Mycology Proficiency Testing Program

Test Event Critique
January 2013

Wadsworth Center
NEW YORK STATE DEPARTMENT OF HEALTH
Mycology Laboratory

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Mycology Laboratory

Mycology Laboratory at the Wadsworth Center, New York State Department of Health (NYSDOH) is a reference diagnostic laboratory for the fungal diseases. The laboratory services include testing for the dimorphic pathogenic fungi, unusual molds and yeasts pathogens, antifungal susceptibility testing including tests with research protocols, molecular tests including rapid identification and strain typing, outbreak and pseudo-outbreak investigations, laboratory contamination and accident investigations and related environmental surveys. The Fungal Culture Collection of the Mycology Laboratory is an important resource for high quality cultures used in the proficiency-testing program and for the in-house development and standardization of new diagnostic tests.

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

Mycology Laboratory Staff and Contact Details

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Mycology Proficiency Testing Program (PTP)

CATEGORY DESCRIPTION

COMPREHENSIVE: This category is for the laboratories that examine specimens for the pathogenic molds and yeasts encountered in a clinical microbiology laboratory. These laboratories are expected to identify fungal pathogens to the genus and species level (for detail, please see mold and yeast master lists). Laboratories holding this category may also perform antifungal susceptibility testing, antigen detection, molecular identification or other tests described under any of the categories listed below.

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

> **Identification yeast only:** This category is for laboratories that isolate and identify pathogenic yeasts or yeast-like fungi to genus and species level (for detail, please see yeast master list). Laboratories holding this category may also perform susceptibility testing on yeasts. These laboratories are expected to refer mold specimens to another laboratory holding Mycology – Comprehensive permit.
>
> **Antigen detection:** This category is for laboratories that perform direct antigen detection methods.
>
> **Molecular methods:** This category is for laboratories that use FDA-approved or lab-developed molecular methods for detection, identification, typing, characterization or determination of drug resistance against fungal pathogens. Laboratories using molecular methods under another Restricted permit category (e.g. Restricted: Antigen detection) or those holding a Comprehensive category permit are exempt from this-category.

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

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

Comprehensive

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

Restricted

Identification Yeast Only

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

Antigen Detection

- Antigen detection of *Cryptococcus neoformans**

Molecular Methods

- No proficiency testing is offered at this time.

TEST SPECIMENS & GRADING POLICY

Test Specimens

At least two strains of each mold or yeast species are examined for inclusion in the proficiency test event. The colony morphology of molds is studied on Sabouraud dextrose agar. The microscopic morphologic features are examined by potato dextrose agar slide cultures. The physiological characteristics such as cycloheximide sensitivity and growth at higher temperatures are investigated with appropriate test media. The strain that best demonstrates the morphologic and physiologic characteristics typical of the species is included as a test analyte. Similarly, two or more strains of yeast species are examined for inclusion in the proficiency test. The colony morphology of all yeast strains is studied on corn meal agar with Tween 80 plates inoculated by Dalmau or streak-cut method. Carbohydrate assimilation is studied with the API 20C AUX identification kit (The use of brand and/or trade names in this report does not constitute an endorsement of the products on the part of the Wadsworth Center or the New York State Department of Health). The fermentations of carbohydrates, i.e., glucose, maltose, sucrose, lactose, trehalose, and cellobiose, are also documented using classical approaches. Additional physiologic characteristics such as nitrate assimilation, urease activity, and cycloheximide sensitivity are investigated with the appropriate test media. The strain that best demonstrates the morphologic and physiologic characteristics of the proposed test analyte is included as test analyte. The morphologic features are matched with molecular identification using PCR and nucleotide sequencing of ribosomal ITS1 – ITS2 regions.

Grading Policy

A laboratory's response for each sample is compared with the responses that reflect 80% agreement of 10 referee laboratories and/or 80% of all participating laboratories. The referee laboratories are selected at random from among hospital laboratories participating in the program. They represent all geographical areas of New York State and must have a record of excellent performance during the preceding three years. The score in each event is established by total number of correct responses submitted by the laboratory divided by the number of organisms present plus the number of incorrect organisms reported by the laboratory multiplied by 100 as per the formula shown on the next page.

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

For molds and yeast specimens, a facility can elect to process only those analytes that match the type of clinical materials included within the scope of the facility's standard operating procedures (SOP). Similarly, the participating laboratory can elect to provide only genus level identification if it reflects the SOP for patient testing in the concerned facility. In all such instances, a maximum score of 100 will be equally distributed among the number of test analytes selected by the laboratory. The rest of the score algorithm will be similar to the aforementioned formula.

Acceptable results for antifungal susceptibility testing are based on the consensus/reference laboratories' MIC values within +/- 2 dilutions and the interpretation per CLSI (NCCLS) guidelines or related, peer-reviewed publications. One yeast species is to be tested against following drugs: amphotericin B, anidulafungin, caspofungin, flucytosine, fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. The participating laboratories are free to select any number of antifungal drugs from the test panel based upon test practices in their facilities. A maximum score of 100 is equally distributed to account for the drugs selected by an individual laboratory. If the result for any drug is incorrect then laboratory gets a score of zero for that particular test component or set.

For *Cryptococcus neoformans* antigen test, laboratories are evaluated on the basis of their responses and on overall performance for all the analytes tested in the Direct Detection category. The maximum score for this event is 100. Appropriate responses are determined by 80% agreement among participant responses. Target values and acceptable ranges are mean value +/- 2 dilutions; positive or negative answers will be acceptable from laboratories that do not report antigen titers. When both qualitative and quantitative results are reported for an analyte, ten points are deducted for each incorrect result. When only qualitative OR quantitative results are reported, twenty points are deducted from each incorrect result.

A failure to attain an overall score of 80% is considered unsatisfactory performance. Laboratories receiving unsatisfactory scores in two out of three consecutive proficiency test events may be subject to 'cease testing'.

TEST ANALYTE MASTER LISTS

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 neither include all molds that might be encountered in a clinical laboratory nor is 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 relevant 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 neither includes all yeasts that might be encountered in a clinical laboratory nor is it intended to be used for the competency assessment of the laboratory personnel in diagnostic mycology

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

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	Other acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
M-1	*Exserohilum* species	**(Not validated)**		49/63 (78%)
M-2	*Phialophora* species	*Phialophora* species	*Phialophora verrucosa*	60/63 (95%)
M-3	*Chrysosporium* species	*Chrysosporium* species		56/63 (89%)
M-4	*Fusarium* species	*Fusarium* species	*Fusarium oxysporum* species complex *Fusarim solani* species complex	58/63 (92%)
M-5	*Rhizopus* species	*Rhizopus* species	*Rhizopus oryzae*	62/63 (98%)

Mycology – Yeast Only

	Specimen key	Validated specimen	Other acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
Y-1	*Rhodotorula mucilaginosa*	*Rhodotorula mucilaginosa*		48/54 (890%)
Y-2	*Trichosporon asahii*	*Trichosporon asahii*	*Trichosporon* species	55/55 (100%)
Y-3	*Candida glabrata*	*Candida glabrata*		55/55 (100%)
Y-4	*Candida albicans*	*Candida albicans*		55/55 (100%)
Y-5	*Geotrichum candidum*	*Geotrichum candidum*	*Geotrichum* species *Geotrichum klebahnii*	54/55 (98%)

Mycology – Direct detection (*Cryptococcus* Antigen Test)

	Specimen key (Titer)	Validated specimen	Correct responses / Total laboratories (% correct responses)	
			Qualitative	Quantitative
Cn-Ag-1	Positive (1:64)	Positive (1:64)	68/68 (100%)	NA
Cn-Ag-2	Negative	Negative	66/68 (97%)	NA
Cn-Ag-3	Positive (1:4)*	Negative	68/68 (100%)	NA
Cn-Ag-4	Negative	Negative	67/68 (99%)	NA
Cn-Ag-5	Positive (1:16)	Positive (1:16)	67/68 (99%)	NA

*Artificial CSF spiked with very low titer of *Cryptococcus* antigen produced discrepant results and therefore, both positive and negative results were accepted in this event.

Antifungal Susceptibility Testing for Yeast (S-1: *Candida parapsilosis* M958)

Drugs	Acceptable MIC (μg/ml) Range	Acceptable interpretation	Laboratories with acceptable responses/ Total laboratories (% correct responses)
Amphotericin B	0.125 – 1	Susceptible / No interpretation	20/20 (100%)
Anidulafungin	0.25 – 2	Susceptible	16/16 (100%)
Caspofungin	0.125 – 2	Susceptible	20/21 (95%)
Flucytosine (5-FC)	0.016 – 0.25	Susceptible	24/24 (100%)
Fluconazole	0.06 – 2	Susceptible	30/30 (100%)
Itraconazole	0.008 – 0.125	Susceptible	28/28 (100%)
Ketoconazole	0.006 – 0.125	Susceptible / No interpretation	4/4 (100%)
Micafungin	0.25 – 2	Susceptible	16/16 (100%)
Posaconazole	0.008 – 0.125	Susceptible / No interpretation	15/15 (100%)
Voriconazole	0.008 – 0.03	Susceptible	24/24 (100%)

Commercial Device Usage Statistics:

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

Device	**No. laboratories**
Yeast Identification*	
AMS Vitek	1
API 20C AUX	44
Bruker MicroFlex LT Biotyper	1
Dade Behring MicroScan Rapid Yeast Identification Panel	4
Remel RapID Yeast Plus System	4
Vitek2	28
Antifungal Susceptibility*	
Disk diffusion	1
Etest	1
Vitek II	1
YeastOne – Mold	2
YeastOne – Yeast	26
CLSI Microbroth dilution method – Yeast	2
CLSI Microbroth dilution method – Mold	2
Cryptococcal antigen	
Immuno-Mycologics Latex Cryptococcus Antigen Detection System	9
Immuno-Mycologics CrAg Lateral Flow Assay	2
Meridien BioScience Cryptococcal Antigen Latex Agglutination System (CALAS)	44
Meridien BioScience Premier Cryptococcal Antigen Detection (EIA)	3
Remel Cryptococcal Antigen Latex Test	10

*Include multiple systems used by some laboratories

MOLD DESCRIPTIONS

M-1 *Exserohilum* species

Source: Tissue / Corneal / Skin
Clinical Significance: *Exserohilum* species are dematiaceous ('darkly pigmented') fungi commonly found in soil and on grasses as pathogens. Three species – *E. longirostratum*, *E. mcginisii,* and *E. rostratum* are known to cause humans disease both in immunocompetent individuals and in immunocompromised patients. The clinical spectrum includes cutaneous and subcutaneous lesions, keratitis, nasal polyps, and disseminated infections. *Exserohilum rostratum* has been identified as one of the predominant pathogens in the multistate outbreak of fungal meningitis and other fungal infections associated with contaminated steroid injections from May 2012.

Colony: *Exserohilum* spp. colonies grow moderately fast, velvety to wooly, grey, brownish-black to black on Sabouraud's dextrose agar at 25°C (Figure 1).

Microscopy: Lactophenol - Cotton blue mount shows septate brown hyphae. Conidia are brown, large, and cylindrical with multiple septa and have a strong protruding, truncate hilum and the septum above the hilum is usually thickened and dark (Figure 1). It sporulates well on Tape water agar.

Differentiation: *Exserohilum* spp. can be differentiated from *Bioplaris* and *Drechslera* species by its conidia with strongly protruding hilum.

Molecular test: Internal transcribed spacer (ITS) regions of ribosomal DNA can be used for the identification of *Exserohilum* spp. Real-time PCR assay has been developed to rapidly identify *E. rostratum* from clinical specimens.

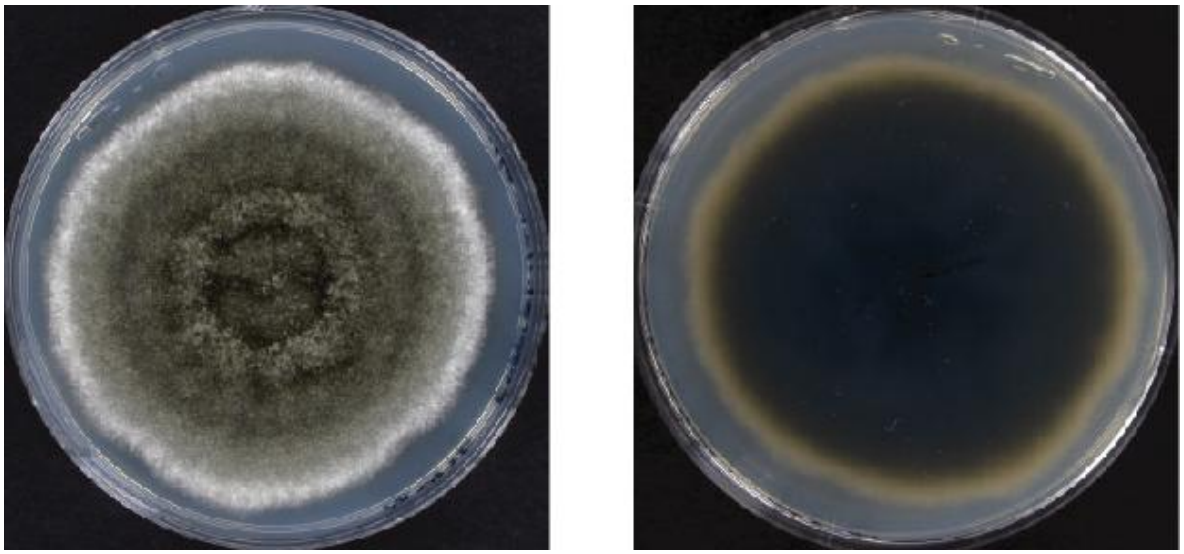
Antifungal susceptibility: In general, all the antifungal drugs tested showed relatively low MICs against *Exserohilum* isolates, with only a few exceptions for echinocandins. Caspofungin, micafungin, and anidulafungin showed relatively high MICs against a few isolates.

Participant performance:

Referee Laboratories with correct ID:	08
Laboratories with correct ID:	49
Laboratories with incorrect ID:	14
(*Bioplaris* species)	(7)
(*Scytalidium* species)	(3)
(*Alternaria* species)	(1)
(*Drechslera* species)	(1)
(*Pithomyces* species)	(1)

Illustrations:

Figure 1. Seven-day-old, velvety to wooly colony of *Exserohilum* species on Sabouraud's dextrose agar; the reverse of colony appears black (upper panel). Microscopic morphology of *Exserohilum* species showing large multiseptated conidia with strong protruding hilum (lower panel; bar = 50 µm).



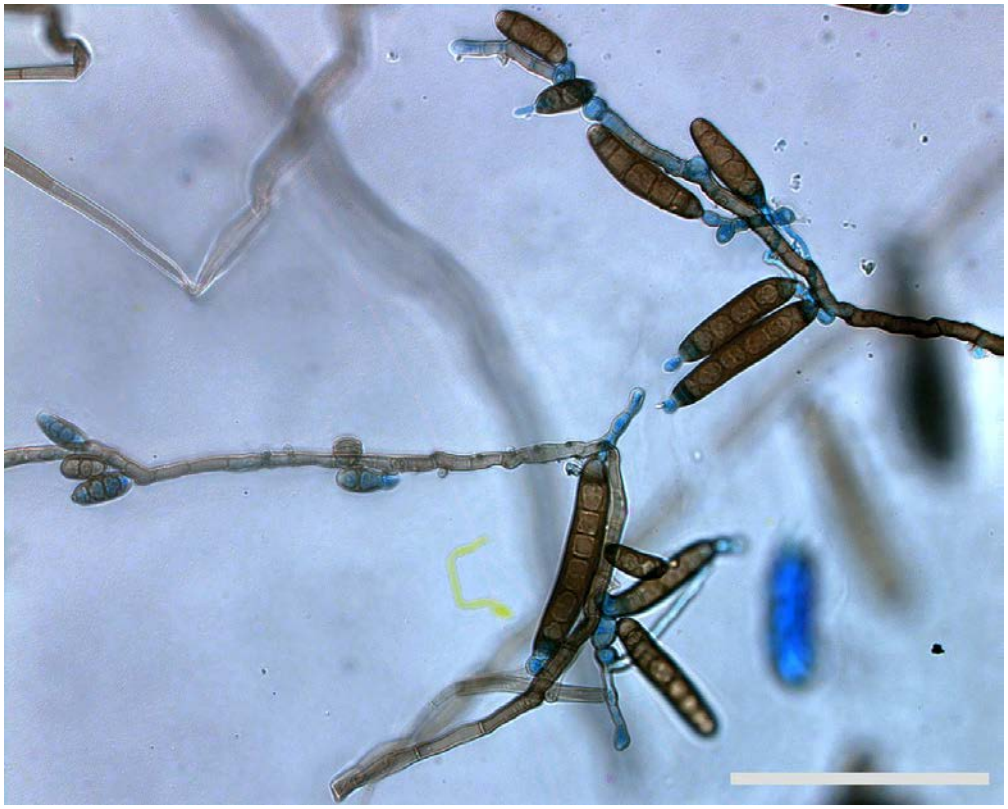
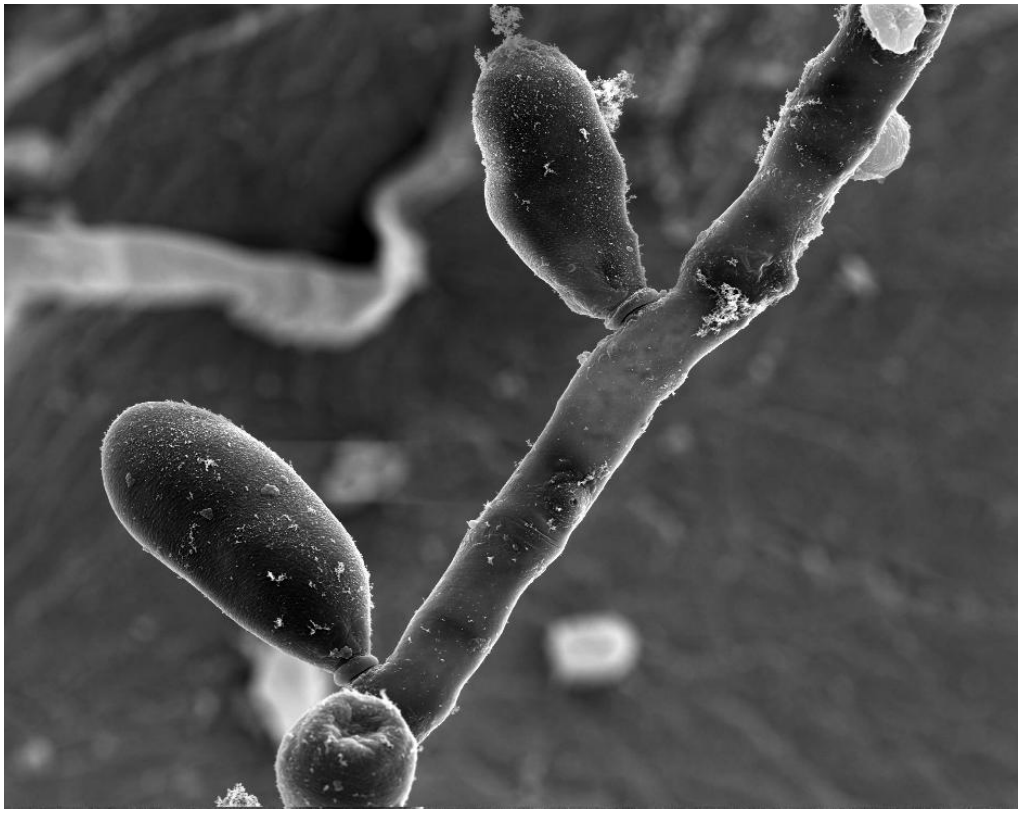
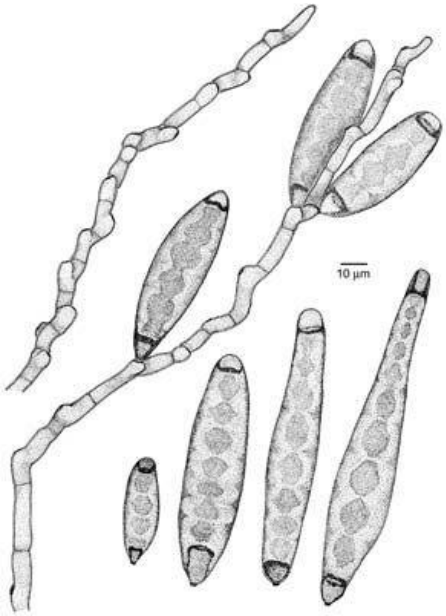


Figure 1A. Scanning electron micrograph of *Exserohilum* species.





http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=3808

Further reading:

Casadevall A, Pirofski LA. 2013. *Exserohilum rostratum* fungal meningitis associated with methylprednisolone injections. *Future Microbiol*. 8: 135-137.

Centers for Disease Control and Prevention. Health Hazards Associated with Laundry Detergent Pods — United States, May–June 2012. 2012. MMWR 61: 825-829.

da Cunha KC, Sutton DA, Gené J, Capilla J, Cano J, Guarro J. 2012. Molecular identification and in vitro response to antifungal drugs of clinical isolates of *Exserohilum*. *Antimicrob Agents Chemother*. 56: 4951-4954.

Joseph NM, Kumar MA, Stephen S, Kumar S. 2012. Keratomycosis caused by *Exserohilum rostratum*. *Indian J Pathol Microbiol*. 55: 248-249.

Juhas E, Reyes-Mugica M, Michaels MG, Grunwaldt LJ, Gehris RP. 2012. *Exserohilum* Infection in an Immunocompromised Neonate. *Pediatr Dermatol*. [Epub ahead of print]

Lin SC, Sun PL, Ju YM, Chan YJ. 2009. Cutaneous phaeohyphomycosis caused by *Exserohilum rostratum* in a patient with cutaneous T-cell lymphoma. *Int J Dermatol*. 48: 295-298.

Lyons JL, Gireesh ED, Trivedi JB, Bell WR, Cettomai D, Smith BR, Karram S, Chang T, Tochen L, Zhang SX, McCall CM, Pearce DT, Carroll KC, Chen L, Ratchford JN, Harrison DM, Ostrow LW, Stevens RD. 2012. Fatal *Exserohilum* meningitis and central nervous system vasculitis after cervical epidural methylprednisolone injection. *Ann Intern Med*. 157: 835-836.

Saint-Jean M, St-Germain G, Laferrière C, Tapiero B. 2007. Hospital-acquired phaeohyphomycosis due to *Exserohilum rostratum* in a child with leukemia. *Can J Infect Dis Med Microbiol*. 18: 200-202.

Zhao Y, Petraitiene R, Walsh TJ, Perlin DS. 2013. A real-time PCR assay for rapid detection and quantification of *Exserohilum rostratum*, a causative pathogen of fungal meningitis associated with injection of contaminated methylprednisolone. *J Clin Microbiol*. 51: 1034-1036.

M-2 *Phialophora* species

Source: Finger / Bronchial Wash

Clinical significance: *Phialophora verrucosa* causes chromoblastomycosis and phaeohyphomycosis, which include cutaneous infections, subcutaneous cysts, keratitis, endocarditis, arthritis, osteomyelitis, cerebral infection, fatal hemorrhage, and disseminated infection.

Colony: *Phialophora verrucosa* grows slowly. The colony is wooly to velvety, initially white and later becoming dark grey-green or black on Sabouraud's dextrose agar at 25°C for 7 days. The reverse is iron gray to black (Figure 2).

Microscopy: Lactophenol - Cotton blue mount shows septate hyphae, phialides, and conidia. The hyphae are branched, and hyaline to brown. The phialides are flask-or bottle-shaped, and are terminally or laterally located on the hyphae. Phialides of *Phialophora* typically have clearly visible collarettes at their tips. The shape of the collarette varies in different species of *Phialophora*. Conidia produced on collarettes are unicellular, hyaline or brown, smooth, and round, oval or cylindrical in shape. These conidia accumulate in masses at the apices of the phialides with collarettes, giving the appearance of a vase of flowers (Figure 2).

Differentiation: *Phialophora* spp. differ from *Exophiala* spp. by having phialides, while *Exophiala* spp. form annellides. *Phialophora* spp. differ from *Wangiella* spp. by having phialides with collarettes.

Molecular test: Identification of *Phialophora* spp. by large subunit ribosomal DNA D1/D2 domain sequence analysis was reported.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *P. verrucosa* isolate WM04.477 (GenBank accession no. AJ853749.1).

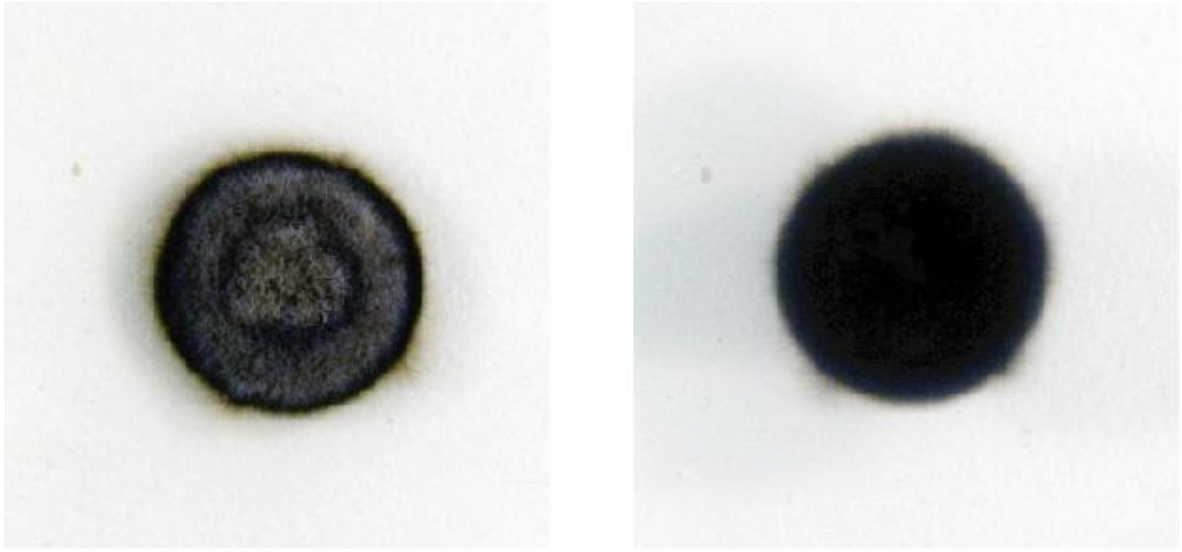
Antifungal susceptibility: *Phialophora* spp. are susceptible to amphotericin B, terbinafine, itraconazole, and voriconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	60
Laboratories with incorrect ID:	03
(*Cladosporium* species)	(1)
(*Exophiala* species)	(1)
(*Fonsecaea* species)	(1)

Illustrations:

Figure 2. Seven-day-old, black colony of *Phialophora verrucosa* on Sabouraud's dextrose agar. at 25℃ (upper panels). Microscopic morphology of *Phialophora verrucosa* showing the phialides with vase-shaped collarette and conidia accumulating at the tip of phialides (bar = 25 µm; lower panel).



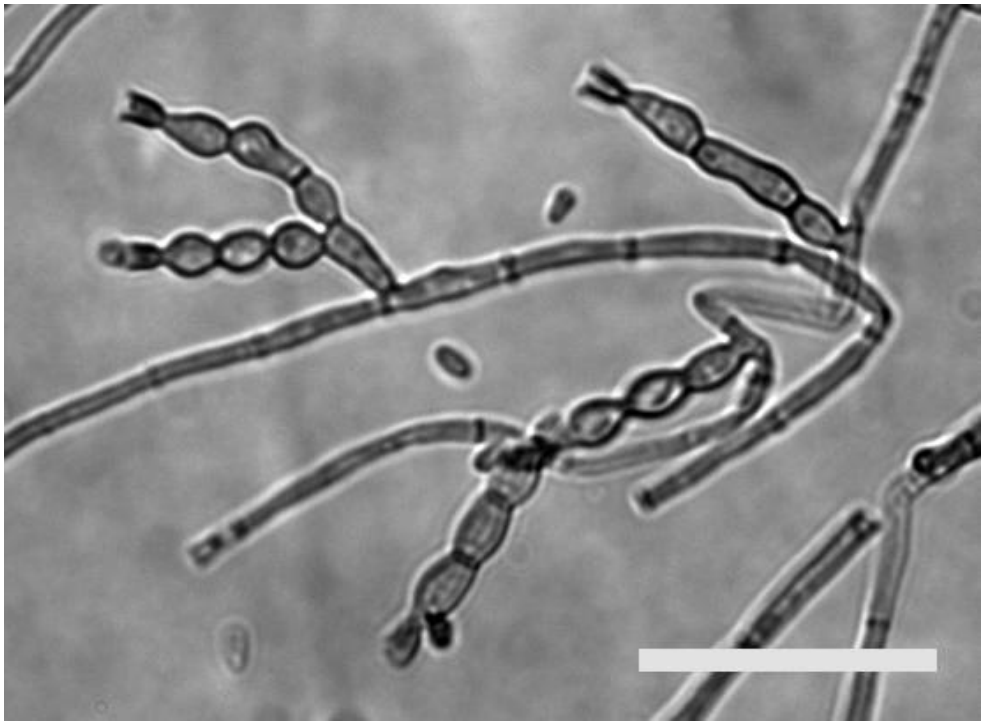
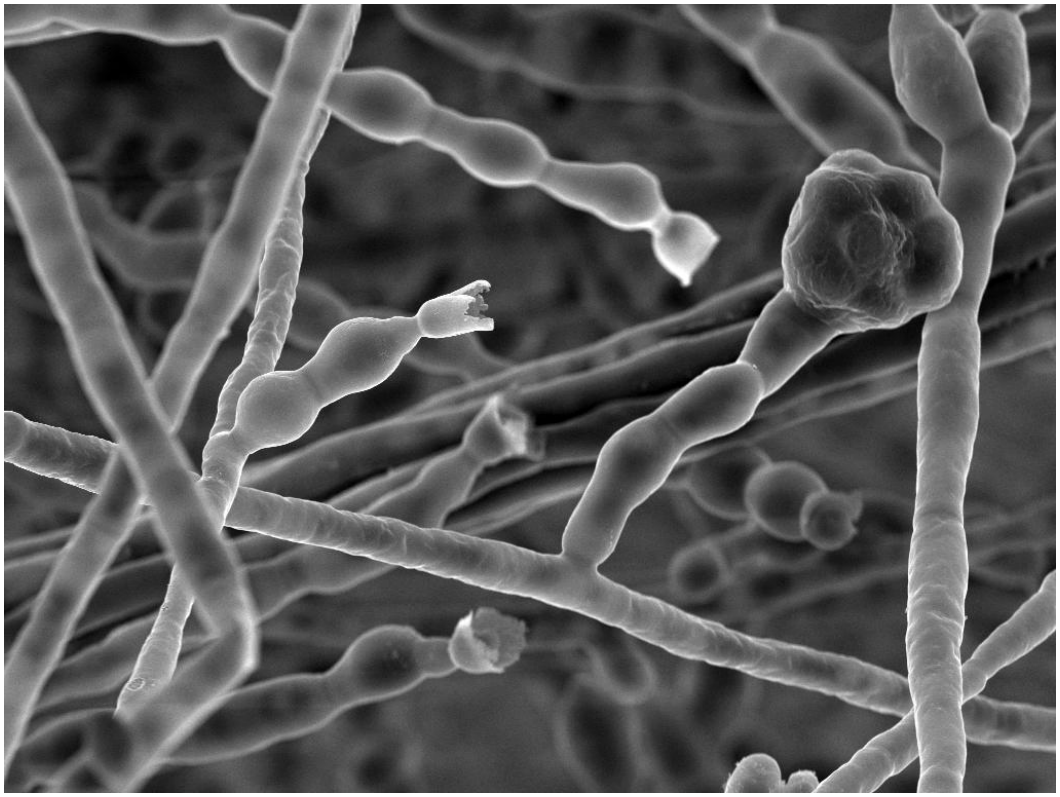
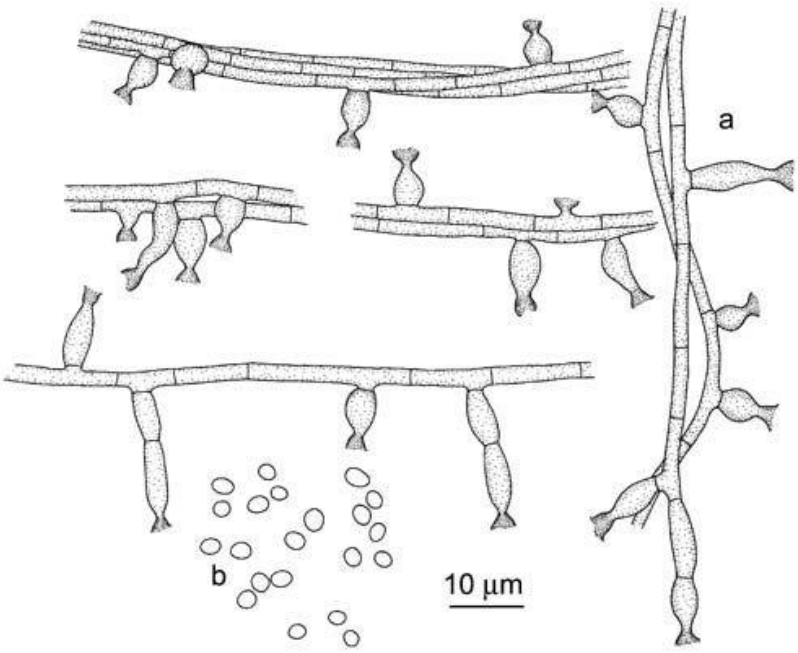


Figure 2A. Scanning electron micrograph of vase-shaped collarette and conidia of *Phialophora verrucosa* on Sabouraud's dextrose agar (upper panel). Line drawings of *Phialophora verrucosa* (lower panel).





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M-3 *Chrysosporium* species

Source: Nail / Chest

Clinical significance: *Chrysosporium* sp is occasionally reported from skin and nail infection. Invasive *Chrysosporium* infection of the nose and paranasal sinuses in an immunocompromised host has also been reported.

Colony: *Chrysosporium* sp. grows moderately fast. Colony is white to cream color on the surface and powdery to granular texture on Sabouraud's dextrose agar at 25°C. Reverse yellow or buff (Figure 3).

Microscopy: Lactophenol - Cotton blue mount shows hyaline septate hyphae. Ovoid or club-shaped conidia with broad truncated bases are seen either singly or in short chains borne directly on hyphae, or in short conidiophores (Figure 3).

Differentiation: *Chrysosporium* sp. is distinct from *Emmonsia* sp. in not developing adiaspores at 37°C. It does not display thermal dimorphism and is negative with specific nucleic acid probe, which serves to differentiate it from *Blastomyces dermatititidis*. *Chrysosporium* sp. grows on the media with cycloheximide and is urease-positive, which distinguished it from *Sporotrichum* sp. Please refer to Table 1 for details.

Molecular test: Internal transcribed spacer (ITS) regions can be used for *Chrysosporium* sp. identification.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Chrysosporium articulatum* UAMH 4320 (Genebank accession number: AJ007841).

Antifungal susceptibility: Limited information is available. In general, *Chrysosporium* sp. is susceptible to amphotericin B, itraconazole, ketoconazole, and voriconazole. Fluconazole had higher MIC to *Chrysosporium* sp.

Participant performance:

Referee Laboratories with correct ID:	09
Laboratories with correct ID:	56
Laboratories with incorrect ID:	07
(*Scedosporium apiospermum* species complex)	(2)
(*Scedosporium* species)	(2)
(*Epidermophyton floccosum*)	(1)
(*Fusarium* species)	(1)
(*Scedosporium apiospermum*)	(1)

TABLE 1: Differentiation of *Chrysosporium* species from some related fungi.

Characteristic	*Chrysosporium* sp.	*Emmonsia parva* var. *parva* and var. *crescens*	*Sporotrichum* sp.	*Blastomyces dermatitidis*
Growth on cycloheximide medium (25°C)	No growth	Growth	No growth	Growth
Chlamydospores (25°C)	Absent	Absent	Spherical, up to 60 μm	Absent
at 37°C	No adiaspores No yeast form	Adiaspores (40 – 200 μm) No yeast form	Chlamycospores No yeast form	Yeast form with broad-based budding (8 –30 μm)
B. dermatitidis GenProbe	Negative	Negative	Negative	Positive

Illustrations:

Figure 3. *Chrysosporium* sp. white to cream colored powdery to granular on Sabouraud's dextrose agar, 25°C; the reverse is pale to yellow (upper panel). Microscopic morphology of *Chrysosporium* sp. showing hyaline septate hyphae, ovoid or club-shaped conidia with broad truncated bases singly or in short chains borne directly on hyphae or in short conidiophores (lower panel; bar = 25 μm).

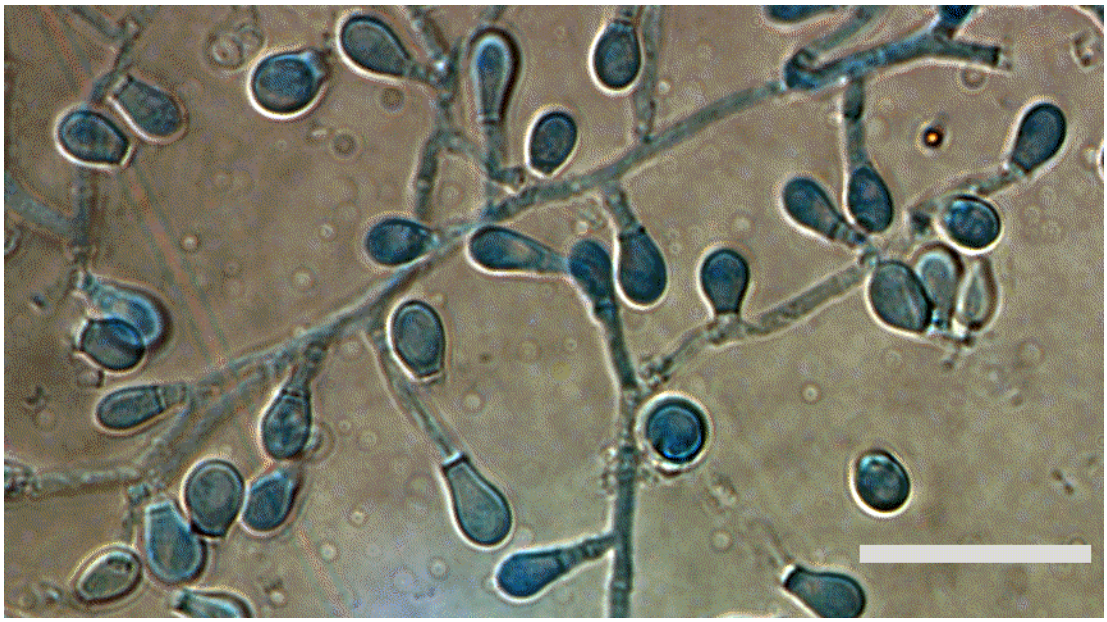
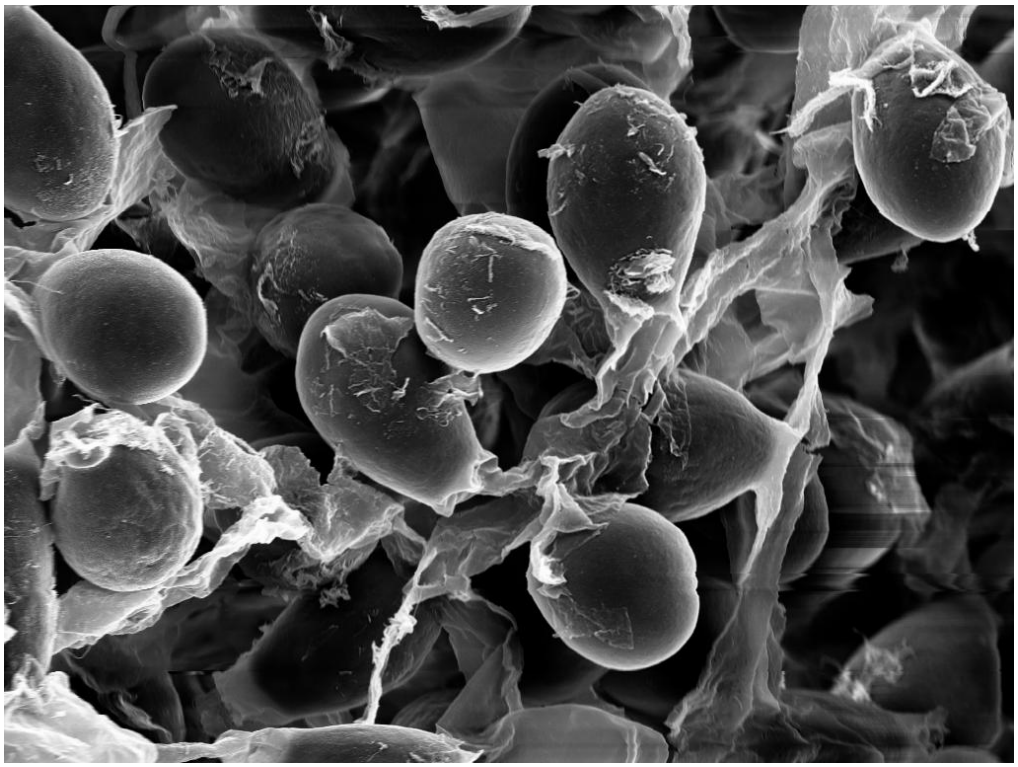
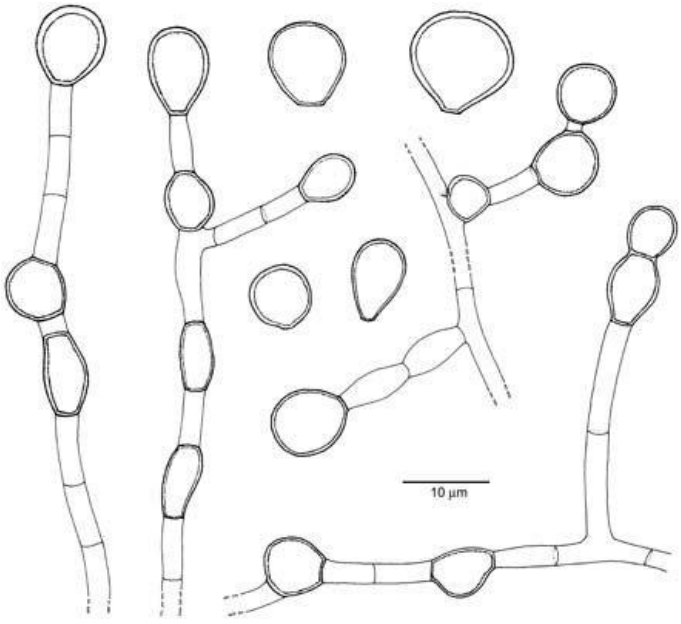


Figure 3A. Scanning electron micrograph of *Chrysosporium* sp. (upper panel). Line drawing with details of *Chrysosporium inops* (lower panel).





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M-4 *Fusarium* species

Source: Sputum / Toenail / Tissue

Clinical significance: A frequent casual agent of keratitis, endophthalmitis, and onychomycosis in healthy individuals. It has been reported from peritonitis and disseminated infection in immunocompromised patients. Most common etiologic agents of human infections are *F. oxysporum* species complex (FOSC) and *F. solani* species complex (FSSC).

Colony: *Fusarium* grows fast. Colony is white, pinkish to purplish in color, wooly with orange, to red–violet reverse on Sabouraud's dextrose agar (Figure 4).

Microscopy: Lactophenol - Cotton blue mount shows septate hyphae, with short or long phialides. Microconidia are ovoid, and macroconidia are septate and curved-boat/banana-shaped (Figure 4).

Differentiation: *Fusarium* species produce curved, septate macroconidia along with single-cell microconidia, which distinguish them from other hyphomycetes, especially *Acremonium* species.

Molecular test: PCR method for rapid detection and identification of *Fusarium* species from culture and clinical samples was described. Pan-fungal PCR, followed by nested PCR with species–specific primers was reported for rapid detection of *Fusarium* DNA in ocular samples.

Antifungal susceptibility: Most clinical isolates are susceptible to amphotericin B. Some isolates are variably susceptible to azoles.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	58
Laboratories with incorrect ID:	06
(*Acremonium* species)	(4)
(*Trichophyton* species)	(1)

Illustrations:

Figure 4. Wooly, orange to pinkish colony of *Fusarium* sp. on Sabouraud's dextrose agar, 25°C (upper panel). Microscopic morphology of *Fusarium* sp. with curved microconidia (bar = 25 μm; lower panel)

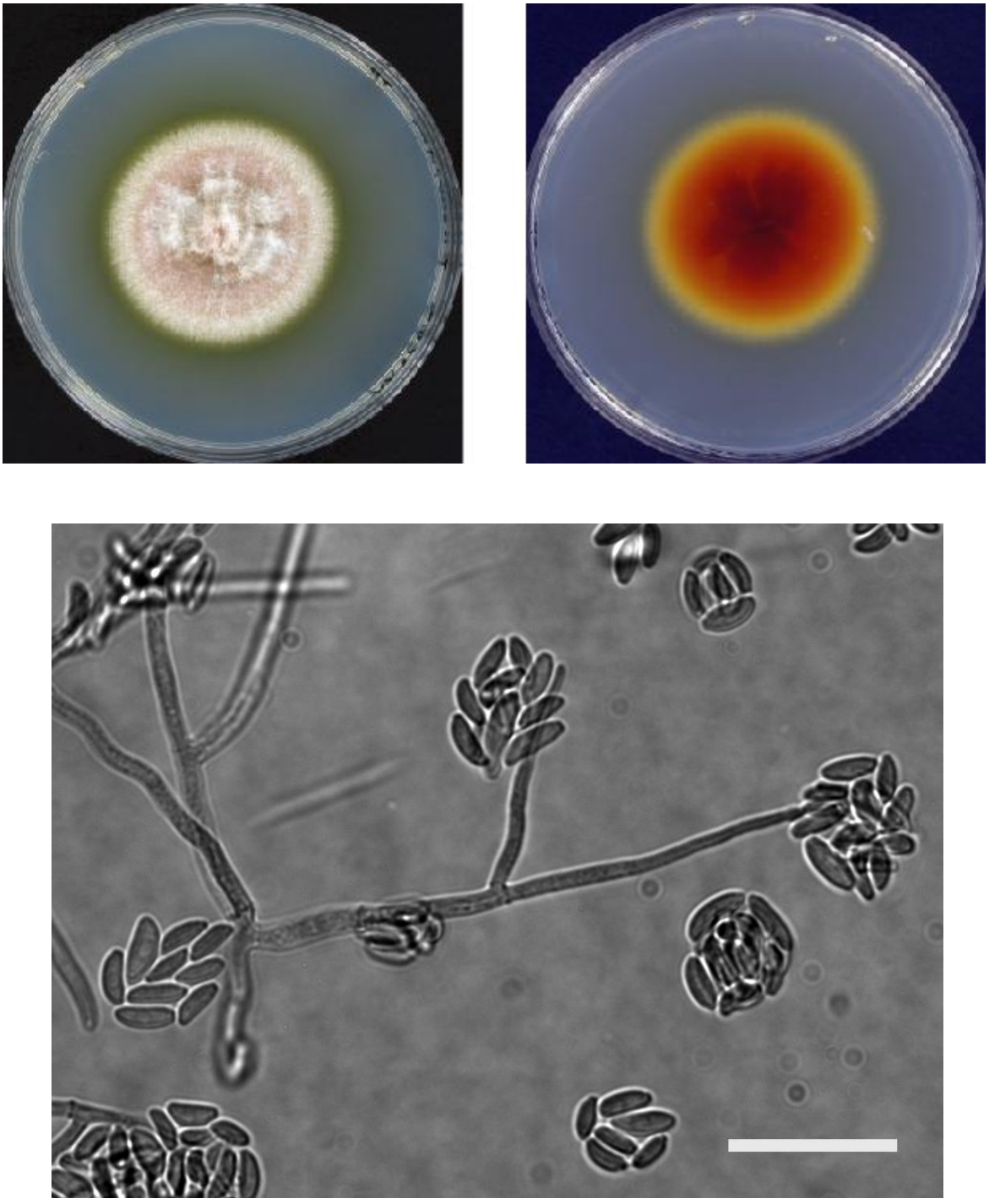
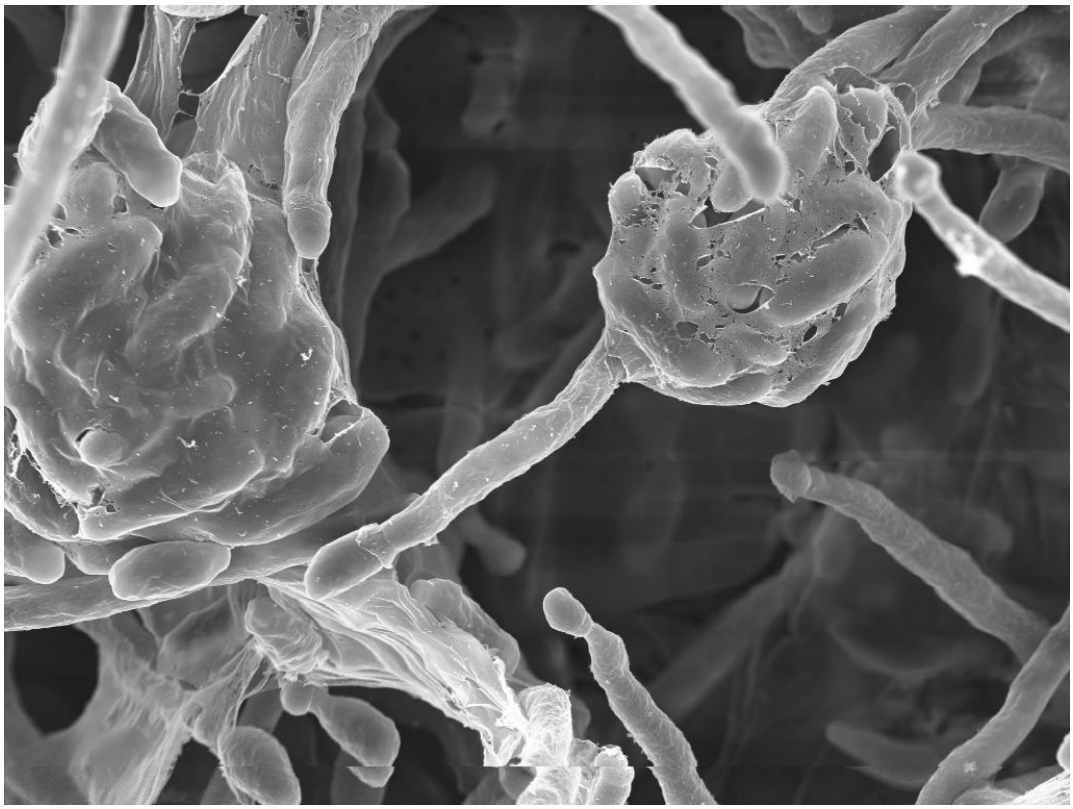
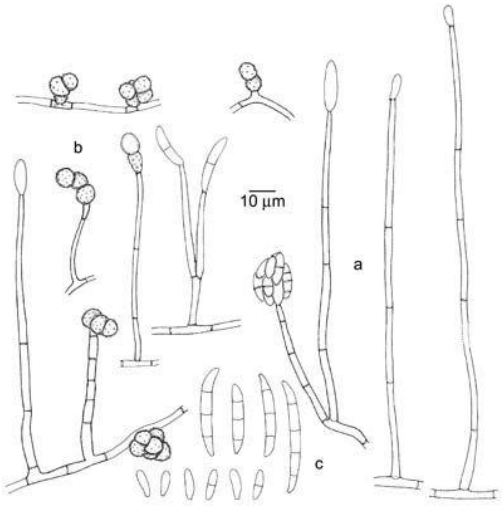


Figure 4A. Scanning electron micrograph of *Fusarium* spceies highlighting characteristic macroconidida (upper panel). Line drawing with details of *Fusarium solani* (lower panel).





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M-5 *Rhizopus oryzae*

Source: Lung / Nail / Urine

Clinical significance: *Rhizopus oryzae* is one the most common organisms isolated from patients with zygomycosis. It causes angioinvasion, thrombosis, infarction, and necrosis of the involved tissues. The sites of infection most often involved are sinuses and rhinocerebral structures. Disseminated disease can involve virtually any organ in the body, with the skin, central nervous system, liver, spleen, and kidney being most common.

Colony: *R. oryzae* grows rapidly with floccose aerial mycelia covers whole plate on Sabouraud's dextrose agar within a few days at 25°C, grayish in color on surface and yellow to light brown on reverse (Figure 5).

Microscopy: Lactophenol cotton blue mount shows broad, aseptate hyphae, either single or tufts of brown sporangiophores (conidiophores) arising from hyphae (stolons) opposite well-developed rhizoids (root like structures). Sporangiophores end in sporangia with a round columella (vesicle, enlarged at the apex), producing round to oval sporangiospores or sexual spores (Figure 5).

Differentiation: *R. oryzae* is distinguished from other zygomycetes by the presence of well-developed rhizoids situated opposite sporangiophores. Sporangiophores are unbranched and in tufts unlike in *Mucor, Rhizomucor,* and *Absidia*. *Rhizopus* spp. produces striated or grooved sporangiospores, which is useful in differentiating of *Rhizopus* from *Absidia, Mucor,* and *Thamnidum* spp., all of which produce smooth sporangiospores. Please refer to Table 2 for details.

Molecular test: PCR assay for the rapid and accurate identification of the agents of mucormycosis has been reported.

Antifungal susceptibility: Most clinical isolates are susceptible to amphotericin B.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	62
Laboratories with incorrect ID:	01
(*Mucor* species)	(1)

Illustrations:

Figure 5. Grayish color colony of *Rhizopus oryzae* on Sabouraud's dextrose agar, 25°C; the reverse of the colony is yellow to light brown (upper panel). Microscopic morphology of *Rhizopus oryzae* showing collumella and rhizoids present and ovoid sporangiospores (bar = 50 μm; lower panel).

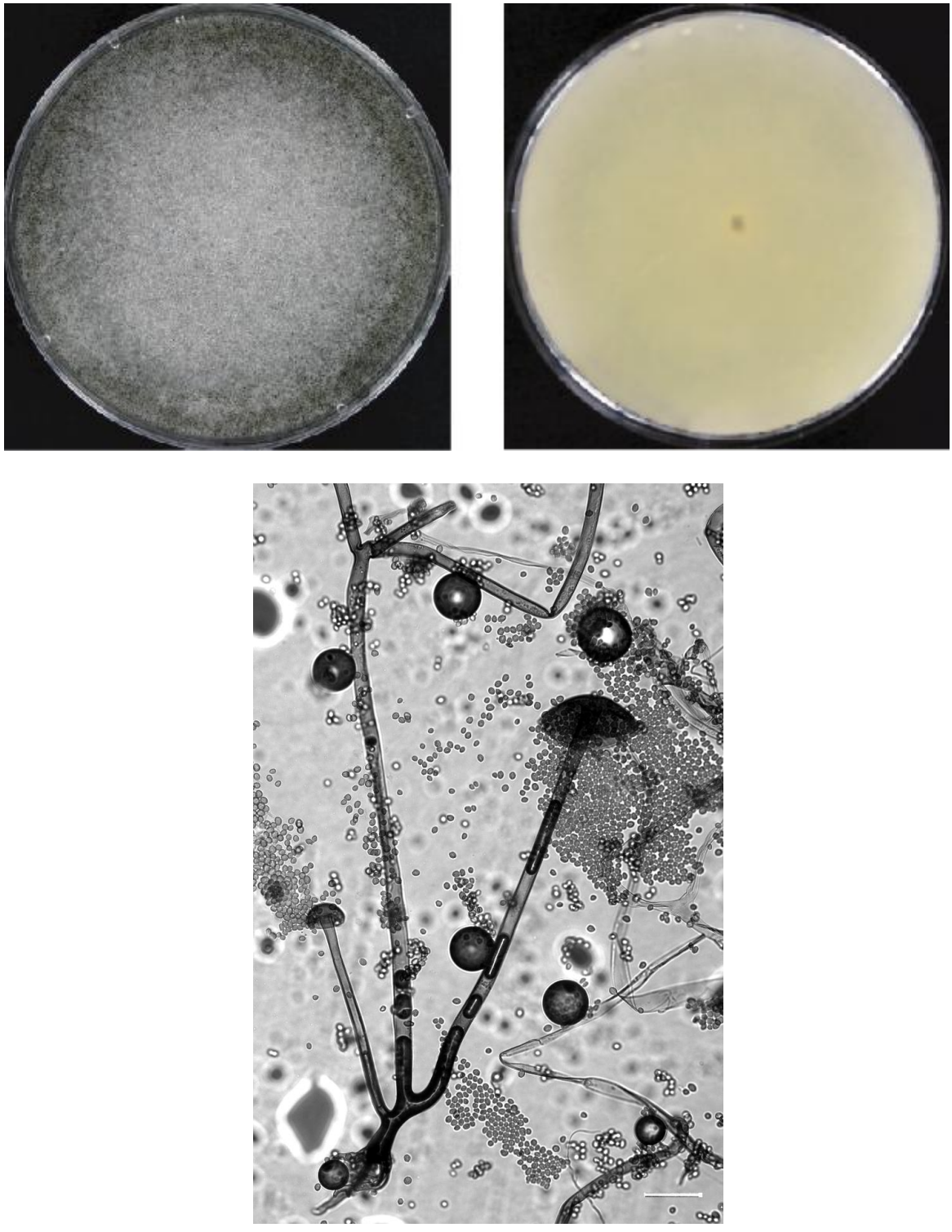

Figure 5A. Scanning electron micrograph of *Rhizopus oryzae* (upper panel). Line drawing with details of *Rhizopus oryzae* (lower panel).

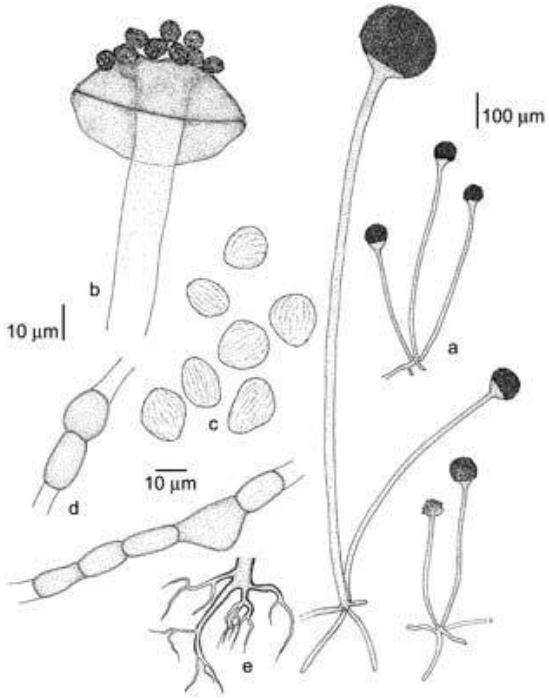

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Table 2. Scheme for differentiation of various genera of zygomycetes pathogenic for humans and animals

Genus	Rhizoids	Conidiophores	Sporangia	Columella	Apophysis	Conidia
Absidia	Present	Branched	Pyriform	Hemi-spherical	Present	Globose, smooth
Mucor	Absent	Branched – single or Multiple	Globose	Various forms – globose, elongated	Absent	Globose - cylindrical
Rhizopus	Present	Single or group	Globose, gray – brown	Sub-globose	Present, but inconspicuous	Angular, striated
Rhizomucor	Present	Sympodial	Globose, gray	Sub-globose, brown	Absent	Sub-globose, small

YEAST DESCRIPTIONS

Y-1 *Rhodotorula mucilaginosa*

Source: Blood / Nail / Stool

Clinical significance: *Rhodotorula mucilaginosa* is an uncommon cause of catheter-associated fungemia, dialysis-related peritonitis, and post surgery ventriculitis, endocarditis and meningitis.

Colony: *R. mucilaginosa* colony is smooth, moist, soft, pink to coral red on Sabouraud's dextrose agar at 25°C (Figure 6).

Microscopy: *R. mucilaginosa* forms oval to round yeast cells, sometimes in short chains on corn meal agar with Tween 80. Rarely, a faint capsule and rudimentary pseudohyphae are also observed (Figure 6).

Differentiation: *R. mucilaginosa* does not ferment any carbohydrate, grows at 37ºC, but does not grow on media containing cycloheximide. It forms pink pigment, thereby differentiating it from other yeast species. It does not produce ballistoconidia, thus distinguishing it from *Sporobolomyces* species. *R. mucilaginosa* does not assimilate nitrate or nitrite, which distinguishes it from *R. glutinis*.

Molecular test: Using species-specific oligonucleotide primers, PCR identification of the basidiomycetous yeasts *Cryptococcus neoformans*, *Trichosporon cutaneum*, and *R. mucilaginosa* can be done from single and mixed yeast populations.

The ribosomal ITS1 region of the test isolate showed 100% nucleotide identity with *Rhodotorula mucilaginosa* S22834 (Genebank accession number: EU871493).

Antifungal susceptibility: *R. mucilaginosa* is susceptible to amphotericin B and 5-fluorocytosine variably susceptible to itraconazole, and resistant to fluconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	48
Laboratories with incorrect ID:	06
(*Rhodotorula glutinis*)	(6)

Illustrations:

Figure 6. *Rhodotorula mucilaginosa,* colony smooth, moist, soft, pink to coral red on Sabouraud's dextrose agar, 25°C. Microscopic morphology on corn meal agar with Tween 80, showing oval to round blastoconidia (bar = 25 μm).

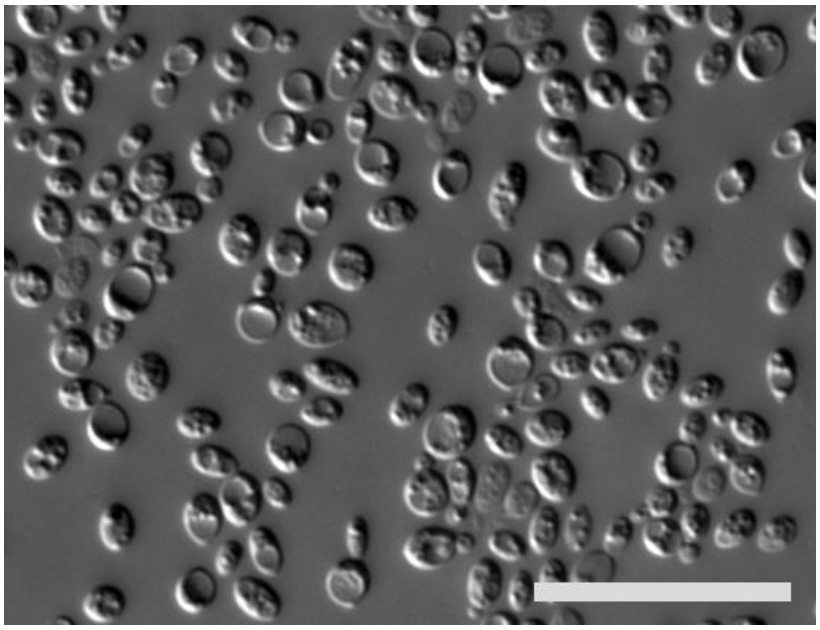
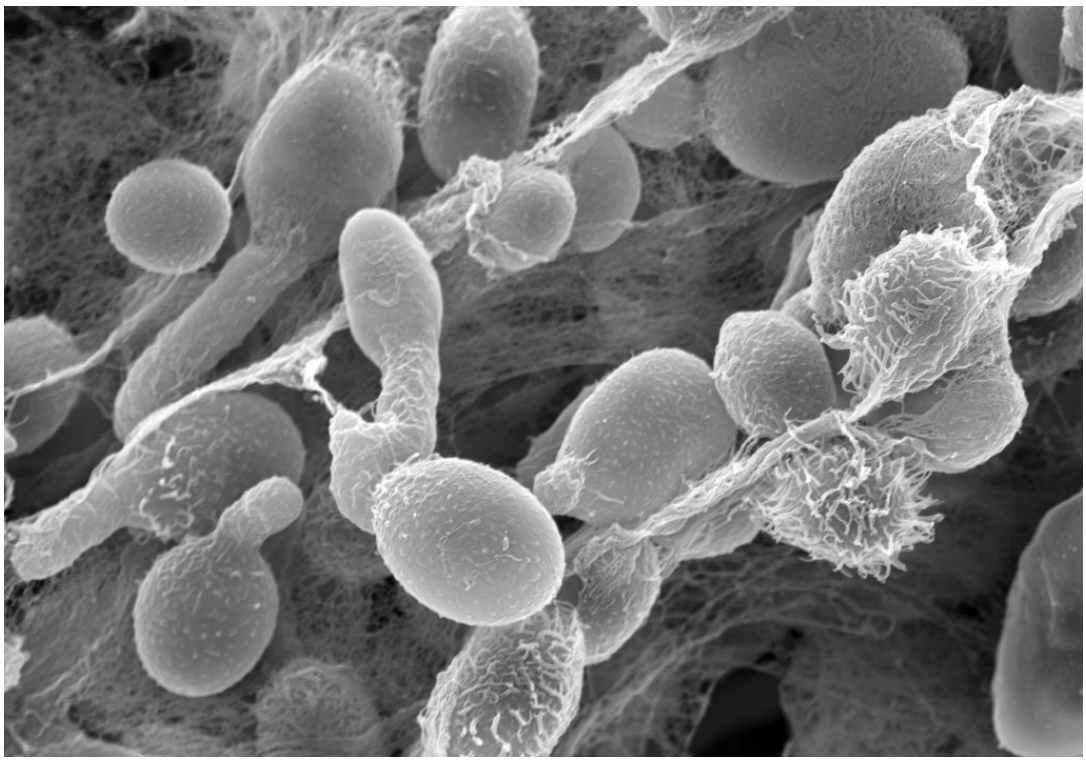


Figure 6A. Scanning electron micrograph of *Rhodotorula mucilaginosa* illustrates blastoconidia.



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Y-2 *Trichosporon asahii*

Source: Catheter / Nail / Urine

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

Colony: *T. asahii* colony is white to yellowish. The surface is wrinkled, velvety on Sabouraud's dextrose agar at 25°C (Figure 7).

Microscopy: – On corn meal agar with Tween 80, *T. asahii* produces true and pseudohyphae with blastoconidia singly or in short chains. Rectangular-to-oval arthroconidia are prominent; they originate by fragmentation of hyphae and hyphal branches (Figure 7).

Differentiation: *T. asahii* is nonfermentative, urease-positive, nitrate-negative, cycloheximide resistant, and metabolically active for assimilation of a wide range of carbohydrates. It can be distinguished from *Geotrichum candidum* by its wooly colony and production of urease.

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

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

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

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	55
Laboratories with incorrect ID:	0

Illustrations:

Figure 7. *Trichosporon asahii*, white to yellowish colony with wrinkled surface on Sabouraud's dextrose agar, 25°C. Microscopic morphology on corn meal agar showing arthroconidia (bar = 25 μm).

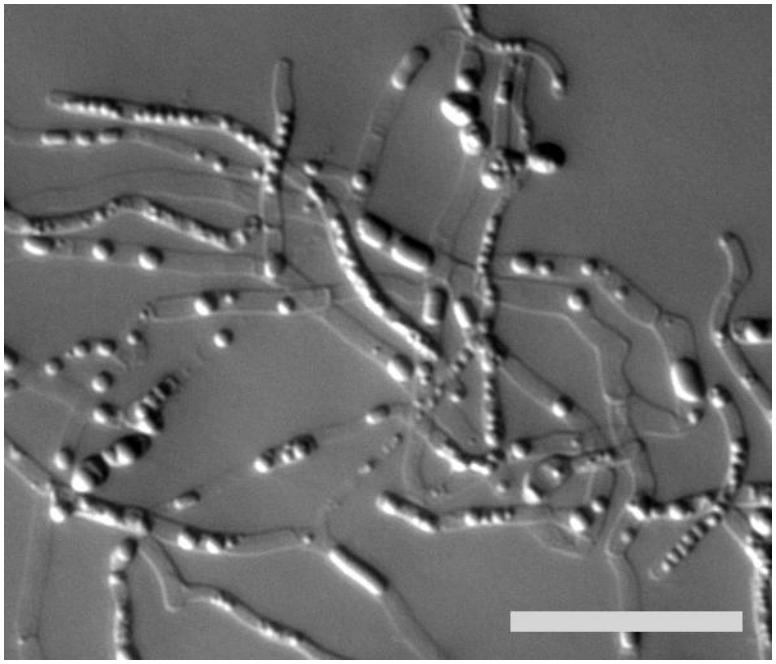
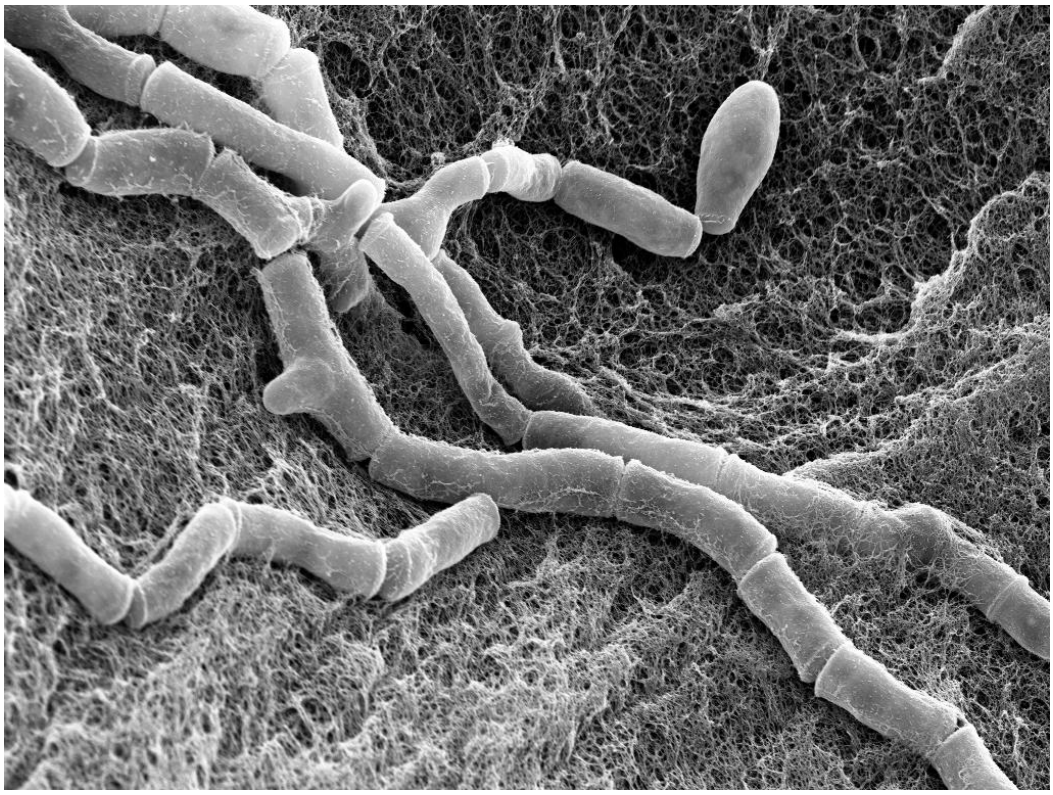


Figure 7A. Scanning electron micrograph illustrates arthroconidia.



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Y-3 *Candida glabrata*

Source: Urine / Blood / Lung wash

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

Colony: *C. glabrata* colony is white to cream, smooth and shiny on Sabouraud's dextrose agar after 5 days at 25°C (Figure 8).

Microscopy: *C. glabrata* shows tiny, round or elliptical shape blastoconidia on corn meal agar with Tween 80 (Figure 8).

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

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

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

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

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	55
Laboratories with incorrect ID:	0

Illustrations:

Figure 8. *Candida glabrata* white and shiny colony on Sabouraud's dextrose agar, 25°C. Microscopic morphology of *Candida glabrata* with small elliptical shaped blastoconidia on corn meal agar with Tween 80 (bar = 25 μm).

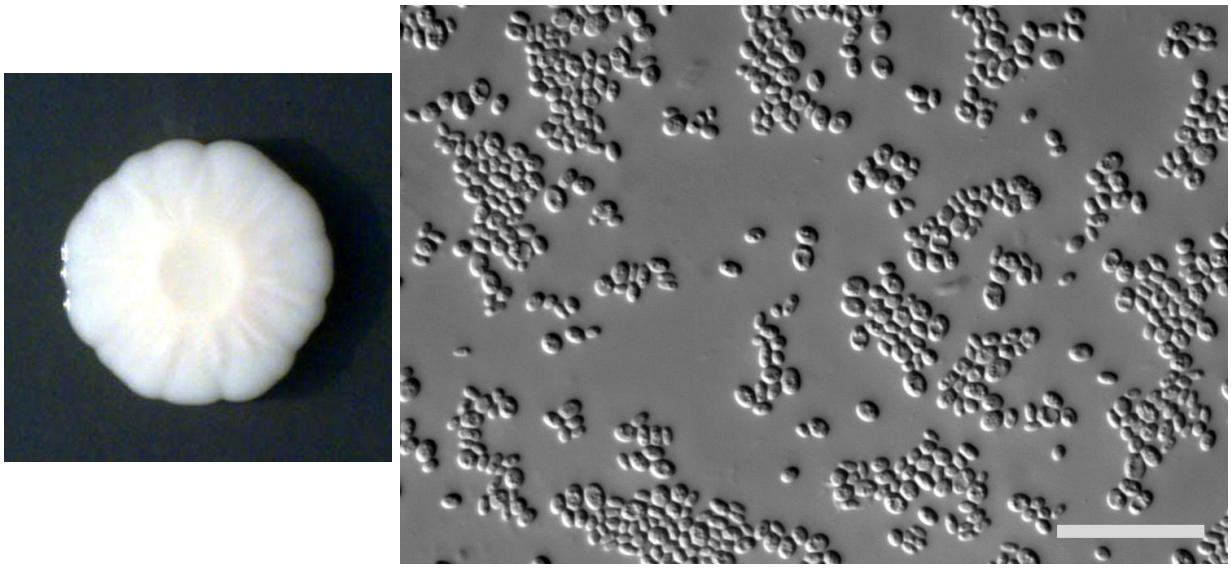
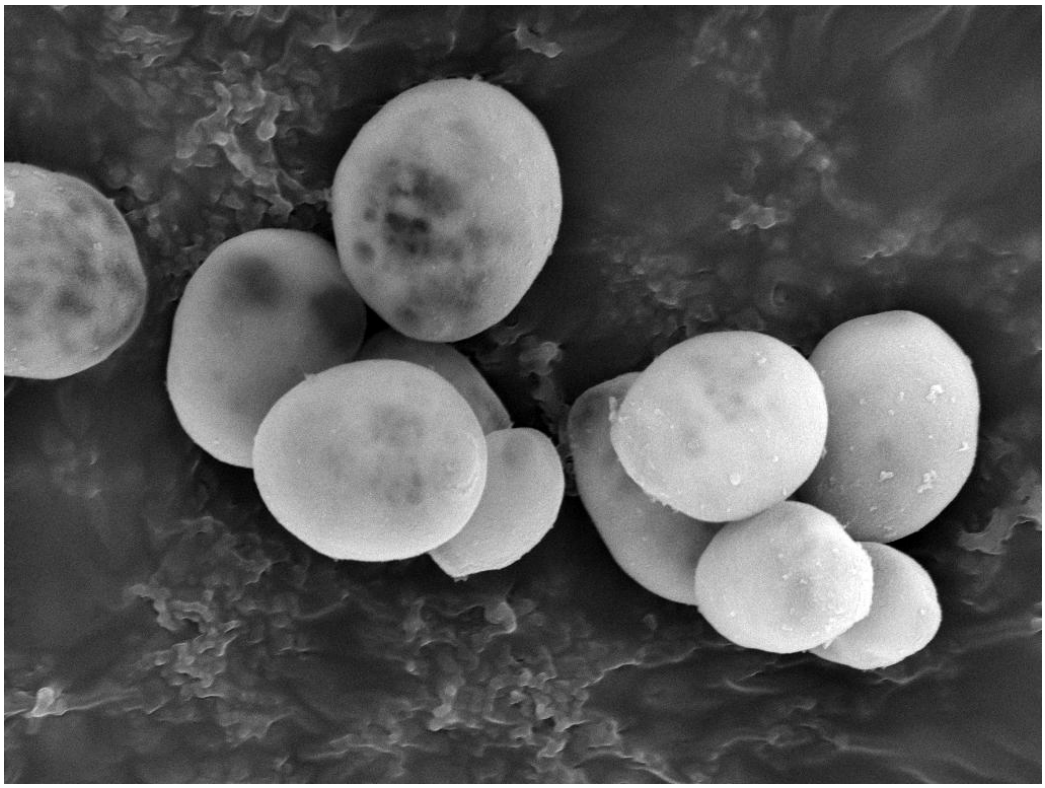


Figure 8A. Scanning electron micrograph with blastoconidia.



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Y-4 *Candida albicans*

Source: CSF / Urine / Vaginal

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

Colony: *C. albicans* colony is white to creamy, glossy, smooth and soft on Sabouraud's dextrose agar at 25°C for 3 to 5 days (Figure 9).

Microscopy: *C. albicans* yeasts are round blastoconidia bunched together with pseudohyphae on corn meal agar with Tween 80. Thick walled, mostly terminal chlamydospores are prominent (Figure 9).

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

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

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

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

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	55
Laboratories with incorrect ID:	0

Illustrations:

Figure 9. *Candida albicans*, glossy and smooth colony on Sabouraud's dextrose agar, 25°C. *Candida albicans* on corn meal agar with Tween 80 showing pseudohyphae with blastoconidia (bar = 25 μm).

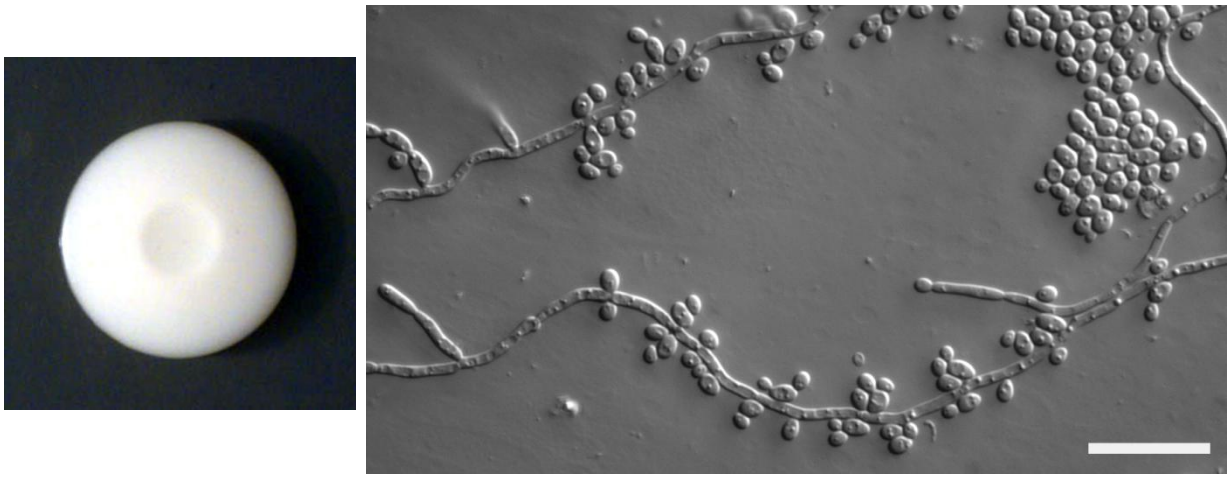
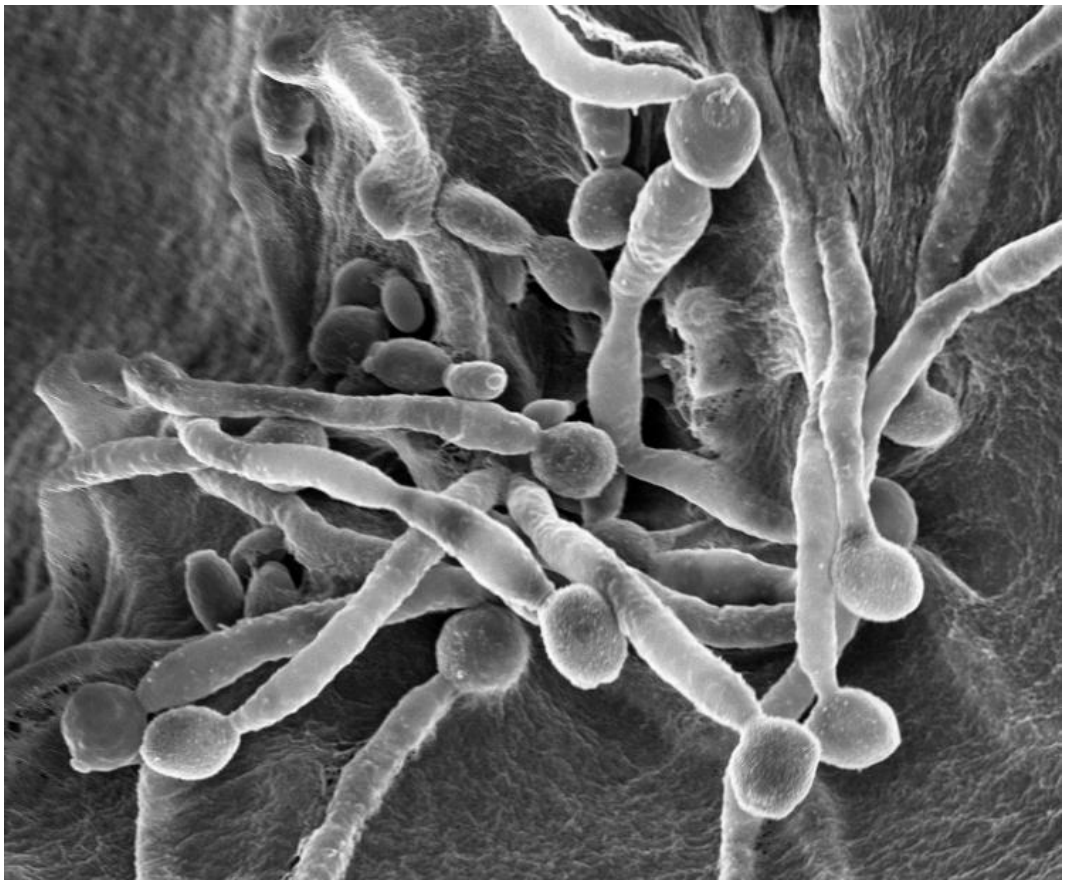


Figure 9A. Scanning electron micrograph illustrating blastoconidia.



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Y-5 *Geotrichum candidum*

Source: Stool / Sputum / Urine

Clinical significance: *Geotrichum candidum* commonly causes pulmonary infections in immunocompromised patients. It also produces lesions in alimentary tract, vagina, and skin. *G. candidum* has also been reported to cause fungemia and disseminated infection.

Colony: *G. candidum* colony grows rapidly. It is white to cream colored, flat with aerial mycelium on Sabouraud's dextrose agar at 25°C (Figure 10).

Microscopy: *G. candidum* has true hyphae with arthroconidia on corn meal agar with Tween 80. Arthroconidia formation is by the fragmentation of hyphae, no disjunctor cells (empty cells between the arthroconidia) and no blastoconidia is formed (Figure 10).

Differentiation: *G. candidum* grows on the media containing cycloheximide, negative on urease reaction, grows sparingly at 37°C. It is differentiated from *Trichosporon* species by absence of blastoconidia, no growth at higher temperatures (40, 42, & 45°C). *Blastoschizomyces capitatus* could be differentiated from *G. candidum* by the lack of growth on a medium containing D-xylose as a carbon source and its growth at 45°C. *G. candidum* is differentiated from arthroconidia forming molds by its colony morphology. Microscopically *Arthrographis* and *Odiodendron* have conidiophores while *Malbranchea* and *Coccidioides immitis* have disjunctor cells.

Molecular test: Randomly amplified polymorphic DNA (RAPD) PCR had been used for the identification of *G. candidum* isolated from cheese. Using DNA/DNA re-association techniques, de Hoog et al (1986 and 1990) found the relatedness between *G. candidum* and its teleomorph (sexual state) *Galactomyces geotrichum*.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Geotrichum candidum* strain ITEM 10460 (GenBank accession no. FN376416.1).

Antifungal susceptibility: Limited studies suggested that most isolates were susceptible to amphotericin B and to azoles like fluconazole and itraconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	55
Laboratories with incorrect ID:	01
(*Blastoschizomyces* species)	(1)

Illustrations:

Figure 10. *Geotrichum candidum*, white and mold like colony on Sabouraud's dextrose agar, 25°C. Microscopic morphology of *Geotrichum candidum* showing arthroconidia on Corn meal agar with Tween 80 (bar = 25 μm).

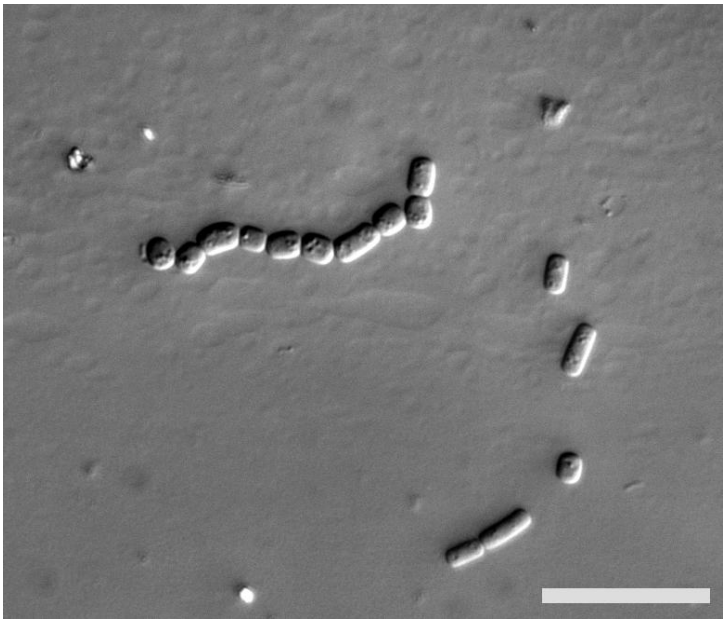
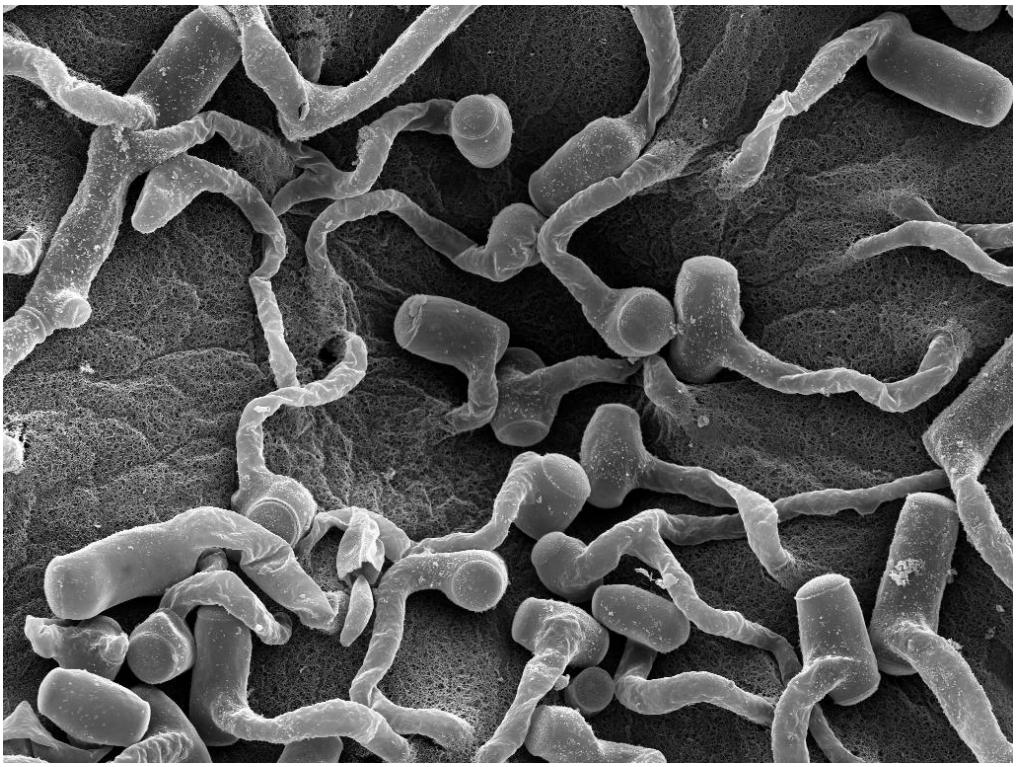
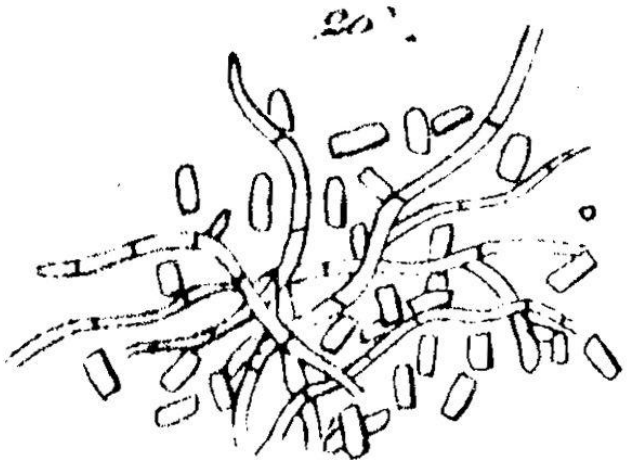


Figure 10A. Scanning electron micrograph with *Geotrichum candidum*.





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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 & Methods: Sixty-eight laboratories participated in the January 30, 2013 direct antigen detection ~~antigen~~ test event. Three positive artificial CSF samples (Cn-Ag-1, Cn-Ag-3, and Cn-Ag-5) with the titer of 1:64, 1:4, and 1:16, respectively for cryptococcal antigen were included. The titers for these samples were accepted in all the ranges.

Results: Overall, the performance of 68 laboratories was satisfactory except one. The consensus results for specimens Cn-Ag-2 and Cn-Ag-4 were negative as expected. There were two laboratories reported Cn-Ag-2 positive and one laboratory reported Cn-Ag-4 positive, which were not acceptable. Cn-Ag-1 and Cn-Ag-5 were reported positive by all the participating laboratories except only one laboratory reported Cn-Ag-5 negative. Of 68 laboratories, 61 reported negative while 7 reported positive for Cn-Ag-3. This specimen was spiked with *Cryptococcus* antigen with the titer of 1:4. The discrepancy in the results could be due to the combination of factors including use of very low antigen titer and interference of components presents in the artificial CSF. This might also be the reason for the reported titers for specimens Cn-Ag-1 and Cn-Ag-5 having a bigger range than usual serum samples. Therefore, we accepted all the reported titers for specimens Cn-Ag-1, Cn-Ag-3, and Cn-Ag-5 in this event. We also accepted the report for specimen Cn-Ag-3 as negative result in this event.

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ANTIFUNGAL SUSCEPTIBILITY TESTING FOR YEASTS

Introduction: Clinical laboratories perform susceptibility testing of pathogenic yeasts to determine their *in vitro* resistance to antifungal drugs. This test is also useful in conducting surveillance for evolving patterns of antifungal drug resistance in a healthcare facility. The results are likely to facilitate the selection of appropriate drugs for treatment. Clinical Laboratory Standards Institute (CLSI) documents of M27-A3, M27-S3, M27-S4, and M44-A, describe the current standard methods for antifungal susceptibility testing of pathogenic yeasts. Another resource for standardized method is the EUCAST Definitive Document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. The FDA approved devices for antifungal susceptibility testing of yeasts include Sensititre YeastOne Colorimetric Panel (Trek Diagnostic Systems Inc. Cleveland, OH) and Etest (bioMérieux, Inc., Durham, NC). The following ten drugs are included in the Mycology Proficiency Test Program - amphotericin B, anidulafungin, caspofungin, flucytosine (5-FC), fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. The participating laboratories are allowed to select any number of antifungal drug(s) from this test panel based upon practices in their facilities.

Materials: *Candida parapsilosis* (S-1) was the analyte in the January 30, 2013 antifungal proficiency testing event. The interpretation of MIC values for antifungal susceptibility testing of yeasts and molds is in a state of constant change. These changes are necessitated by new information emerging from clinical trials and laboratory susceptibility testing. NYSDOH Mycology Laboratory uses latest CLSI and EUCAST documents to score proficiency testing results. However, the participating laboratories are advised to regularly consult these organizations for the latest version of their standard documents.

Comments: Acceptable results were MICs +/-2 dilutions of the reference laboratory results for any single drug. Only 2 of the 30 laboratories participating in this test event tested all 10 antifungal drugs. The reported results were as follows: itraconazole (28 laboratories), flucytosine (24 laboratories), voriconasole (24 laboratories), caspofungin (21 laboratories), amphotericin B (20 laboratories), anidulafungin (16 laboratories), micafungin (16 laboratories), posacoanazole (15 laboratories), and ketocoanzole (4 laboratories). Fluconazole was the only drug tested by all 30 laboratories. One laboratory reported high MIC value for caspofungin, which was not acceptable.

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

S-1: *Candida parapsilosis* (M958)

Drug	No. labs	MIC (µg/ml)									
		0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4
Amphotericin B	20						4	15	1		
Anidulafungin	16							2	12	2	
Caspofungin	21							18	2		1
Flucytosine (5-FC)	24			2	13	7	2				
Fluconazole	30*					3	12	12	2		
Itraconazole	27*		2	14	9	1	1				
Ketoconazole	4*	1			1	1					
Micafungin	16								11	5	
Posaconazole	15	1	6	7	1						
Voriconazole	24	21	2		1						

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

Colors represent the testing method used:

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

Table 4. Antifungal Susceptibility Interpretations Reported by the Participating Laboratories

S-1: *Candida parapsilosis* (M958)

Drug	No. laboratories	Susceptible	Susceptible-dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	20	12					8
Anidulafungin	16	16					
Caspofungin	21	20				1	
Flucytosine	24	24					
Fluconazole	30	30					
Itraconazole	28	28					
Ketoconazole	4	2					2
Micafungin	16	16					
Posaconazole	15	9					6
Voriconazole	24	24					

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR MOLDS (EDUCATIONAL)

Introduction: Clinical laboratories perform susceptibility testing of pathogenic molds to determine their *in vitro* resistance to antifungal drugs. This test is also useful in conducting surveillance for evolving patterns of antifungal drug resistance in a healthcare facility. It is not clear at this juncture if the results of mold susceptibility testing have direct relevance in the selection of appropriate drugs for treatment. Clinical Laboratory Standards Institute (CLSI) document of M38-A2 describes the current standard methods for antifungal susceptibility testing of pathogenic molds. Another resource for standardized method is the EUCAST Technical Note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia-forming moulds. The following nine drugs are included in the antifungal susceptibility panel - amphotericin B, anidulafungin, caspofungin, fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole.

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

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

Table 5. MIC (µg/ml) Values of Mold Antifungal Susceptibility: *Aspergillus fumigatus* M2040

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