron micrograph of conidia and conidiophores of *Trichoderma* spp. on Sabouraud's dextrose agar; (Bar = 10 μm; upper panel). Line drawings of phialides and conidia (lower panel).





http://www.mycobank.org/MycoTaxo.aspx?Link=T&Rec=340299

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.

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M-3 *Fonsecaea* species

Source: Skin

CLINICAL SIGNIFICANCE: *Fonsecaea* spp. include causal agents of chromoblastomycosis. This disease is a chronic localized infection of the skin and subcutaneous tissue that follows from traumatic implantation of the etiologic agent. Other human infections caused by *Fonsecaea* spp. include paranasal sinusitis, keratitis, and fatal brain abscesses resulting from hematogenous dissemination.

COLONY: *Fonsecaea* spp. grew slowly on Sabouraud's dextrose agar, after 10 days at 25°C (Figure 3). The colony was black with 'downy' appearance and dark-brown to black reverse.

MICROSCOPY: Lactophenol cotton blue mount showed dark brown, septate hyphae, cylindrical conidiophores, and sympodially (alternate, lateral emergence of conidia) formed conidia (Figure 3).

DIFFERENTIATION: *Fonsecaea* spp. are differentiated from *Cladophialophora* by production of short conidial chains, which consist of 5 or less conidia. The conidial head with apical, irregular, swollen ends that function as conidiogenous cells distinguishes *Fonsecaea* from *Cladosporium* and *Rhinocladiella*.

MOLECULAR TEST: Duplex PCR of ribosomal DNA internal transcribed spacer regions was used for rapid and more specific identification of the genus *Fonsecaea.* Restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA) has been used for classifying *Fonsecaea* spp. The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Fonsecaea pedrosoi* isolate 7013 (GenBank accession no. HM748581.1)

ANTIFUNGAL SUSCEPTIBILITY: *Fonsecaea* spp. are susceptible to amphotericin B, itraconazole, ketoconazole, ravuconazole, voriconazole, and posaconazole, but resistant to caspofungin, anidulafungin, fluconazole and flucytosine.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	63
Laboratories with incorrect ID:	03
(*Cladosporium* spp.)	(1)
(*Exophiala* spp.)	(1)
(*Phialophora* spp.)	(1)

Illustrations:

FIGURE 3. Ten-day-old, black, 'downy' colony of *Fonsecaea* spp. on Sabouraud's dextrose agar, 25°C; the reverse is dark-brown to black (Upper panel). Microscopic morphology of *Fonsecaea* spp. showing sympodially formed conidia (bar = 5 µm: lower panel).

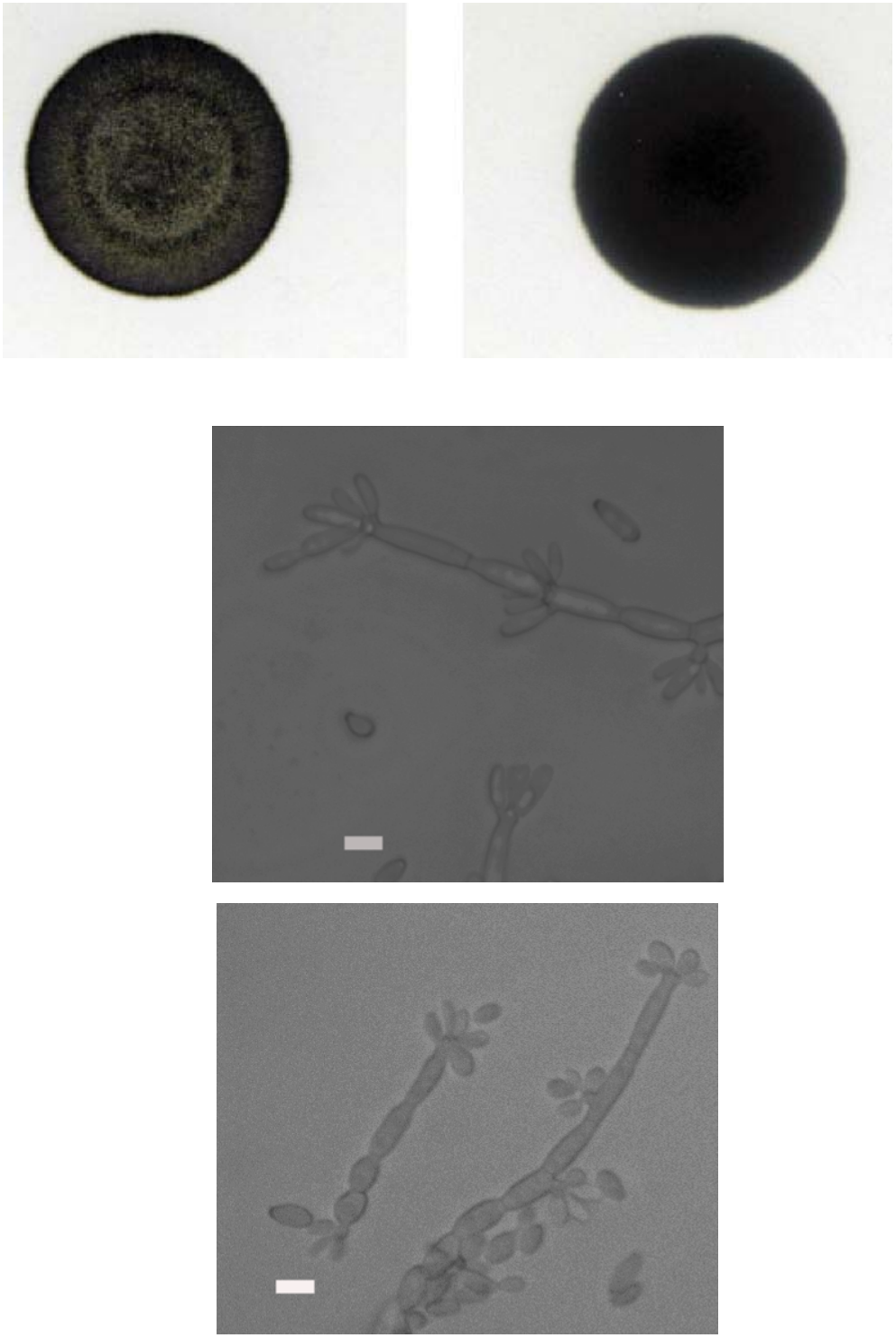
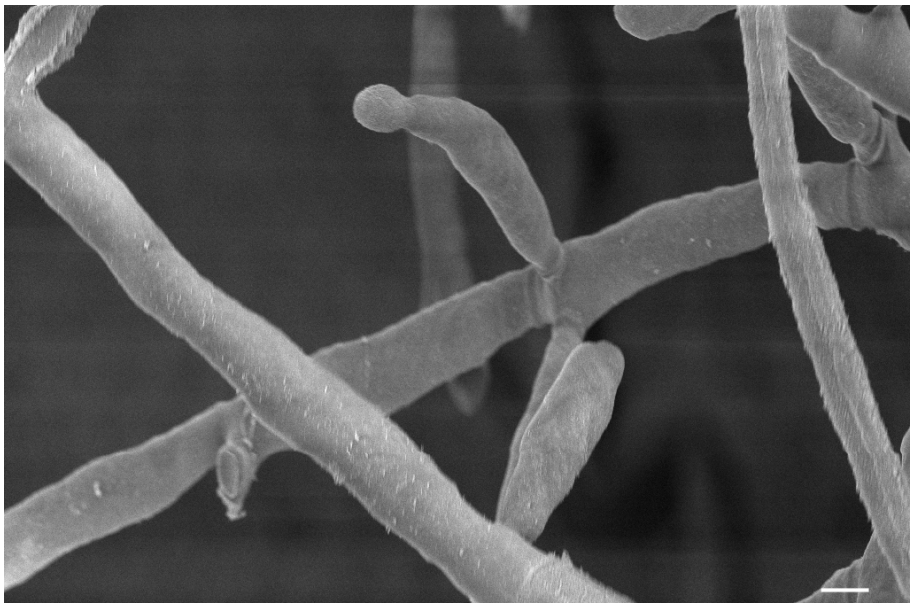
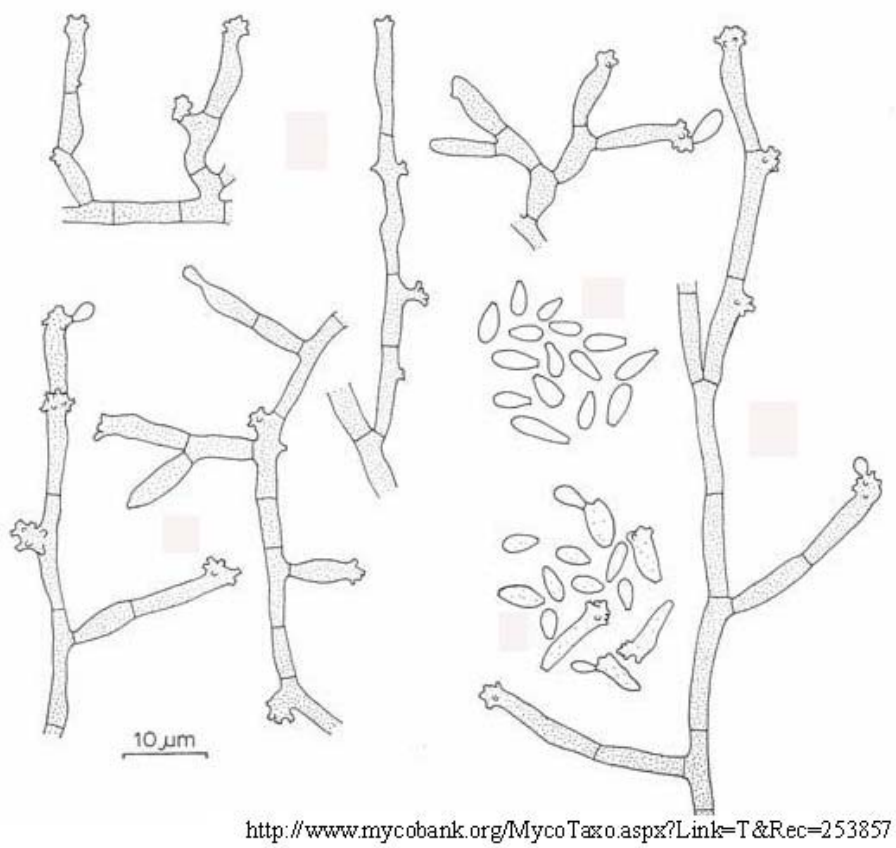


FIGURE 3A. Scanning electron micrograph of *Fonsecaea* spp. with conidium and conidiophores (bar = 2 µm; upper panel). Line drawing with details of conidiogenous cells, attachment and shape of conidia (lower panel).





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M-4 *Trichophyton tonsurans*

Source: Scalp

CLINICAL SIGNIFICANCE: *T. tonsurans* causes hair and skin infections of scalp ('tinea capitis'). Occasionally, the fungus is etiologic agent of infections in nails (onychomycosis) and foot ('tinea pedis'). A sibling species *T. equinum* usually infects horses, but it is rarely implicated in human disease.

COLONY: *T. tonsurans* grew slowly on Sabouraud's dextrose agar. After 7 days at 25°C, the colony was white to pale yellow, powdery to velvety with yellow to dark brown or red-brown pigmentation on reverse (Figure 4). *T. tonsurans* was urease-positive with growth stimulated by thiamine.

MICROSCOPY: Lactophenol cotton blue mount showed abundant microconidia with various shapes such as tear-drop, club-shaped or balloon shaped (Figure 4). Macroconida were rare.

DIFFERENTIATION: *T. tonsurans* is differentiated from *T. mentagrophytes* and *T. rubrum* by its microconidia of diverse shapes and sizes, requirement of thiamine for growth, and rare macroconidia. *T. tonsurans* is urease positive but *T. rubrum* is urease negative. The differentiation of *T. equinum* from *T. tonsurans* requires morphological, physiological and ribosomal ITS1-ITS2 sequencing.

MOLECULAR TEST: Restriction fragment length polymorphism (RFLP*)* analysis of PCR amplified ribosomal DNA including ITS was reported as a rapid tool for identification of *T. tonsurans*. The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Trichophyton tonsurans* isolate UAMH 8552 (GenBank accession no. AF170479)

ANTIFUNGAL SUSCEPTIBILITY: *T. tonsurans* is susceptible to amphotericin B, fluconazole, itraconazole, ketoconazole, and terbinafine.

PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	9
Laboratories with correct ID:	54
Laboratories with incorrect ID:	12
(*Trichophyton mentagrophytes* species complex)	(4)
(*Trichophyton rubrum*)	(3)
(T*richophyton* sp.)	(3)
(*Trichophyton interdigitale*)	(1)
(*Trichophyton terrestre*)	(1)

Illustrations:

FIGURE 4. Seven-day old *Trichophyton tonsurans* white to pale yellow colony on Sabouraud's dextrose agar, 25°C; the reverse is dark brown or reddish-brown. (Upper panel) Microscopic morphology of *Trichophyton tonsurans* showing plenty of tear-drop, club-shaped, or balloon shaped microconidia (bar = 5 µm; lower panel)

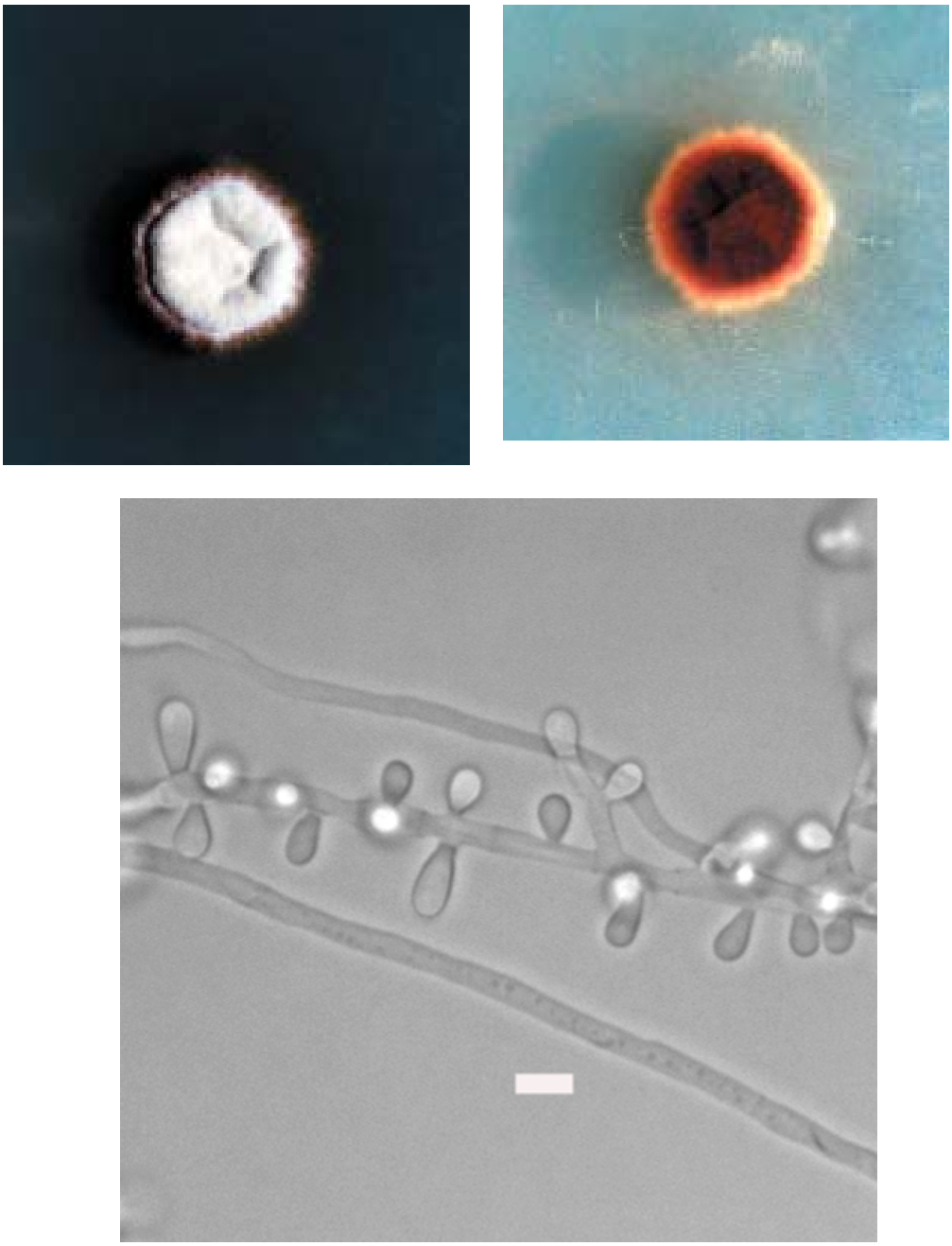
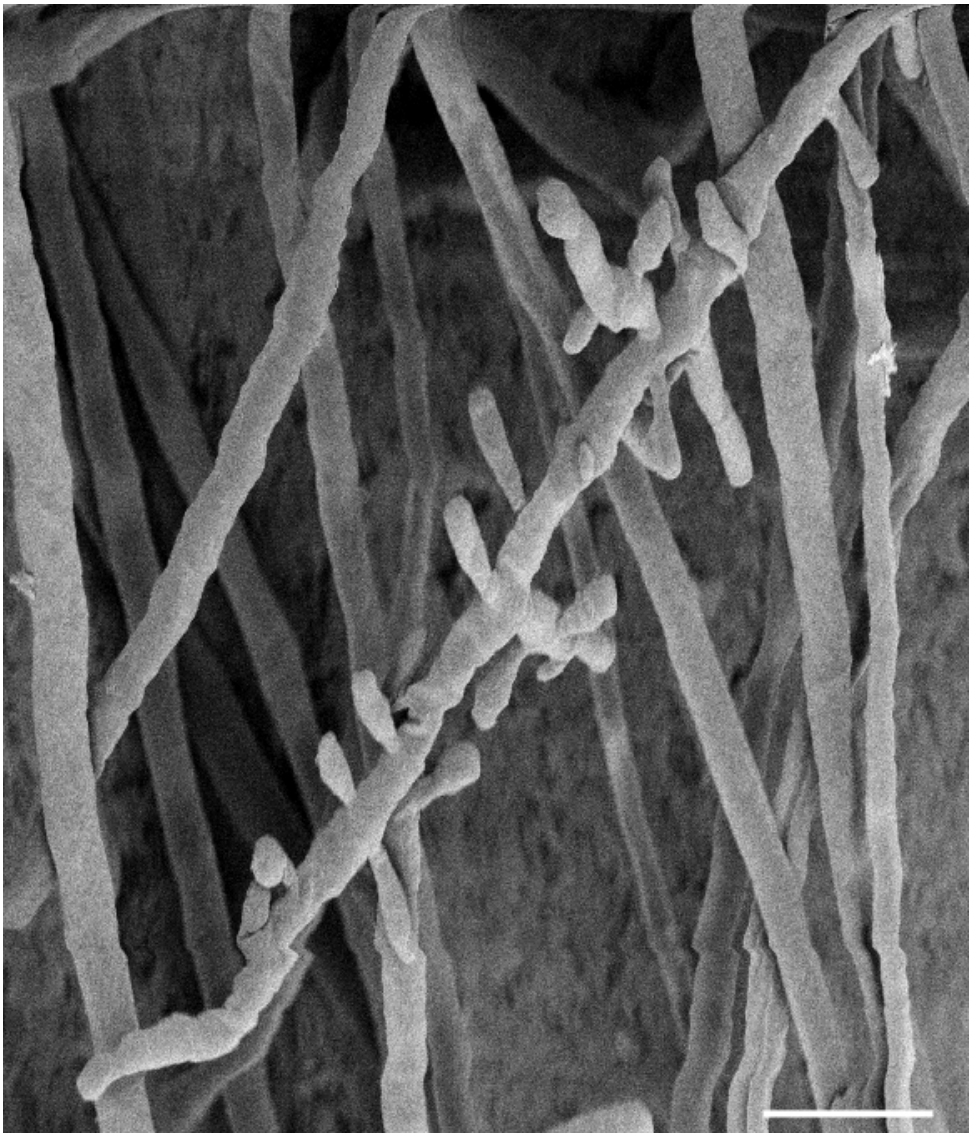


FIGURE 4A. Scanning electron micrograph of *Trichophyton tonsurans* highlighting tear-drop, club-shaped, or balloon shaped microconidia (bar = 10 µm).



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M-5 *Rhizomucor* species

Source: Blood

CLINICAL SIGNIFICANCE: *Rhizomucor* spp. rarely cause serious infections in humans. Cutaneous, pulmonary, rhinofacial, and disseminated mucormycosis due to *Rhizomucor* spp. have been reported in patients with underlying debilitating diseases.

COLONY: *Rhizomucor* spp. grew rapidly, filling the Petri dish in 4 days at 25°C. The colony was wooly, white initially that turned grayish black over time. The reverse was white to pale (Figure 5).

MICROSCOPY: Lactophenol cotton blue mount showed nonseptate or sparsely septate, broad hyphae, sporangiophores, sporangia, and sporangiospores. Sporangiophores were irregularly branched with sporangia at the top. Sporangia were brown in color and round in shape. Apophysis was absent. Columellae were prominent and spherical to pyriform in shape. Sporangiospores were small, unicellular, and round to ellipsoidal in shape (Figure 5).

DIFFERENTIATION: *Rhizomucor* spp. can be distinguished from *Mucor* species by the presence of rhizoids and stolons. It can be differentiated from *Rhizopus* species by the presence of branched sporangiophores and rhizoids not arising opposite the sporangiophores. *Rhizomucor* spp. are distinct from *Absidia* by the presence of globose sporangia and sporangiophores that are not swollen where they merge with the columellae.

MOLECULAR TEST: PCR-nucleotide sequencing has been used to confirm identity of *Rhizomucor* species. The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Rhizomucor pusillus* isolate SC-A1 (Genebank accession no. HQ404246.1).

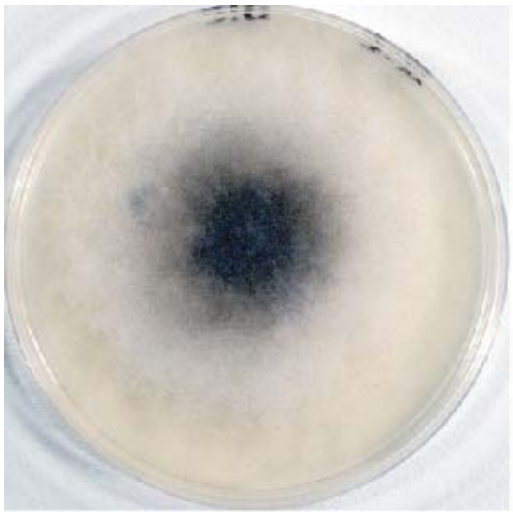
ANTIFUNGAL SUSCEPTIBILITY: *Rhizomucor* spp. are susceptible to amphotericin B and caspofungin.

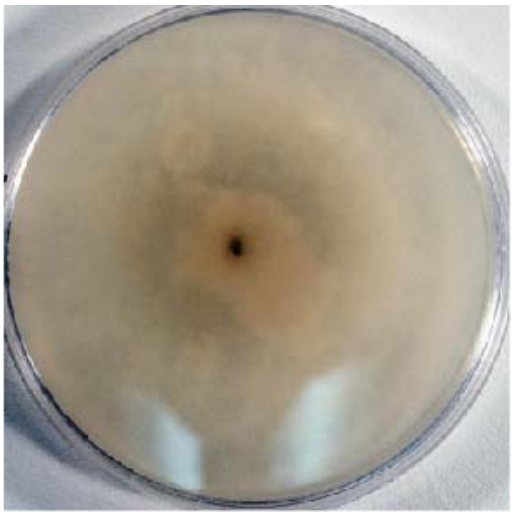
PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	09
Laboratories with correct ID:	43
Other Acceptable Answers:	
Rhizomucor pusillus	14
Laboratories with incorrect ID:	09
(*Mucor plumbeus*)	(2)
(*Mucor* spp.)	(7)

Illustrations:

FIGURE 5. Seven-day old *Rhizomucor* spp. colony filling the Petri dish, Sabouraud's dextrose agar, 25°C; the reverse of the colony is white to pale (upper panel). Microscopic morphology of *Rhizomucor* sp. showing nonseptate hyphae, irregularly branched sporangiophores, round sporangia, and pyriform columellae (bar = 50 µm; lower panel).





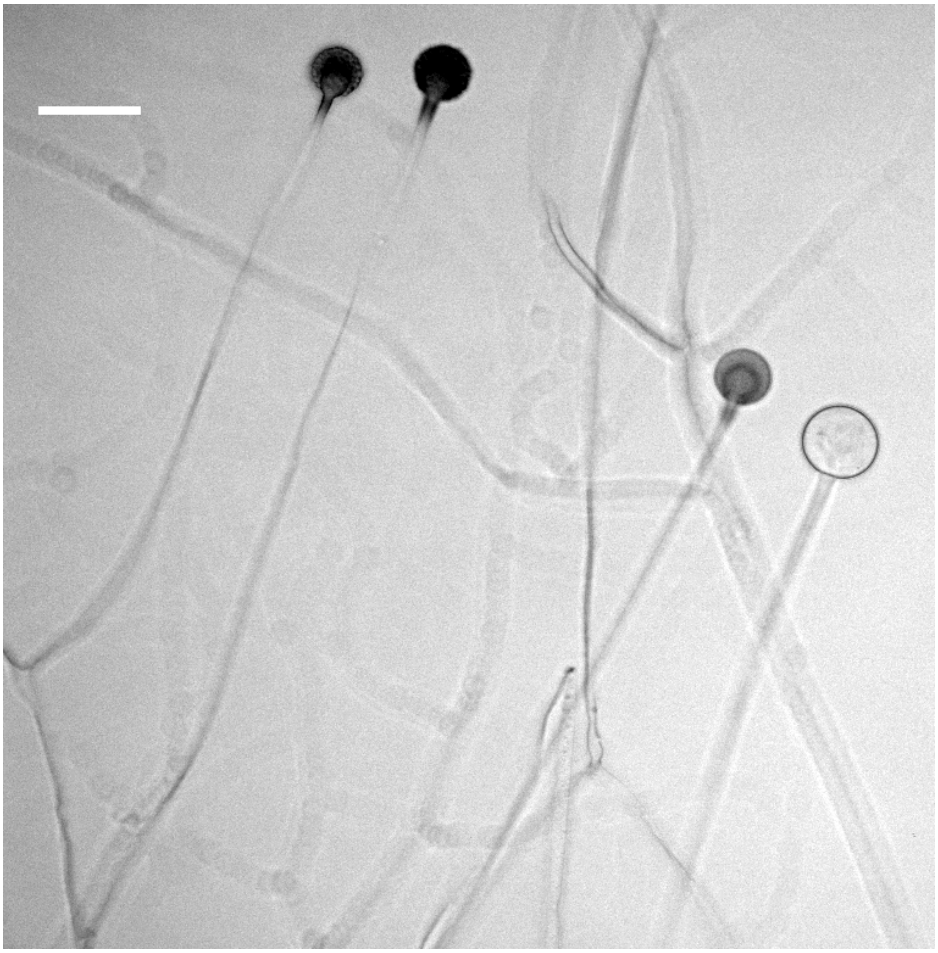
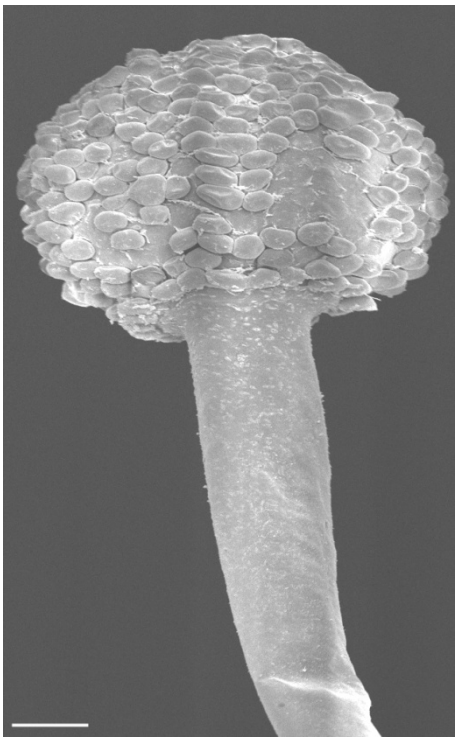
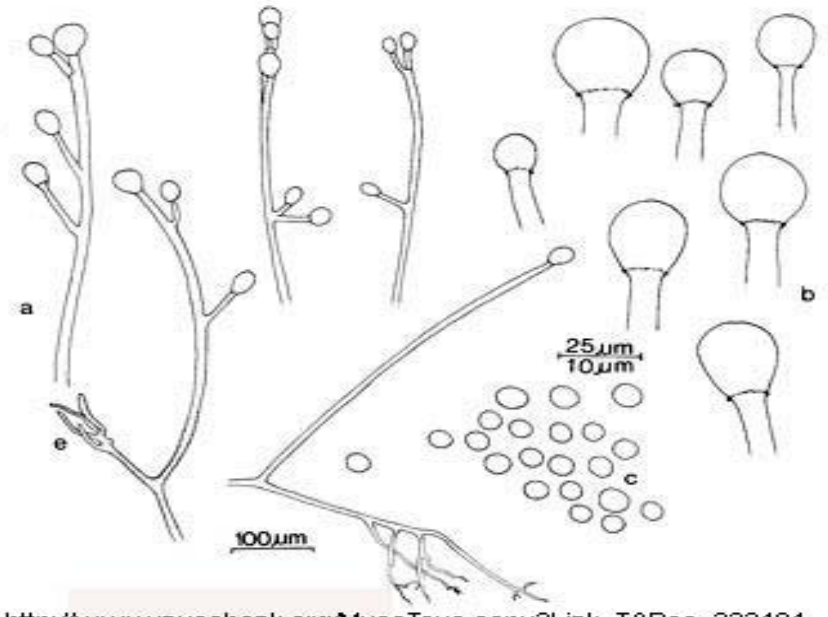


FIGURE 5A. Scanning electron micrograph of *Rhizomucor* spp. showing sporangiophore with sporangia (bar = 2 µm, upper panel); line drawings of nonseptate hyphae, irregularly branched sporangiophores, round sporangia, and pyriform columellae and round to elliposoidal sporangiospores.





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YEAST DESCRIPTIONS

Y-1 *Candida tropicalis*

Source: Bronchial wash / Urine / Sputum

CLINICAL SIGNIFICANCE: *Candida tropicalis* causes sepsis, wound infections, and disseminated infections in immunocompromised patients.

COLONY: *C. tropicalis* colony was smooth to wrinkled, cream-colored and rapid-growing on Sabouraud's dextrose agar after 7 days at 25°C, (Figure 6).

MICROSCOPY: *C. tropicalis* showed long true hyphae and pseudohyphae, with either single or small clusters of blastoconidia on Corn meal agar with Tween 80 (Figure 6).

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 was 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 was 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)

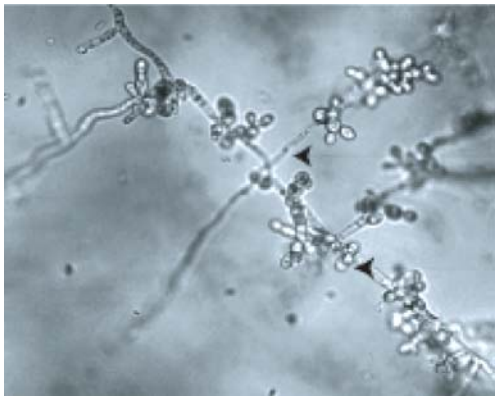
ANTIFUNGAL SUSCEPTIBILITY: *C. tropicalis* is generally susceptible to azoles and echinocandins, but variably susceptible to flucytosine. Few strains of *C. tropicalis* have been reported with high amphotericin B MIC.

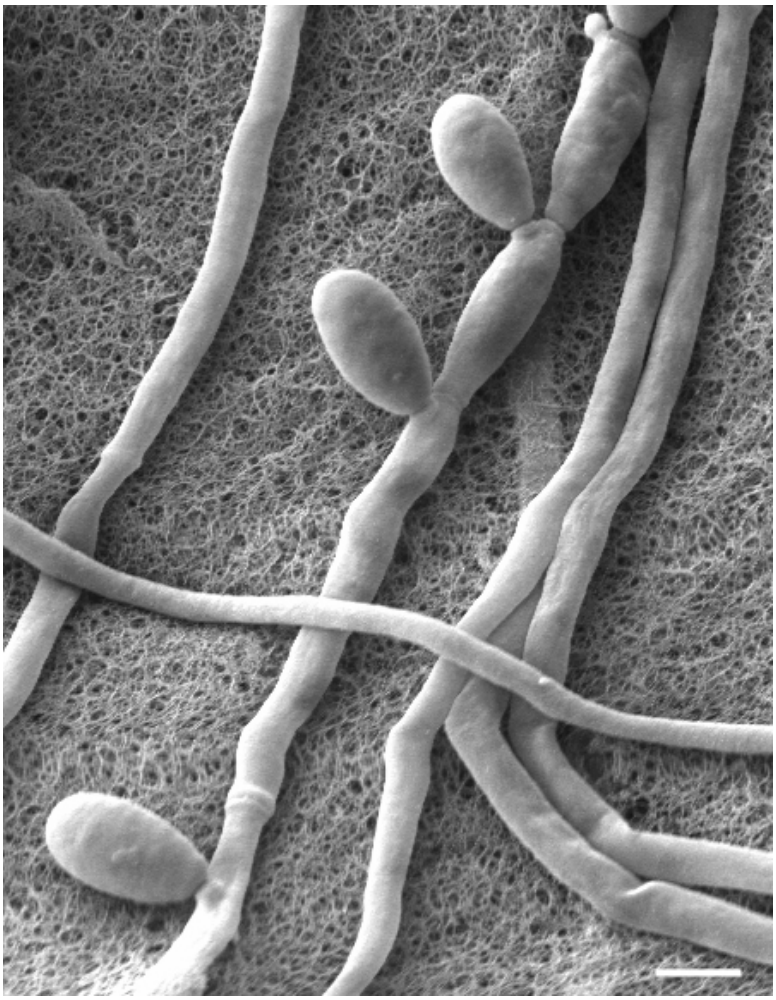
PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	56
Laboratories with incorrect ID:	0

Illustrations:

FIGURE 6. *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 (400× magnification). Scanning electron micrograph illustrates true and pseudohyphae (with constrictions) and blastoconidia (bar = 2 μm)





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Y-2 *Candida krusei*

Source: CSF / Tissue / Urine

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 was soft, cream to buff, glassy and wrinkled on Sabouraud's dextrose agar, after 7 days at 25°C (Figure 7).

MICROSCOPY: *C. krusei* showed branched pseudohyphae with elongated blastoconidia on Corn meal agar with Tween 80 (Figure 7).

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 were 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 were developed for the detection of clinically important yeasts in tissue specimens. PFGE, RFLP, and RAPD procedures were 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.

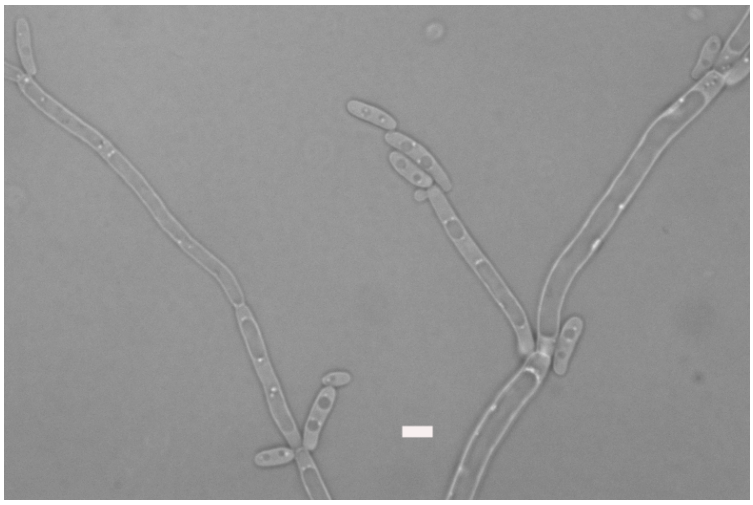
ANTIFUNGAL SUSCEPTIBILITY: *C. krusei* is susceptible to amphotericin B and flucytosine. *C. krusei* is innately resistant to fluconazole and variably resistant to other azoles such as itraconazole and ketoconazole, but not voriconazole. *C. krusei* is also susceptible to anidulafungin, micafungin and caspofungin.

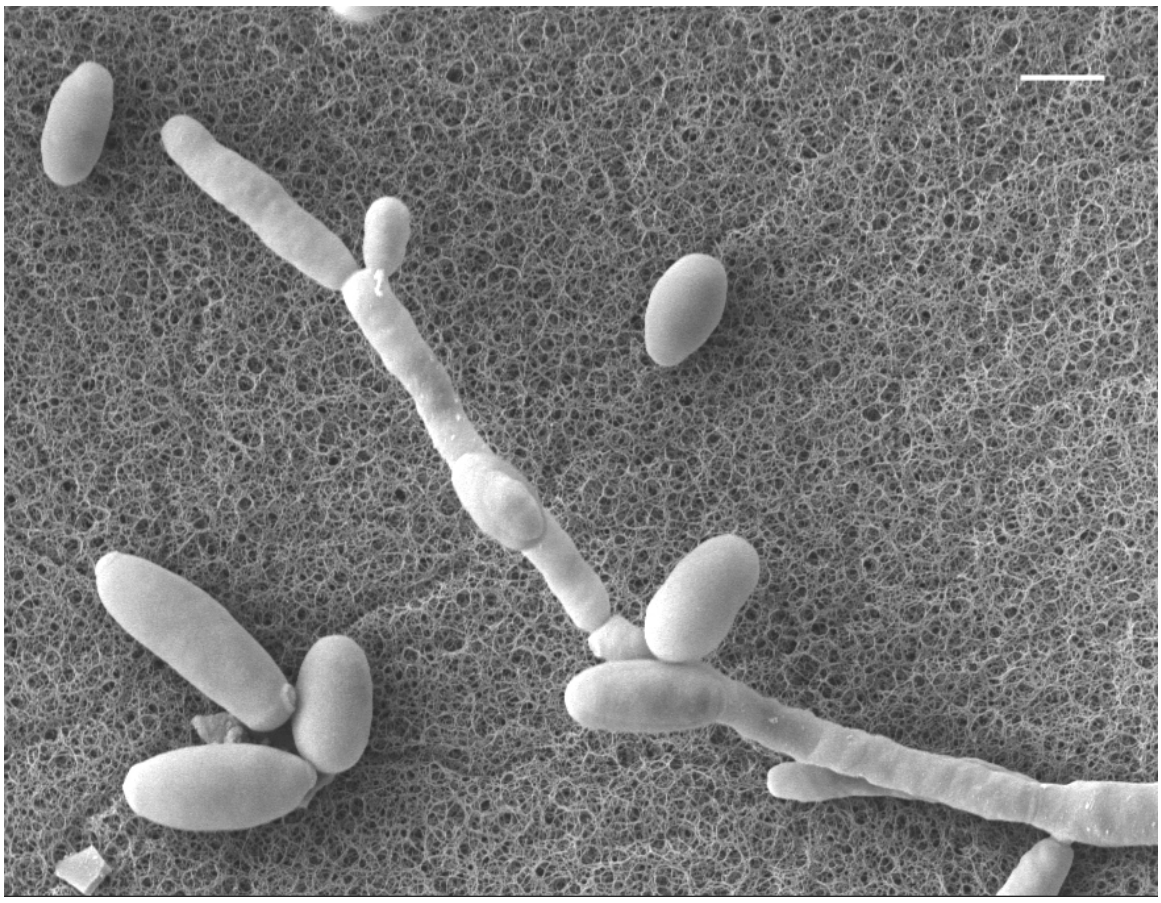
PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	51
Laboratories with incorrect ID:	05
(*Candida norvegensis*)	(2)
(*Candida kefyr*)	(1)
(*Candida lipolytica*)	(1)

Illustrations:

FIGURE 7. *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-3 *Candida kefyr*

Source: Vaginal Swab / Urine

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 appeared smooth, creamy, and soft on Sabouraud's dextrose agar after 3 to 5 days, 25°C (Figure 8).

MICROSCOPY: *Candida kefyr* showed abundant long pseudohyphae, and oval to elongated blastoconidia on Corn meal agar with Tween 80 (Figure 8). Ascospores within asci were 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 cycloheximide containing media. *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)

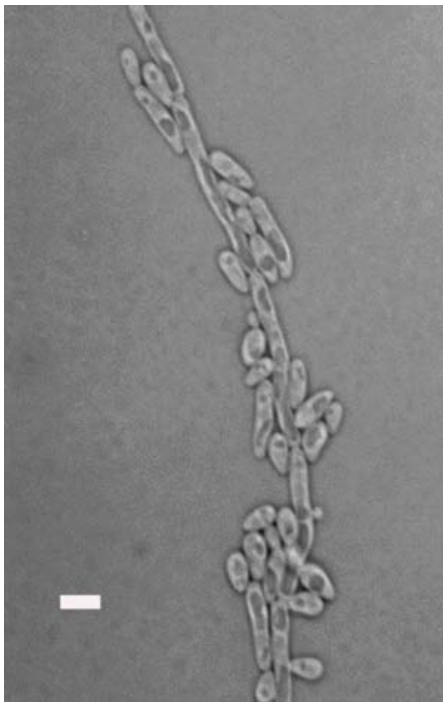
ANTIFUNGAL SUSCEPTIBILITY: *C. kefyr* is susceptible to amphotericin B, caspofungin, different azoles, and 5-fluorocytosine.

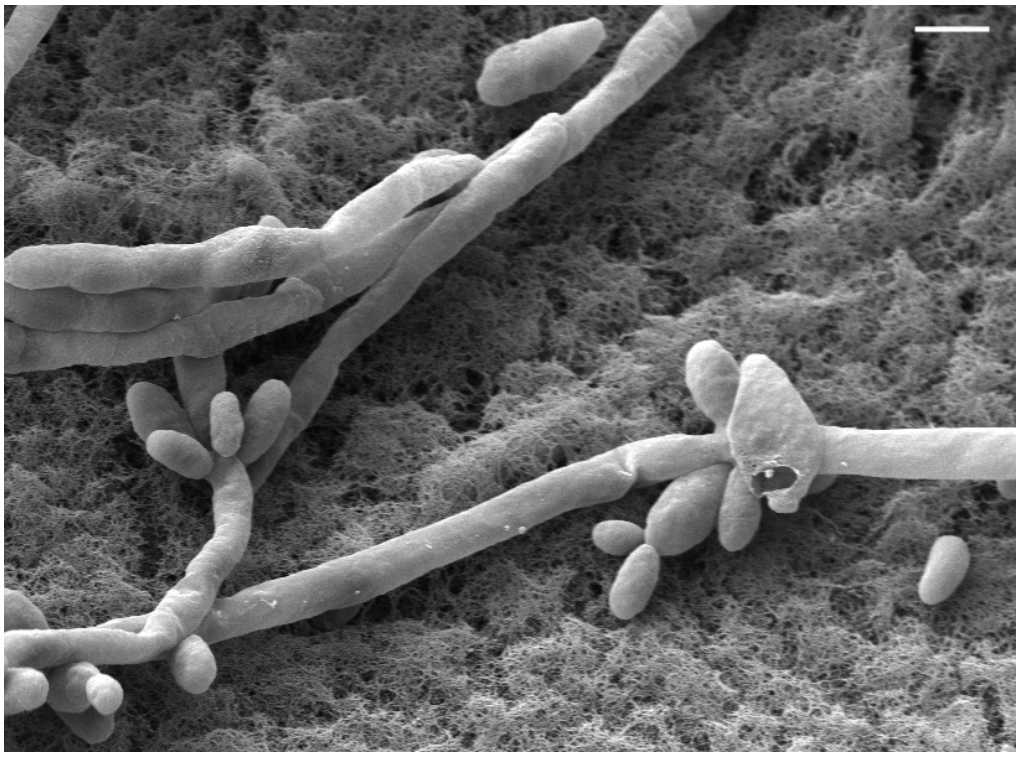
PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	55
Laboratories with incorrect ID:	01
(*Candida krusei*)	(1)

Illustrations:

FIGURE 8. *Candida kefyr* creamy, smooth colony 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).





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Y-4 *Candida glabrata*

Source: Wound / Sinus / 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. There is also a noticeable increase in antifungal resistant *C. glabrata* among hospitalized patients.

COLONY: *Candida glabrata* colony was white to cream, smooth and shiny on Sabouraud's dextrose agar, 3 to 5 days, 25°C (Figure 9).

MICROSCOPY: *Candida glabrata* blastoconidia were tiny, round or elliptical in shape on Cornmeal agar with Tween 80 (Figure 9).

DIFFERENTIATION: *C. glabrata* grows at 42°C, but it 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 (GenBank Accession no. AY198398).

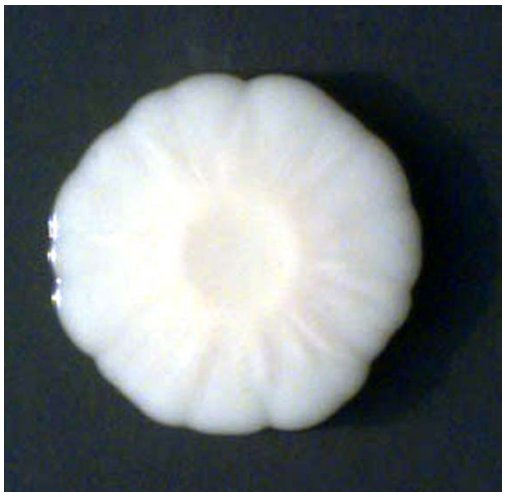
ANTIFUNGAL SUSCEPTIBILITY: *C. glabrata* is susceptible to amphotericin B, caspofungin, and 5-FC but resistant to azoles such as fluconazole and itraconazole.

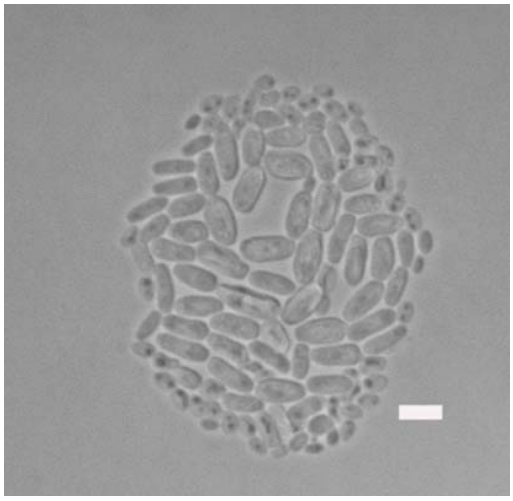
PARTICIPANT PERFORMANCE:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	56
Laboratories with incorrect ID:	0

Illustrations:

FIGURE 9. *Candida glabrata* colony white to cream, smooth and shiny on Sabouraud's dextrose agar, 4 days, 25°C. Microscopic morphology of *Candida glabrata* with elliptical-shaped blastoconidia on Corn meal agar with Tween 80 (bar = 5 μm).





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Y-5 *Candida parapsilosis*

Source: Blood / Urine / Nail

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

COLONY: *Candida parapsilosis* colony was white to cream, dull with smooth surface on Sabouraud's dextrose agar after 5 days at 25°C (Figure 10).

MICROSCOPY: *Candida parapsilosis* showed long, multibranched pseudohyphae, together with small elongated blastoconidia on corn meal agar with Tween 80 (Figure 10).

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

MOLECULAR TEST: PCR assay of ITS regions of rDNA was used to identify *C. parapsilosis* in clinical specimens. Chromosome length polymorphism and RAPD procedures were used to characterize the genetic diversity of this organism. The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with a reference strain of *C. parapsilosis* CBS 604 (Genebank accession no: AY391843).

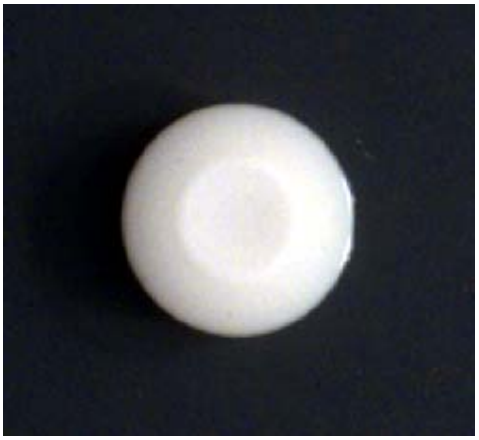
ANTIFUNGAL SUSCEPTIBILITY: *C. parapsilosis* is susceptible to amphotericin B, 5-flucytosine, caspofungin, and azoles such as fluconazole, ketocoanzole, itraconazole, and voriconazole. A few clinical isolates are reported as resistant to fluconazole.

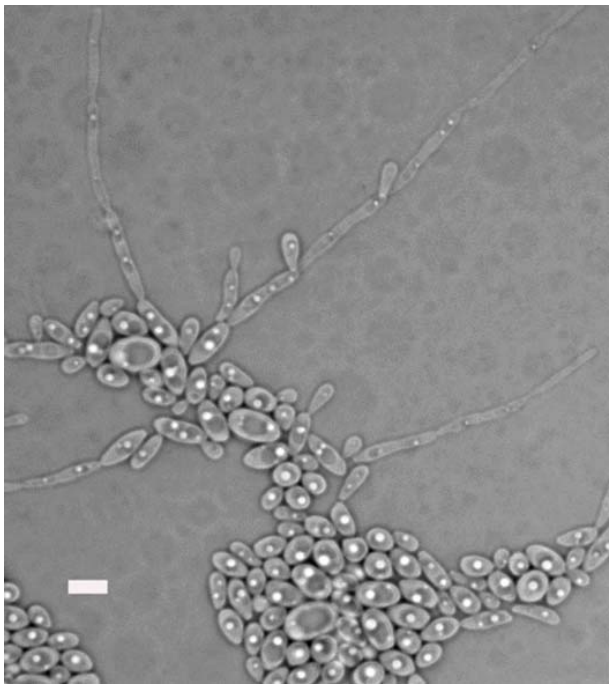
PARTICIPANT PERFORMANCE:

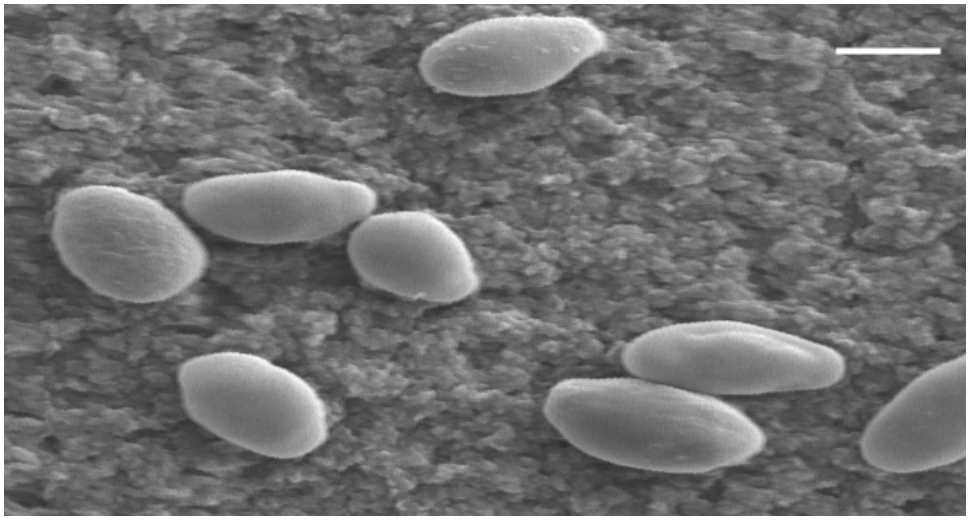
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	56
Laboratories with incorrect ID:	0

Illustrations:

FIGURE 10. *Candida parapsilosis* white to cream, smooth colony on Sabouraud's dextrose agar, seven-day-old, 25°C. Microscopic morphology of *Candida parapsilosis* with long, multibranched pseudohyphae together with small cluster of elongated blastoconidia on Corn meal agar with Tween 80 (bar = 5 µm). Scanning electron micrograph with blastoconidia (bar = 2 µm).







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DIRECT DETECTION (*Cryptococcus neformans* ANTIGEN TEST)

INTRODUCTION: In early 1960s, a simple, sensitive latex test, capable of detecting the capsular polysaccharide of C. *neoformans* in serum, was described and proven to be superior in sensitivity to the India ink mount. 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 for point-of-care testing of cryptococcosis from serum.

MATERIALS & METHODS: Sixty-eight laboratories participated in the September 27, 2011 direct detection antigen test event. One positive serum sample (Cn-Ag-4) with the titer of 1:256 for cryptococcal antigen was included. Titers within ± 2 dilutions of the reference and/or consensus results were the acceptable results for this event.

RESULTS: Overall, the performance of 68 laboratories was satisfactory in this test event. The consensus results for specimens Cn-Ag-1, Cn-Ag-2, Cn-Ag-3, and Cn-Ag-5 were negative as expected. Cn-Ag-4 was reported positive by majority of the participating laboratories with the acceptable titer ranges 1:64 - 1:1014. One laboratory each reported false positive result for specimen Cn-Ag-1 and Cn-Ag-5, respectively. One laboratory reported lower titer than the acceptable range for specimen Cn-Ag-4 (Table 1).

TABLE 1. Summary of laboratory performance for semi-quantitative detection of cryptococcal antigen

Method		Cn-Ag-4 Titers							
No. laboratories		32	64	80	128	200	256	512	1024
Latex Agglutination									
Immuno-Mycologics	8		3		1		3	1	
Meridien Diagnostic	43	1	6	1	10	1	18	5	1
Remel	12		3		5		3	1	
Total	63	1	12	1	16	1	24	7	1

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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 M27-A3, M27-S3 and M44-A, describe the current standard methods for antifungal susceptibility testing of pathogenic yeasts. Another resource for standardized method is the EUCAST Definitive Document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. The FDA approved devices for antifungal susceptibility testing of yeasts include Sensititre YeastOne Colorimetric Panel (Trek Diagnostic Systems Inc. Cleveland, OH) and Etest (bioMérieux, Inc., Durham, NC)). The following ten drugs are included in the Mycology Proficiency Test Program - amphotericin B, anidulafungin, caspofungin, flucytosine (5-FC), fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. The participating laboratories are allowed to select any number of antifungal drug(s) from this test panel based upon current practices in their facilities.

MATERIALS: *Candida parapsilosis* (S-1) was the analyte in the September 27, 2011 antifungal proficiency test event. Thirty laboratories participated in this event. The S-1 isolate was validated by all of the participating laboratories. The acceptable results for antifungal susceptibility testings were based on consensus MIC values or interpretation per NCCLS/CLSI guidelines or other publications.

COMMENTS: Only 5 of the 31 laboratories participating in this test event tested all 10 antifungal drugs and they reported 100% acceptable results. Acceptable results were MICs +/-2 dilutions of the reference laboratory results for any single drug. Fluconazole was the only drug tested by all 31 laboratories, but one laboratory failed to achieve acceptable results. Many laboratory only reported limited testing, but with acceptable results as follows: flucytosine (24 laboratories), amphotericin B (23 laboratories), caspofungin (22 laboratories), posacoanazole (18 laboratories), anidulafungin (17 laboratories), and micafungin (17 laboratories). Eight and six laboratories did not report any interpretation for amphotericin B and posaconazole, respectively.

TABLE 2. Laboratory Performance, September 2011

S- 1: *Candida parapsilosis* (M957)

Drug	Laboratories with acceptable responses / Total Laboratories (% acceptable responses)
Amphotericin B	23/23 (100%)
Anidulafungin	17/17 (100%)
Caspofungin	22/22 (100%)
Flucytosine (5-FC)	26/26 (100%)
Fluconazole	30/31 (97%)
Itraconazole	30/30 (100%)
Ketoconzole	5/5 (100%)
Micafungin	17/17 (100%)
Posaconazole	18/18 (100%)
Voriconazole	24/24 (100%)

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

S-1: *Candida parapsilosis* (M957)

Drug	No. labs	MIC (µg/ml)											
		0.008	0.015	0.03	0.047	0.06	0.12	0.25	0.38	0.5	1	2	4
Amphotericin B	23					1	1	5	1	14	1		
Anidulafungin	17										3	14	
Caspofungin	22							2		12	6	2	
Flucytosine (5-FC)	26			3	14		8	1					
Fluconazole	31						1			7	21	2	
Itraconazole	29			4		6	17	1			1		
Ketoconazole	4			2		1		1					
Micafungin	17									1	1	14	1
Posaconazole	18			6		12							
Voriconazole	24	4	17	3									

TABLE 4. Antifungal Susceptibility Interpretations Reported by the Participating Laboratories

S-1: *Candida parapsilosis* (M957)

Drug	Total No. Laboratories reporting	No. Laboratories reporting			
		Susceptible	Susceptible-dose dependent	Non-susceptible	No interpretation
Amphotericin B	23	15			8
Anidulafungin	17	17			
Caspofungin	22	22			
Flucytosine	26	26			
Fluconazole	32	31	1		
Itraconazole	30	30			
Ketoconazole	5	2			3
Micafungin	17	16		1	
Posaconazole	18	12			6
Voriconazole	24	24			

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR MOLDS

INTRODUCTION: Clinical laboratories perform susceptibility testing of pathogenic molds to determine their in vitro resistance to antifungal drugs. This test is also useful in conducting surveillance for evolving patterns of antifungal drug resistance in a healthcare facility. It is not clear at this juncture if the results of mold susceptibility testing have direct relevance in the selection of appropriate drugs for treatment. Clinical Laboratory Standards Institute (CLSI) document of M38-A2 describes the current standard methods for antifungal susceptibility testing of pathogenic yeasts. Another resource for standardized method is the EUCAST Technical Note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia–forming 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* M2036 was used as test analyte; it was obtained from a reference laboratory. Laboratories were free to choose any number of drugs and preferred test method. Three laboratories used CLSI Microdilution method, another three used YeastOne Colorimetric method. Please refer to Table 5 and 6 for summary.

COMMENTS: Six out of thirty laboratories, which hold antifungal susceptibility testing for yeasts permit, participated in this first ever graded event for molds. Acceptable results were MICs +/-2 dilutions of the reference laboratory results for any single drug. All the participating laboratories reported the MIC values within the acceptable ranges for amphotericin B, anidulafungin, fluconazole, itraconazole, posaconazole, and voriconazole. Only one laboratory reported higher MIC value for caspofungin than the acceptable range. The acceptable values for ketoconazole could not be generated since too few laboratories tested this drug.

TABLE 5. Mold Antifungal Susceptibility: Aspergillus fumigatus M2036.

Drugs	Acceptable MIC (µg/ml) Range	Reference laboratory MIC (µg/ml)	Participating laboratories MIC(µg/ml) range in previous event	Participating laboratories MIC (µg/ml) range in current event
Amphotericin B	0.12 – 2.0	0.5	0.12 – 2.0	0.5 – 1.0
Anidulafungin	0.004 – 0.06	0.015	0.008 – 8.0	0.015 – 0.06
Caspofungin	0.015 – 2.0	0.5	0.06 – 8.0	0.015 – 8.0
Fluconazole	≥ 64	64	64 – 512	64 – 256
Itraconazole	0.12 – 2.0	0.5	0.06 – 1.0	0.12 – 0.5
Ketoconzole	invalidated	1.0	2.0	1.0 – 8.0
Micafungin	0.004 – 0.06	0.015	0.008 – 8.0	0.008 – 0.06
Posaconazole	0.06 – 1.0	0.25	0.03 – 0.25	0.06 – 0.25
Voriconazole	0.12 – 2.0	0.5	0.25	0.12 – 1.0

TABLE 6. 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	8.0	32	64	128	256
Amphotericin B	6							3	3						
Anidulafungin	4		3		1										
Caspofungin	5		2			1		1			1				
Fluconazole	5												2		3
Itraconazole	6					1	3	2							
Ketoconazole	2								1		1				
Micafungin	4	2	1		1										
Posaconazole	5				1	3	1								
Voriconazole	5					2	1	1	1						

COLORS REPRESENT THE TESTING METHOD USED:

CLSI microdilution method

YeastOne Colorimetric method

Both CLSI microdilution and YeastOne Colorimetric methods

Further Reading:

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