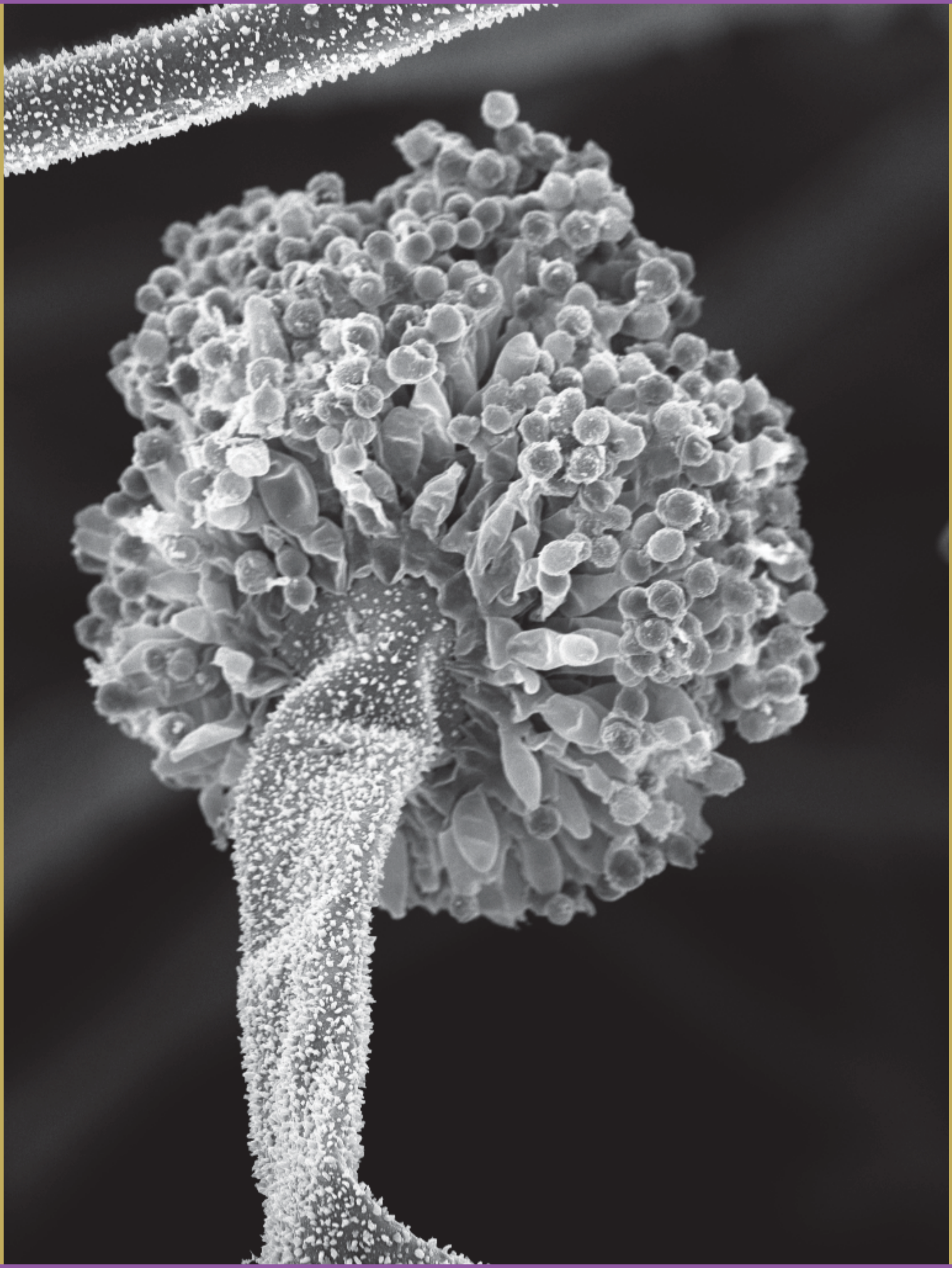
Mycology Proficiency Testing Program



Test Event Critique
September 2012

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

<u>Identification Yeast Only</u>
- Culture and Identification of yeast*
- Susceptibility testing of yeasts and molds

<u>Antigen Detection</u>
- Antigen detection of *Cryptococcus neoformans**

<u>Molecular Methods</u>
- 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% 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 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	*Scytalidium hyalinum*	*Scytalidium hyalinum*	*Scytalidium* species	55/64 (86%)
M-2	*Aspergillus fumigatus*	*Aspergillus fumigatus*	*Aspergillus fumigatus* species complex	62/64 (97%)
M-3	*Absidia corymbifera*	*Absidia corymbifera*	*Abisidia* species	61/64(95%)
M-4	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus oryzae* *Aspergillus flavus* group	58/64(91%)
M-5	*Ulocladium* species	*Ulocladium* species	*Ulocladium chartarum*	63/64 (98%)

Mycology – Yeast Only

	Specimen key	Validated specimen	Other acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
Y-1	*Candida zeylanoides*	*Candida zeylanoides*		55/55 (100%)
Y-2	*Candida lipolytica*	*Candida lipolytica*		52/55 (95%)
Y-3	*Candida parapsilosis* species complex	*Candida parapsilosis* species complex	*Candida parapsilosis*	55/55 (100%)
Y-4	*Cryptococcus laurentii*	*Candida laurentii*		55/55 (100%)
Y-5	*Cryptococcus neoformans*	*Cryptococcus neoformans*	*Cryptococcus neoformans*-*Cryptococcus gattii* species complex	57/58 (98%)

Mycology – Direct detection (*Cryptococcus* Antigen Test)

	Specimen key (Titer)	Validated specimen	Correct responses / Total laboratories (% correct responses)	
			Qualitative	**Quantitative**
Cn-Ag-1	Positive (1:16/1:32)	Positive (1:16/1:32)	66/68 (97%)	63/63 (100%)
Cn-Ag-2	Positive (1:64/1:128)	Positive (1:64/1:128)	67/68 (99%)	61/63 (96%)
Cn-Ag-3	Negative	Negative	67/68 (99%)	NA
Cn-Ag-4	Negative	Negative	68/68 (100%)	NA
Cn-Ag-5	Negative	Negative	68/68 (100%)	NA

Antifungal Susceptibility Testing for Yeast (S-1: *Candida tropicalis* M2698)

Drugs	Acceptable MIC (μg/ml) Range	Acceptable interpretation	Laboratories with acceptable responses/ Total laboratories (% correct responses)
Amphotericin B	0.06 – 1	Susceptible / No interpretation	20/20 (100%)
Anidulafungin	0.015 – 0.125	Susceptible	16/16 (100%)
Caspofungin	0.03 – 0.25	Susceptible	21/21 (100%)
Flucytosine (5-FC)	0.03 – 0.125	Susceptible	24/24 (100%)
Fluconazole	0.25 – 4.0	Susceptible	29/29 (100%)
Itraconazole	0.06 – 0.25	Susceptible / Susceptible-Dose Dependent	28/28 (100%)
Ketoconzole	0.06 – 0.125	No interpretation	5/5 (100%)
Micafungin	0.008 – 0.03	Susceptible	16/16 (100%)
Posaconazole	0.06 – 0.25	Susceptible / No interpretation	15/15 (100%)
Voriconazole	0.03 – 0.25	Susceptible	23/24 (96%)

Antifungal Susceptibility Testing for Mold - Educational (MS-1-Edu: *Aspergillus fumigatus* M2036)

Drugs	Acceptable MIC (µg/ml) Range	Laboratories within MIC range / Total laboratories (%)
Amphotericin B	0.25 – 1.0	5/5 (100%)
Anidulafungin	0.008 – 0.12	4/4 (100%)
Caspofungin	0.008 – 1.0	4/4 (100%)
Fluconazole	≥ 64	4/4 (100%)
Itraconazole	2.0 – 16	6/6 (100%)
Ketoconzole	4.0 – 16	2/2 (100%)
Micafungin	0.008 – 0.12	4/4 (100%)
Posaconazole	0.06 – 0.5	5/5 (100%)
Voriconazole	0.12 – 1.0	4/4 (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	2
API 20C AUX	45
Biolog Microbial ID System	1
Microscan	5
Remel RapID Yeast Plus System	4
Vitek2	26
Antifungal Susceptibility*	
Disk diffusion	1
Etest	2
YeastOne - Mold	2
YeastOne - Yeast	25
Others[†] - Yeast	3
Others[†] - Mold	3
Cryptococcal antigen	
Immuno-Mycologics	11
Meridien Diagnostics	48
Remel	9

*Include multiple systems used by some laboratories
[†]Include laboratories using CLSI Microbroth dilution method

MOLD DESCRIPTIONS

M-1 *Scytalidium hyalinum*

Source: Foot / Toe

Clinical Significance: *Scytalidium hyalinum* is an agent of dermatomycosis and onychomycosis. This fungus can also rarely cause invasive infection.

Colony: *S. hyalinum* colonies grow rapidly, velvety to wooly, white to cream with reverse yellow to brown on Sabouraud's dextrose agar at 25°C. *S. Hyalinum* does not grow on media containing cycloheximide (Figure 1).

Microscopy: Lactophenol - Cotton blue mount shows hyaline hyphae with single-celled, oval, ellipsoidal or cylindrical arthroconidia. Conidiophores are absent (Figure 1).

Differentiation: *Scytalidium* spp. can be differentiated from *Geotrichum* by its wooly colonies; from *Arthrographis* by its absence of conidiophores; from *Malbranchea* by formation of non-alternate arthroconida.

Molecular test: Internal transcribed spacer (ITS) regions of ribosomal DNA can be used for the identification of *Scytalidium* spp.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Scytalidium hyalinum* strain ATCC 38906 (GenBank accession no. AY213688.1).

Antifungal susceptibility: *Scytalidium* spp. are susceptible to amphotericin B, fluconazole, itraconazole, voriconazole, terbinafine, and anidulafungin, but they are resistant to 5-flucytosine.

Participant performance:

Referee Laboratories with correct ID:	09
Laboratories with correct ID:	55
Laboratories with incorrect ID:	09
(*Arthrographis* species)	(5)
(*Malbranchea* species)	(2)
(*Chaetomium* species)	(1)
(*Chrysonilia sitophila*)	(1)

Illustrations:

Figure 1. Seven-day-old, powdery to velvet, white to grey colony of *Scytalidium hyalinum* on Sabouraud's dextrose agar; the reverse of colony appears yellow to brown (upper panel). Microscopic morphology of *Scytalidium hyalinum* showing arthroconidia in chain (lower panel; bar = 25 μm).

Figure 1A. Scanning electron micrograph of arthroconidia of *Scytalidium hyalinum* on Sabouraud's dextrose agar.

Further reading:

Lacroix C, de Chauvin MF. 2008. *In vitro* activity of amphotericin B, itraconazole, voriconazole, posaconazole, caspofungin and terbinafine against *Scytalidium dimidiatum* and *Scytalidium hyalinum* clinical isolates. *J Antimicrob Chemother.* 61: 835-837.

Hay RJ. 2002. *Scytalidium* infections. *Curr Opin Infect Dis.* 15: 99-100.

Machouart-Dubach M, Lacroix C, de Chauvin MF, Le Gall I, Giudicelli C, Lorenzo F, Derouin F. 2001. Rapid discrimination among dermatophytes, *Scytalidium* spp., and other fungi with a PCR-restriction fragment length polymorphism ribotyping method. *J Clin Microbiol.* 39: 685-690.

Madrid H, Ruíz-Cendoya M, Cano J, Stchigel A, Orofino R, Guarro J. 2009. Genotyping and *in vitro* antifungal susceptibility of *Neoscytalidium dimidiatum* isolates from different origins. *Int J Antimicrob Agents.* 34: 351-354.

Sriaroon C, Vincent AL, Silapunt S, Chandler A, Houston SH, Greene JN. 2008. Successful treatment of subcutaneous *Scytalidium hyalinum* infection with voriconazole and topical terbinafine in a cardiac transplant patient. *Transplantation.* 85: 780-782.

Xavier AP, Oliveira JC, Ribeiro VL, Souza MA. 2010. Epidemiological aspects of patients with ungual and cutaneous lesions caused by *Scytalidium* spp. *An Bras Dermatol.* 85: 805-810.

M-2 *Aspergillus fumigatus*

Source: Chest / Nail / Bronchial Wash

Clinical significance: *Aspergillus fumigatus* is the most frequent etiologic agent of aspergillosis in humans. It causes pulmonary, sinus, cerebral, bone, ocular, cardiovascular, and other organ diseases especially in immunocompromised host. The three major manifestations are: allergy (allergic broncho-pulmonary aspergillosis), colonization of the pre-existent air cavities (aspergilloma) and systemic infections (invasive aspergillosis). *Aspergillus fumigatus* has a pronounced tendency to invade blood vessels (angioinvasion), which often results in fatal outcome.

Colony: *A. fumigatus* grows rapidly. The colony shows powdery, blue – green surface pigmentation with pale yellow reverse on Sabouraud's dextrose agar at 25°C. This fungus is capable of growth at 45°C, which is useful for differentiation (Figure 2).

Microscopy: Lactophenol - Cotton blue mount shows septate hyphae with smooth walled conidiophores. Conidiophore terminates in vesicle. The vesicle is subglobose with its upper half covered (columnar) with single series of sterigmata (uniseriate). Conidia produced from these sterigmata are round, smooth and in chains (Figure 2).

Differentiation: *A. fumigauts* can be differentiated from other *Aspergillus* species by blue – green colonies, columnar conidial heads with uniseriate sterigmata and good growth at 45°C. Please see table 1, p. 25 for a comparison of diagnostic features of common pathogenic species of *Aspergillus*.

Molecular test: For the molecular epidemiology of *A. fumigatus,* many typing methods have been used. The notable methods are multi – locus enzyme electrophoresis, random amplified polymorphic DNA, and sequence – specific DNA primers. A real-time PCR assay to detect *Aspergillus* spp. in clinical samples has been reported.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Aspergillus fumigatus* isolate A2S4_D54 (GenBank accession no. JX501388.1).

Antifungal susceptibility: *A. fumigatus* isolates are variably susceptible to amphotericin B and itraconazole, but highly susceptible to caspofungin, voriconazole and posaconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	62
Laboratories with incorrect ID:	02
(*Aspergillus* sp.)	(2)

<u>Illustrations</u>:

Figure 2. Five-day-old, blue-green colony of *Aspergillus fumigatus* with powdery surface, on Sabouraud's dextrose agar, 25°C; the reverse is pale to black (upper panels). Microscopic morphology of *A. fumigatus* showing typical, columnar conidiophores consisting of subglobose vesicle with uniseriate sterigmata and large chains of round conidia (bar = 25 µm; lower panel).

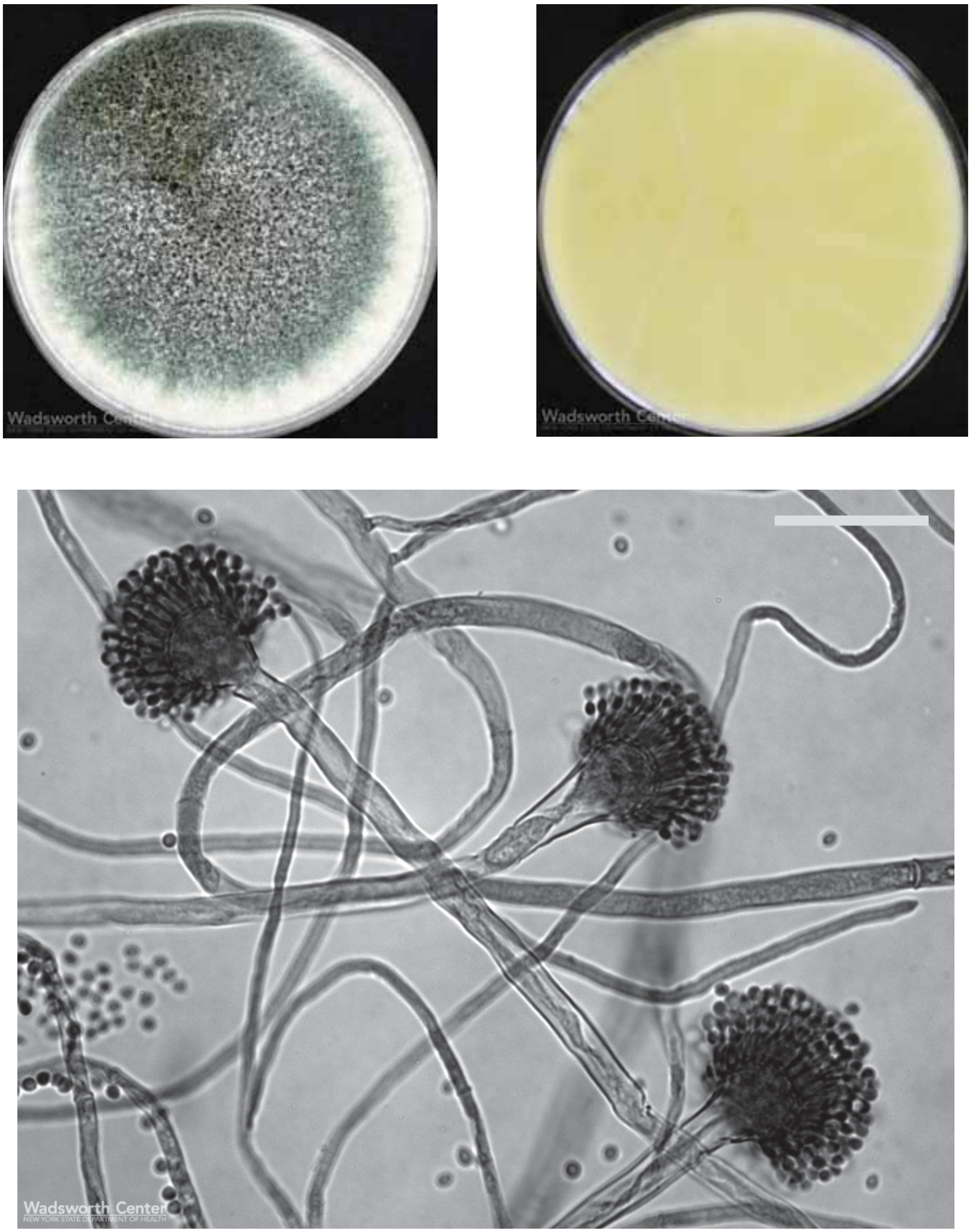
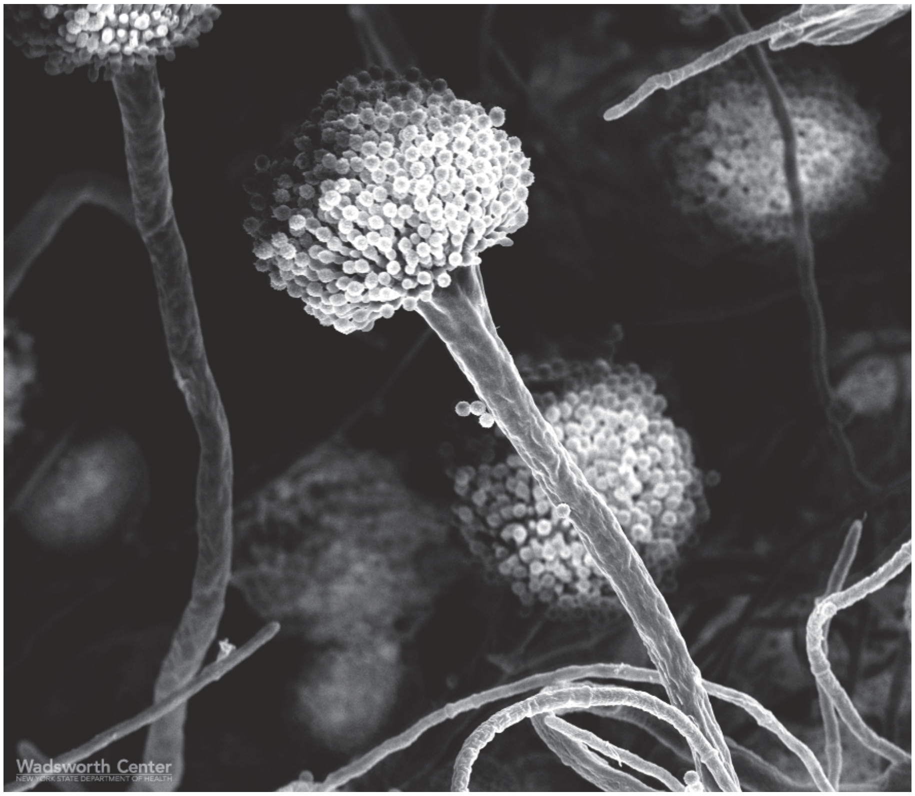
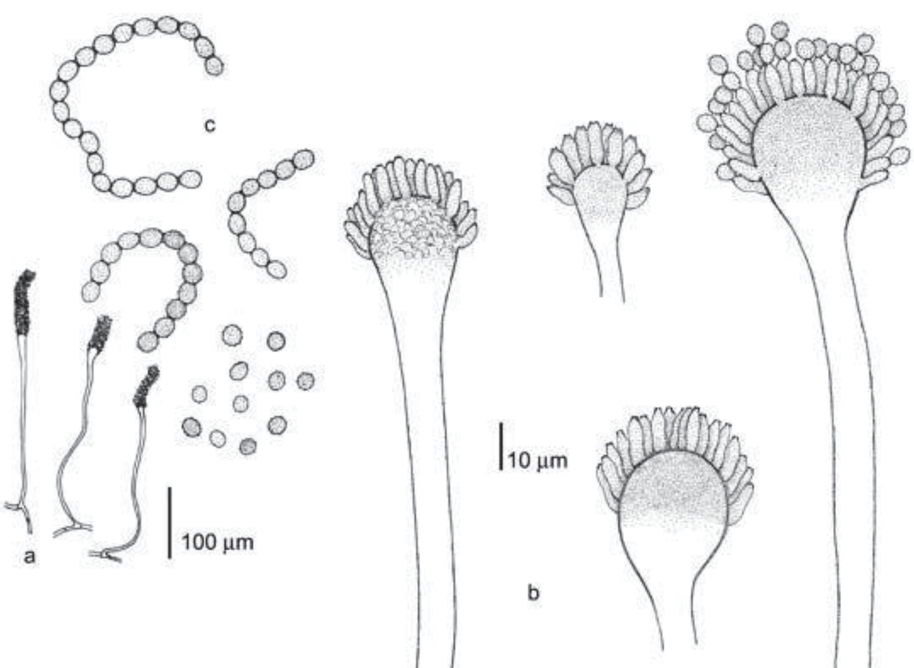


Figure 2A. Scanning electron micrograph of conidia and conidiophores of *Aspergillus fumigatus* on Sabouraud's dextrose agar (upper panel). Line drawings of *Aspergillus fumigatus* (lower panel).





http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=3660

Further reading:

Araujo R, Coutinho I, Espinel-Ingroff A. 2008. Rapid method for testing the susceptibility of *Aspergillus fumigatus* to amphotericin B, itraconazole, voriconazole and posaconazole by assessment of oxygen consumption. *J Antimicrob Chemother.* 62: 1277-1280.

Badiee P, Kordbacheh P, Alborzi A, Ramzi M, Shakiba E. 2008. Molecular detection of invasive aspergillosis in hematologic malignancies. *Infection.* 36: 580-584.

Barberan J, Alcazar B, Malmierca E, Garcia de la Llana F, Dorca J, Del Castillo D, Villena V, Hernandez-Febles M, Garcia-Perez FJ, Granizo JJ, Gimenez MJ, Aguilar L. 2012. Repeated *Aspergillus* isolation in respiratory samples from non-immunocompromised patients not selected based on clinical diagnoses: colonisation or infection? *BMC Infect Dis.* 12: 295. [Epub ahead of print]

Barberan J, Sanz F, Hernandez JL, Merlos S, Malmierca E, Garcia-Perez FJ, Sanchez-Haya E, Segarra M, Garcia de la Llana F, Granizo JJ, Gimenez MJ, Aguilar L. 2012. Clinical features of invasive pulmonary aspergillosis vs. colonization in COPD patients distributed by gold stage. *J Infect.* 65: 447-452.

Chakrabarti A, Chatterjee SS, Das A, Shivaprakash MR. 2011. Invasive aspergillosis in developing countries. *Med Mycol.* 49 Suppl 1: S35-47.

Martínez-Ramos M, Claros-B JA, Vale-Oviedo MA, Siso-Villarroel E, Padilla R, Santiago A, Simón JA. 2008. Effect of the vehicle on the topical itraconazole efficacy for treating corneal ulcers caused by *Aspergillus fumigatus*. *Clin Experiment Ophthalmol.* 36: 335-338.

Pasqualotto AC. 2008. Differences in pathogenicity and clinical syndromes due to *Aspergillus fumigatus* and *Aspergillus flavus*. *Med Mycol.* 25:1-10.

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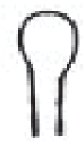
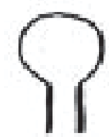
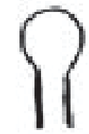
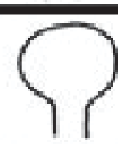
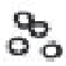
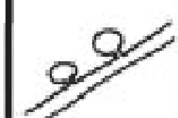
Table 1. Scheme for differentiation of *Aspergilli* most commonly involved in human diseases.

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

M-3 *Absidia corymbifera*

Source: Nail / Sinus

Clinical significance: Infections with *Absidia corymbifera* usually occur in immunocompromised hosts. Rare *A. corymbifera* infections in immucocompetent hosts have also been reported.

Colony: *A. corymbifera* grows rapidly. The colonies are grey with wooly texture on surface with reverse pale to yellowish on Sabouraud's dextrose agar at 25°C (Figure 3).

Microscopy: Lactophenol - Cotton blue mount shows branched sporangiophores with a funnel-shaped swelling (apophysis) under the pyriform ('pear shaped') sporangium (Figure 3). Rhizoids are rare.

Differentiation: *A. corymbifera* has typical funnel-shaped apophysis, which distinguishes it from *Mucor, Rhizomucor,* and *Rhizopus. A. corymbifera* assimilates lactose and nitrate, but does not assimilate ethanol. Please see table 2, p30 for more details.

Molecular test: PCR ITS regions is used for rapid and specific identification.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Lichtheimia corymbifera* (Synonym of *Absidia corymbifera*) strain KACC 45830 (GenBank accession no. JN315001.1).

Antifungal susceptibility: *A. corymbifera* is resistant to fluconazole and 5-flucytosine but susceptible to amphotericin B.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	61
Laboratories with incorrect ID:	03
(*Mucor* species)	(2)
(*Rhizopus* species)	(1)

Illustrations:

Figure 3. Four-day-old, wooly texture of *Absidia corybifera* on Sabouraud's dextrose agar, 25°C; the reverse is pale to yellow (upper panel). Microscopic morphology of *Absidia corybifera* showing sporangiophores with a funnel-shaped swelling (apophysis) under the pyriform sporangium (lower panel; bar = 25 μm).

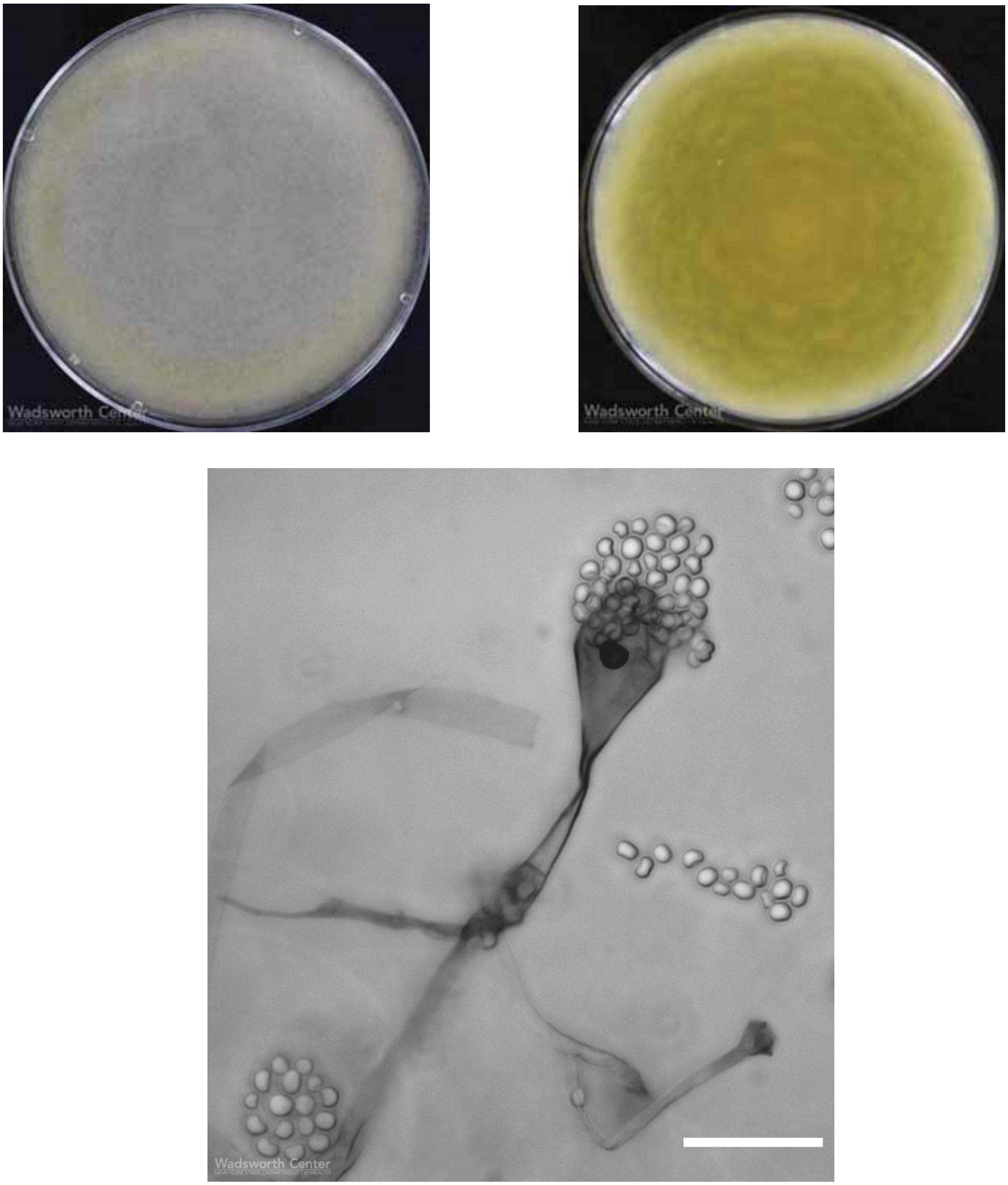
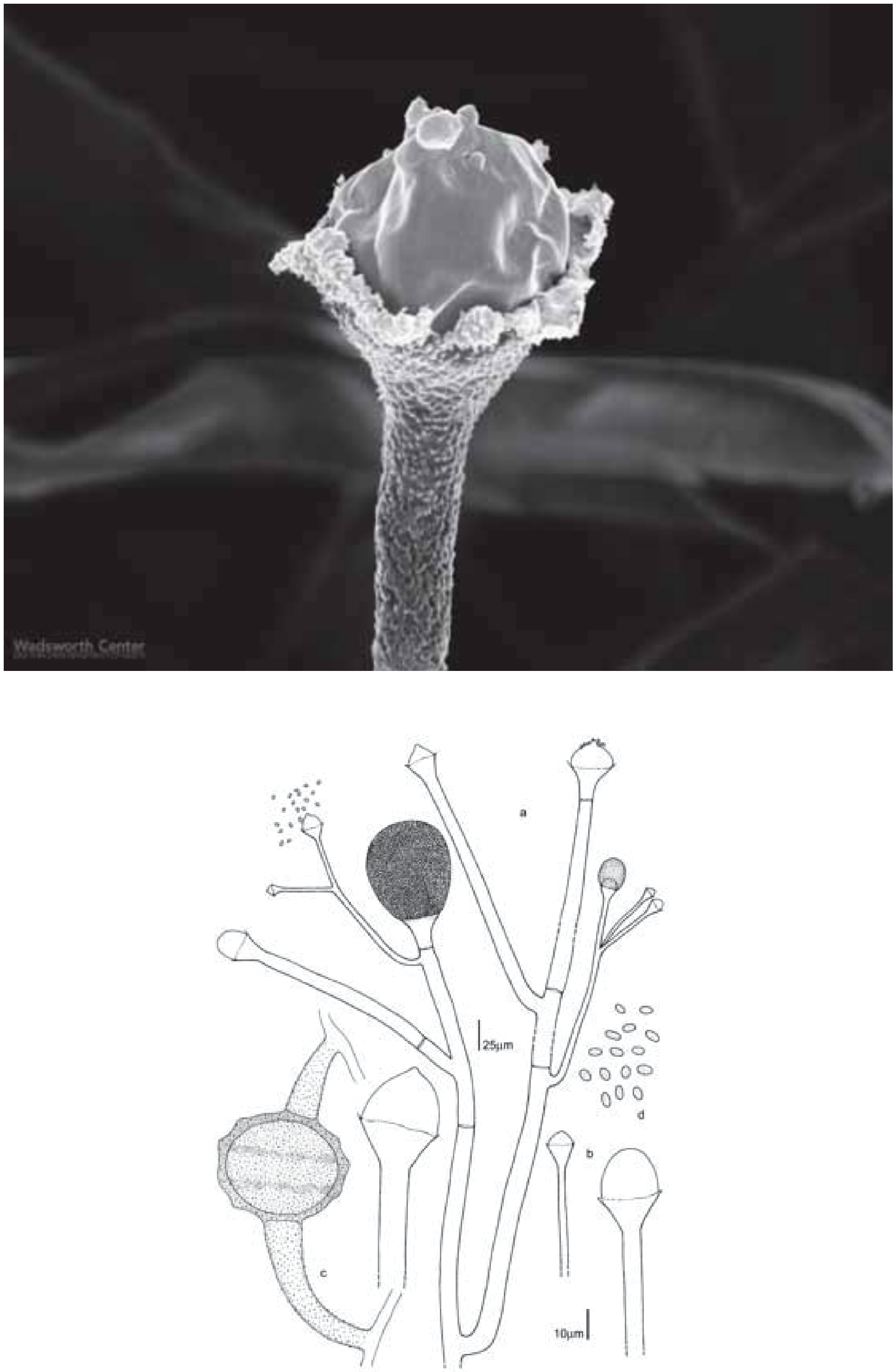


Figure 3A. Scanning electron micrograph of *Absidia corymbifera* (upper panel). Line drawing with details of *Absidia corymbifera* (lower panel).



http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=3547

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Roux BG, Méchinaud F, Gay-Andrieu F, Lortholary O, Dannaoui E, Hoinard D, Corradini N. 2010. Successful triple combination therapy of disseminated *Absidia corymbifera* infection in an adolescent with osteosarcoma. *J Pediatr Hematol Oncol*. 32: 131-133.

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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

M-4 *Aspergillus flavus*

Source: Sputum / Toenail / Tissue

Clinical significance: *Aspergillus flavus* causes pulmonary and disseminated infection in immunocompromised patients. *A. flavus* is angioinvasive, producing extensive damage to blood vessels, leading to infarction and necrosis. Occasionally, this pathogen can cause infection of sinus, eye, ear, and nails. *A. flavus* produces aflatoxins in certain foodstuff like peanuts that can cause mycotoxicosis.

Colony: *A. flavus* colonies grow fast. They are yellow to green, powdery on the surface with pale to yellow reverse on Sabouraud's dextrose agar at 25°C (Figure 4).

Microscopy: Lactophenol - Cotton blue mount shows septate hyphae with rough, colorless conidiophores. Conidiophore terminates in vesicle, which is globose and the entire surface is covered (radiating) with one series or two series of sterigmata (uni- or biseriate). Round, rough-walled conidia, measuring 3 - 6 µm, are arranged in chains on sterigmata (Figure 4).

Differentiation: *A. flavus* can be differentiated from other *Aspergilli* by yellow – green colonies, rough walled conidiophores, radiating conidial heads with uniseriate or biseriate sterigmata. Please see table 1, p25 for more details.

Molecular test: A PCR test targeting alkaline proteases from *A. fumigatus* and *A. flavus* has been reported for direct detection from the respiratory specimens. For molecular epidemiology, RAPD fingerprinting method has been used.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Aspergillus flavus* strain LVPEI.H2168_09 (GenBank accession no. JX868698.1).

Antifungal susceptibility: *A. flavus* shows variable susceptibility to amphotericin B and itraconazole and high susceptibility to voriconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	58
Laboratories with incorrect ID:	06
(*Aspergillus glaucus* group)	(3)
(*Aspergillus* species)	(2)
(*Aspergillus versicolor*)	(1)

Illustrations:

Figure 4. Five-day-old, yellow-green colony of *Aspergillus flavus* on Sabouraud's dextrose agar, 25°C; the reverse of the colony appears pale yellow (upper panel). Microscopic morphology of *Aspergillus flavus* depicting typical radiate heads with globose vesicle, biserate sterigmata, and round conidia (bar = 50 μm; lower panel)

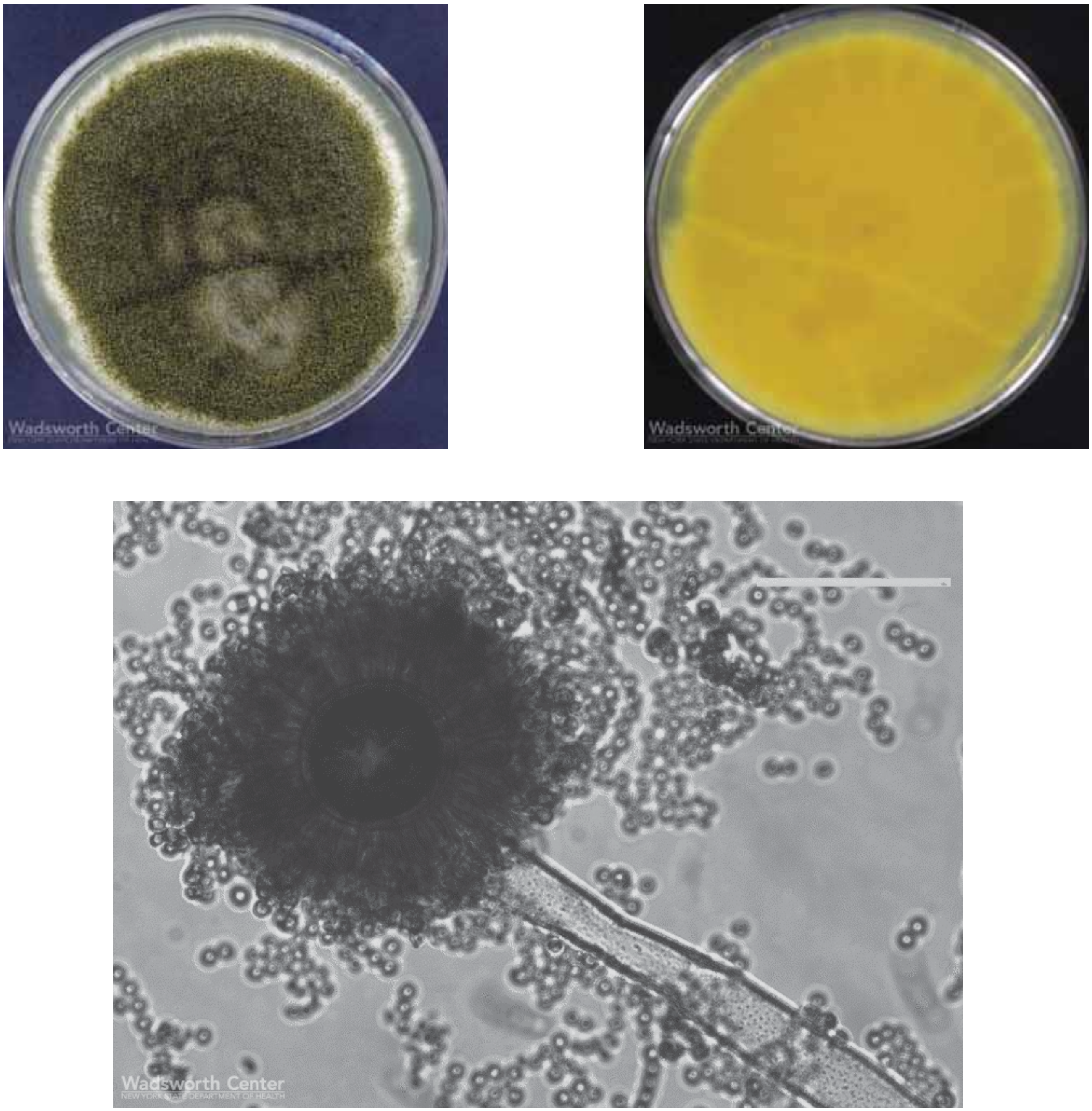
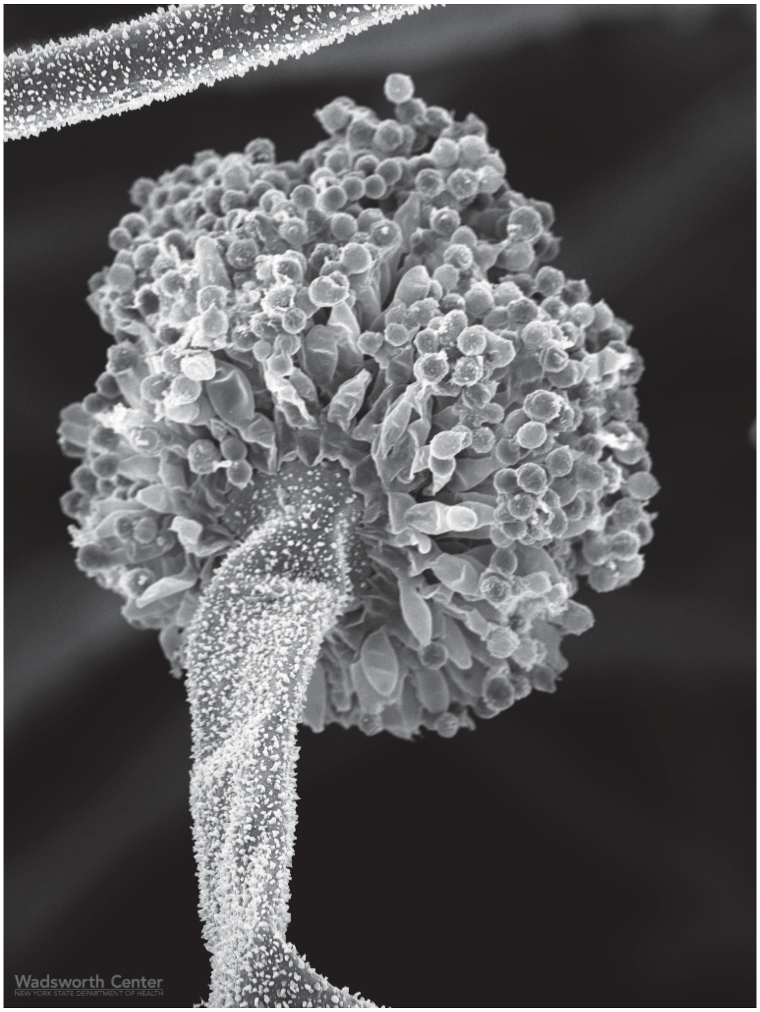
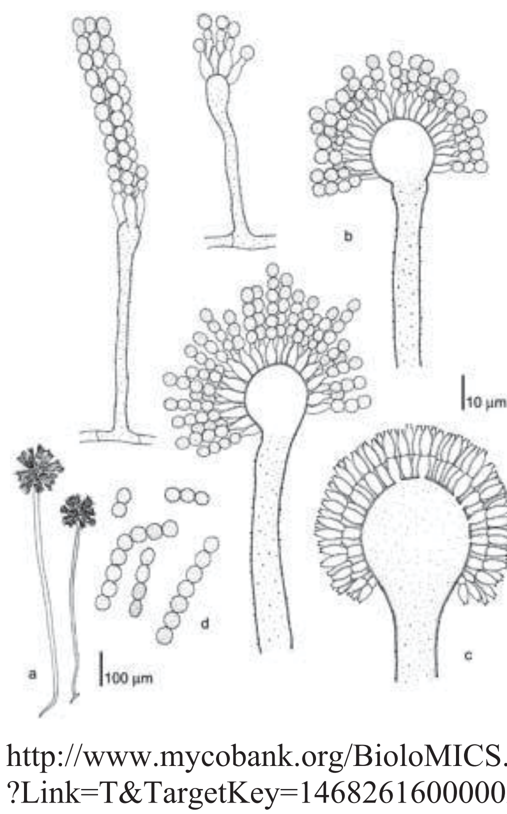


Figure 4A. Scanning electron micrograph of *Aspergillus flavus* highlighting characteristic sporangium and hypha (upper panel). Line drawing with details of *Aspergillus flavus* (lower panel).





http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=3658

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Shivaprakash MR, Geertsen E, Chakrabarti A, Mouton JW, Meis JF. 2011. *In vitro* susceptibility of 188 clinical and environmental isolates of *Aspergillus flavus* for the new triazole isavuconazole and seven other antifungal drugs. *Mycoses.* 54: e583-589.

Steinbach WJ, Marr KA, Anaissie EJ, Azie N, Quan SP, Meier-Kriesche HU, Apewokin S, Horn DL. 2012. Clinical epidemiology of 960 patients with invasive aspergillosis from the PATH Alliance registry. *J Infect.* 65: 453-464.

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M-5 *Ulocladium* species

Source: Toe / Peritoneal fluid

Clinical significance: *Ulocladium* spp. is commonly considered as a contaminant. The fungus may cause phaeohyphomycosis, which manifests as subcutaneous infections.

Colony: *Ulocladium* spp. grows moderately fast on Sabouraud's dextrose agar at 25°C. Colonies are initially white, later becoming brownish black, velvet in texture (Figure 5).

Microscopy: Lactophenol - Cotton blue mount shows brown septate hyphae andmuriform, brown, verrucose conidia (Figure 5).

Differentiation: *Ulocladium* differs from *Alternaria* by its strongly geniculate conidiophores, and the absence of beak-like tapered apex of conidia. It differs from *Bipolaris*, *Curvularia*, and *Drechslera* by producing muriform conidia. *Ulocladium* is differentiated from *Stemphylium* by having geniculate, sympodial conidiophores.

Molecular test: Internal transcribed spacer (ITS) regions can be used for the identification of *Ulocladium* spp.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Ulocladium chartarum* isolate U13-12 (GenBank accession no. JQ585684.1).

Antifungal susceptibility: *Ulocladium* has low MIC for amphotericin B, ketocoanzole, and itraconazole, but high MIC for 5-flucytosine and fluconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	63
Laboratories with incorrect ID:	01
(*Epicoccum* sp.)	(1)

Illustrations:

Figure 5. Seven-day-old, velvet, gray to black colony of *Ulocladium* sp. on Sabouraud's dextrose agar, 25°C; the reverse of the colony is brown to black (upper panel). Microscopic morphology of *Ulocladium* sp. showing hyphae and poroconidia formed sympodially; the condia are slightly curved with transverse septations (bar = 25 μm; lower panel).

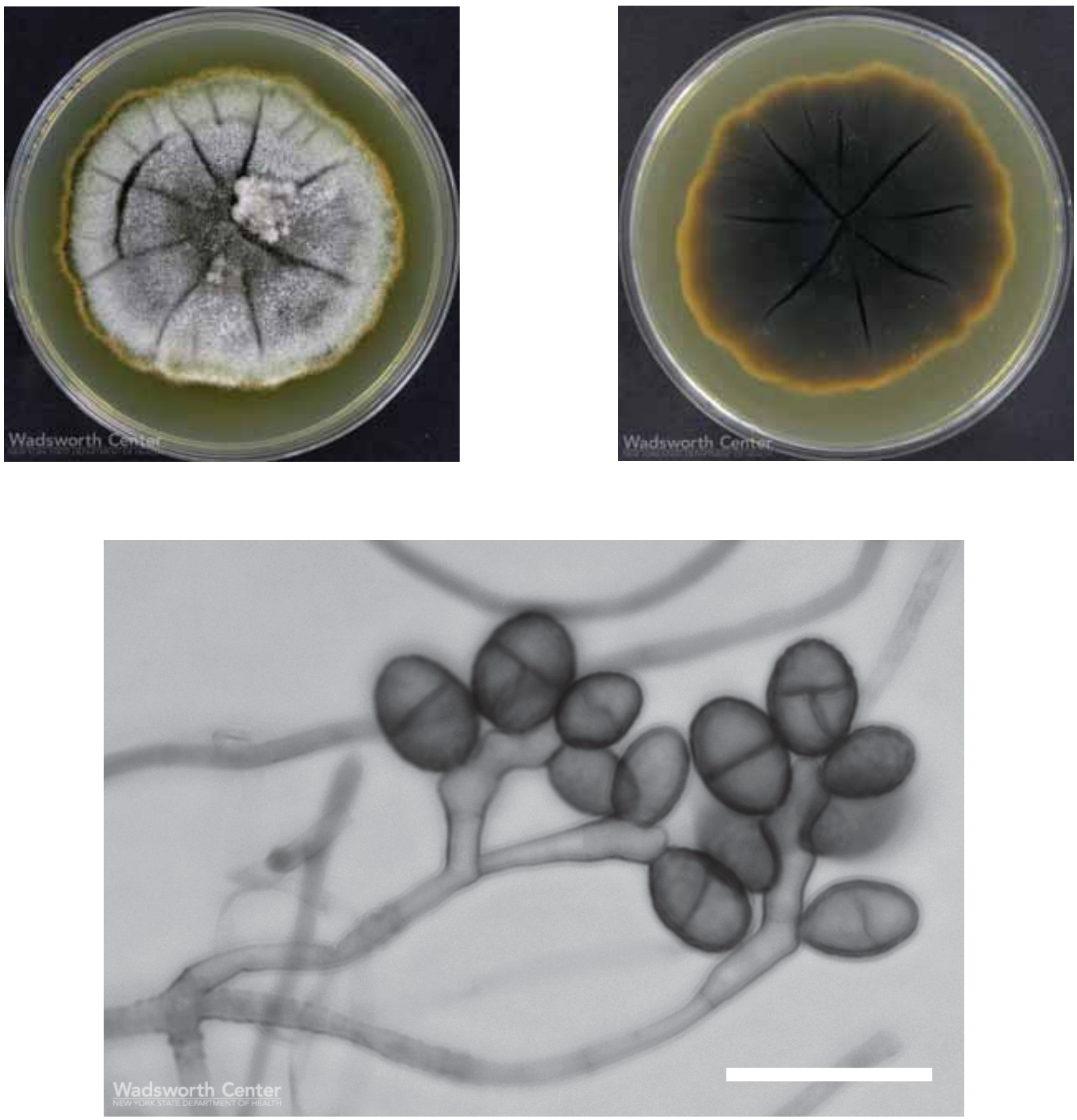

Figure 5A. Scanning electron micrograph of *Ulocladium chartarum* (upper panel). Line drawing with details of *Ulocladium chartarum* (lower panel).

http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=8660

Further reading:

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Knights CB, Lee K, Rycroft AN, Patterson-Kane JC, Baines SJ. 2008. Phaeohyphomycosis caused by *Ulocladium* species in a cat. *Vet Rec* 162: 415-416.

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YEAST DESCRIPTIONS

Y-1 *Candida zeylanoides*

Source: Nail / Urine

Clinical significance: *Candida zeylanoides* is a relatively rare pathogen in humans. In immunocompromised patients, *C. zeylanoides* causes fungemia, endocarditis, and arthritis. In immunocompetent patients, it causes skin and nail infections.

Colony: *C. zeylanoides* colony is smooth, cream-colored, butyrous, and raised on Sabouraud's dextrose agar at 25°C (Figure 6).

Microscopy: *C. zeylanoides* forms long pseudohyphae, with verticillate, ovoid blastoconidia on Corn meal agar with Tween 80. Blastoconidia are produced in whorls around the pseudohyphae (Figure 6).

Differentiation: *C. zeylanoides* does not ferment any carbohydrates, grows at 37°C, grows on media containing cycloheximide, and assimilates limited carbohydrates.

Molecular test: Multiplex PCR using ITS1 and ITS2 was reported for rapid detection and identification of yeast strains of *C. zelanoides*.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Candida zeylanoides* strain TJY13a (GenBank accession no. EF687774.1).

Antifungal susceptibility: *C. zeylanoides* is susceptible to amphotericin B and several azoles.

Participant performance:

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

Illustrations:

Figure 6. *Candida zeylanoides,* colony creamish white, butryous, raised on Sabouraud's dextrose agar, 25°C. Microscopic morphology on corn meal agar with Tween 80, showing long pseudohyphae with verticillate, ovoid blastoconidia (bar = 25 μm).

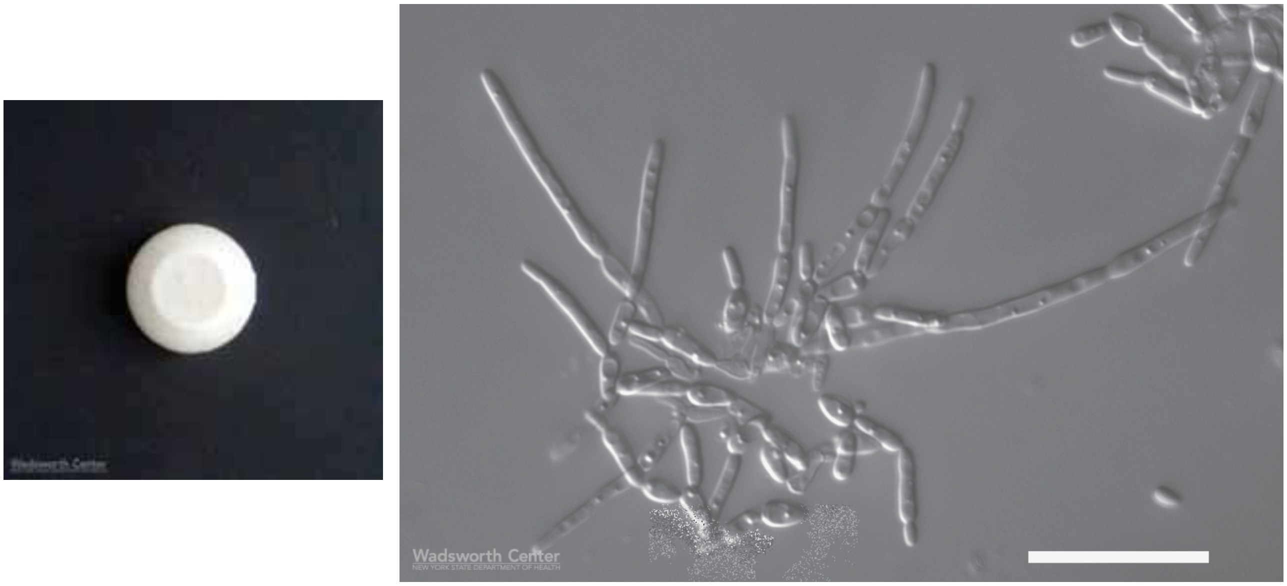
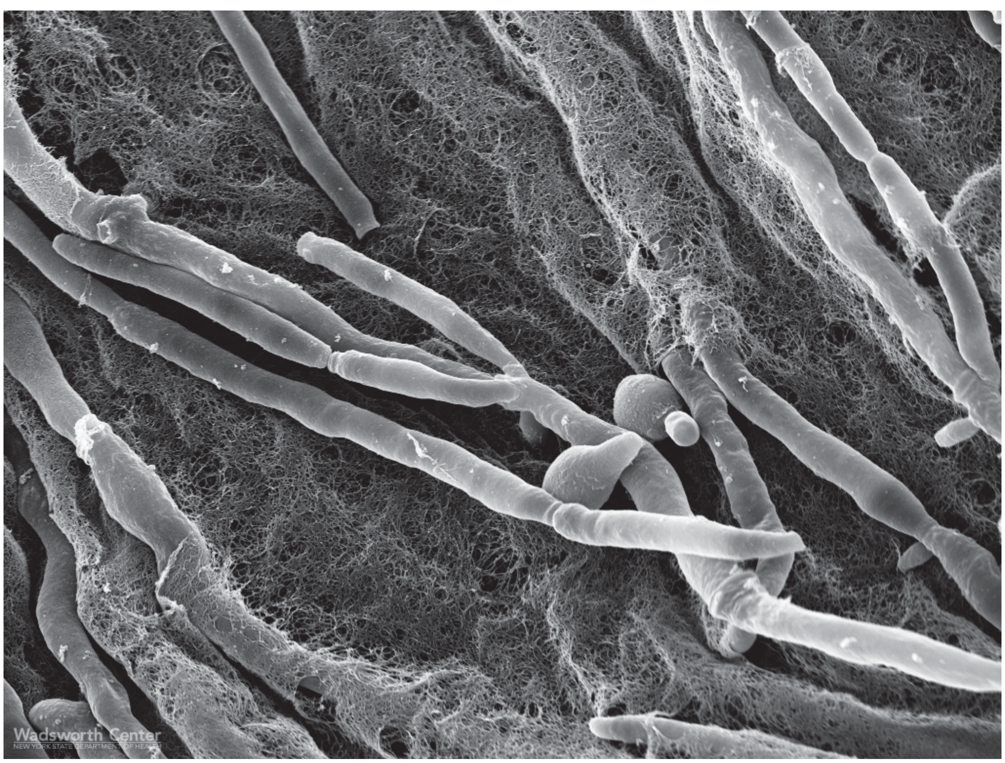


Figure 6A. Scanning electron micrograph of *Candida zeylanoides* illustrates pseudohyphae and blastoconidia.



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Liao W-Q, Li Z-G, Guo M, Zhang J-Z. 1993. *Candida zeylanoides* causing candidiasis as tinea cruris. 1993. *Chinese Medical J.* 106: 542-545.

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Pereira GH, Müller PR, Szeszs MW, Levin AS, Melhem MS. 2010. Five-year evaluation of bloodstream yeast infections in a tertiary hospital: the predominance of non-*C. albicans Candida* species. *Med Mycol.* 2010. 48: 839-842.

Whitby S, Madu EC, Bronze MS. 1996. *Candida zeylanoides* infective endocarditis complicating infection with the human immunodeficiency virus. *Am J Medical Sciences*. 312: 138-139.

Y-2 *Candida lipolytica*

Source: Catheter / Nail / Urine

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

Colony: *C. lypolytica* colony is white to cream, wrinkled on Sabouraud's dextrose agar at 25°C (Figure 7).

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

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

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

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

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

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	52
Laboratories with incorrect ID:	03
(*Candida* species)	(2)
(*Geotrichum candidum*)	(1)

Illustrations:

Figure 7. *Candida lypolytica*, white to cream colony with wrinkled surface on Sabouraud’s dextrose agar, 25°C. Microscopic morphology on corn meal agar showing bushy pseudohyphae (bar = 50 μm).

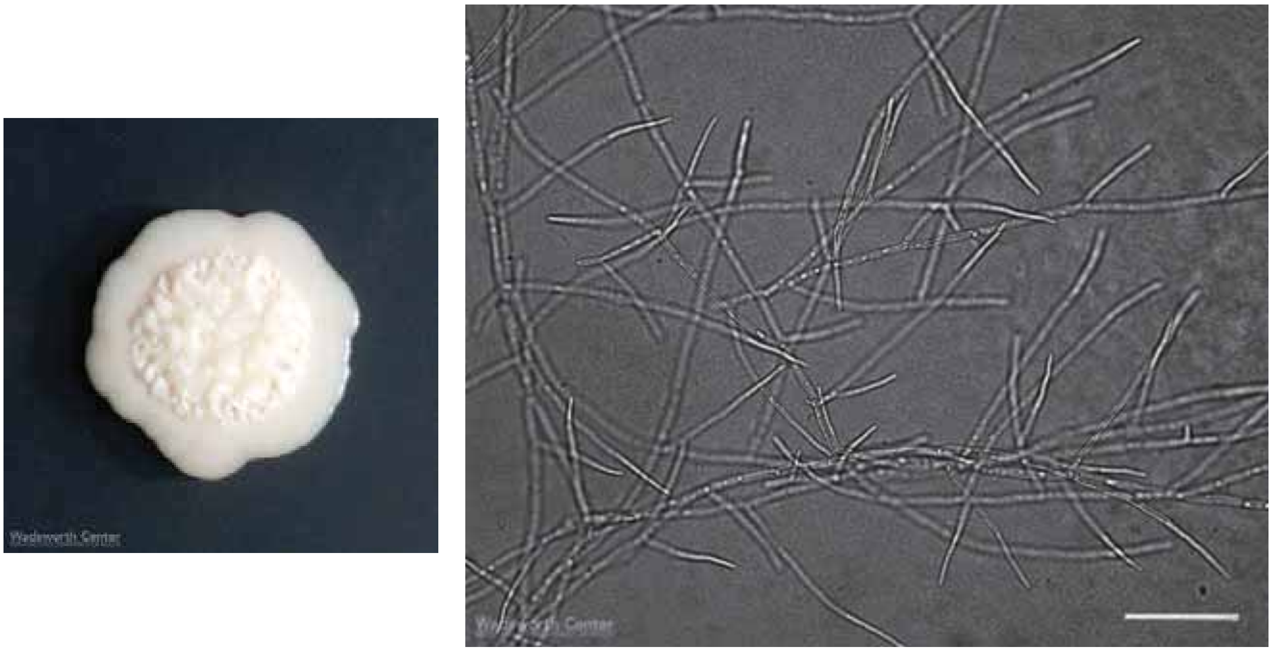
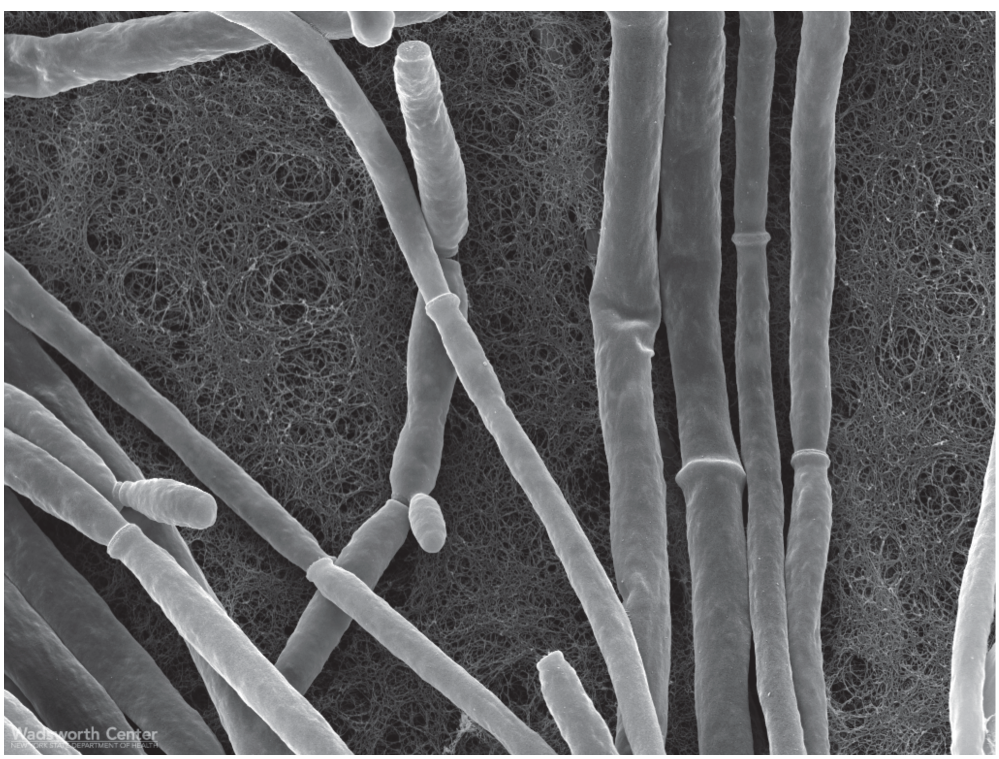


Figure 7A. Scanning electron micrograph illustrates pseudohyphae and blastoconidia.



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Y-3 *Candida parapsilosis*

Source: Urine / Blood / Lung wash

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

Colony: *Candida parapsilosis* colony is white to cream, dull with smooth surface on Sabouraud's dextrose agar after 5 days at 25°C (Figure 8).

Microscopy: *Candida parapsilosis* showes long, multibranched pseudohyphae, together with small elongated blastoconidia on corn meal agar with Tween 80 (Figure 8).

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 form-long pseudohyphae.

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

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with a reference strain of *Candida parapsilosis* CBS 604 (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:	55
Laboratories with incorrect ID:	0

Illustrations:

Figure 8. *Candida parapsilosis* white to cream, smooth colony on Sabouraud's dextrose agar, 25°C. Microscopic morphology of *Candida parapsilosis* with long, multibranched pseudohyphae together with small cluster of elongated blastoconidia on Corn meal agar with Tween 80 (bar = 5 μm).

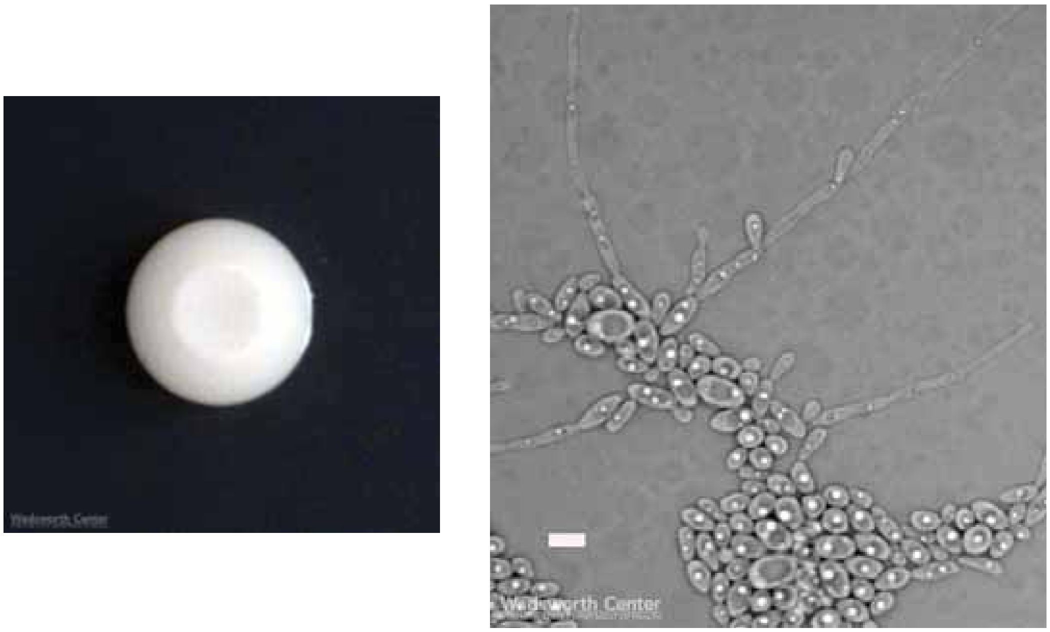
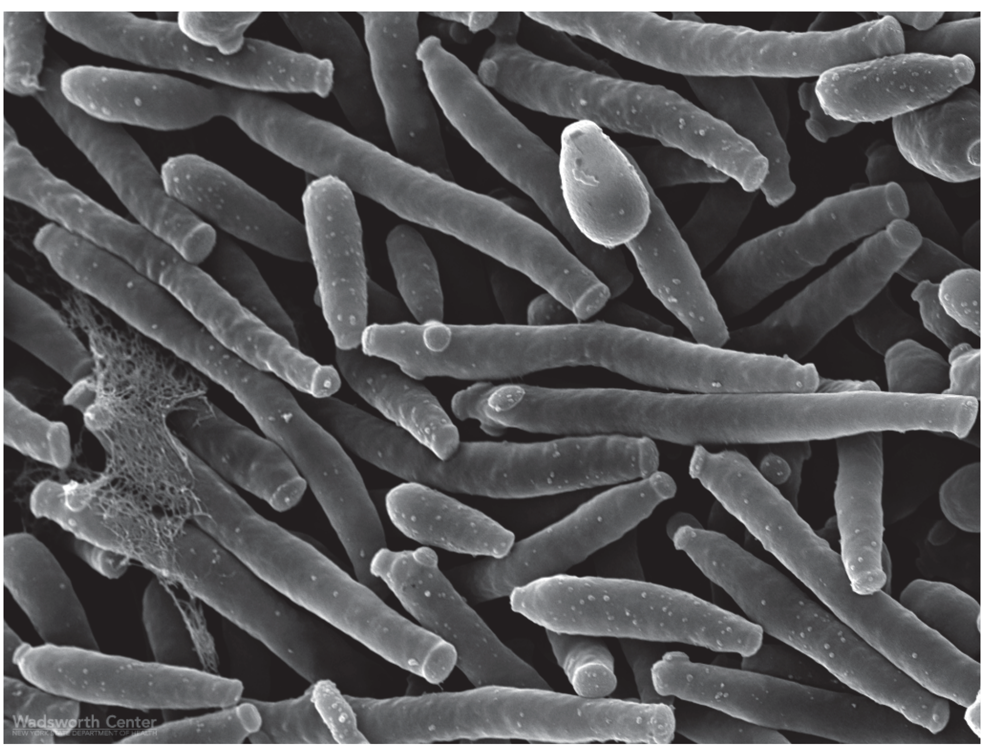


Figure 8A. Scanning electron micrograph with pseudohyphae and blastoconidia.



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Y-4 *Cryptococcus laurentii*

<u>Source</u>: CSF / Urine

<u>Clinical significance</u>: *Cryptococcus laurentii* has been infrequently reported as an etiologic agent of infections in humans. Several cases ranging from fungemia to eye infections have been documented in diabetics and other immunocompromised individuals.

<u>Colony</u>: *C. laurentii* colony ranged from cream, yellow, tan, or pink, and the color intensified as the culture aged (Figure 9).

<u>Microscopy</u>: *C. laurentii* yeasts are round to oval on Corn meal agar with Tween 80. There is no discernible capsule (Figure 9).

<u>Differentiation</u>: *C. laurentii* shares many characteristics with other members of the genus *Cryptococcus*. It produces urease enzyme, assimilates inositol, and does not ferment carbohydrates. It can be differentiated from *C. neoformans* by inability to form brown colonies on Niger Seed Agar.

<u>Molecular test</u>: *C. laurentii*-has been-reported to be a heterogeneous species based on nuclear DNA base composition and whole cell protein electrophoretic fingerprints.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Cryptococcus laurentii* strain ATCC 18803 (GenBank accession no. AY591353.2).

<u>Antifungal susceptibility</u>: In general, non-*neoformans Cryptococcus* species are susceptible to amphotericin B and various azoles. However, some isolates of *C. laurentii* are found to be resistant to fluconazole.

<u>Participant performance</u>:

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

Illustrations:

Figure 9. *Cryptococcus laurentii*, white creamy colony on Sabouraud's dextrose agar, 25°C. *Cryptcoccus laurentii* on corn meal agar with Tween 80 showing blastoconidia (BAR = 50 μm).

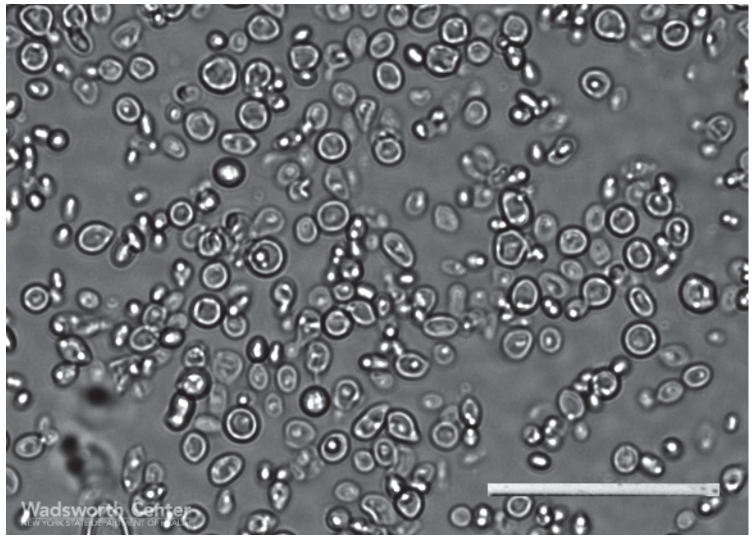
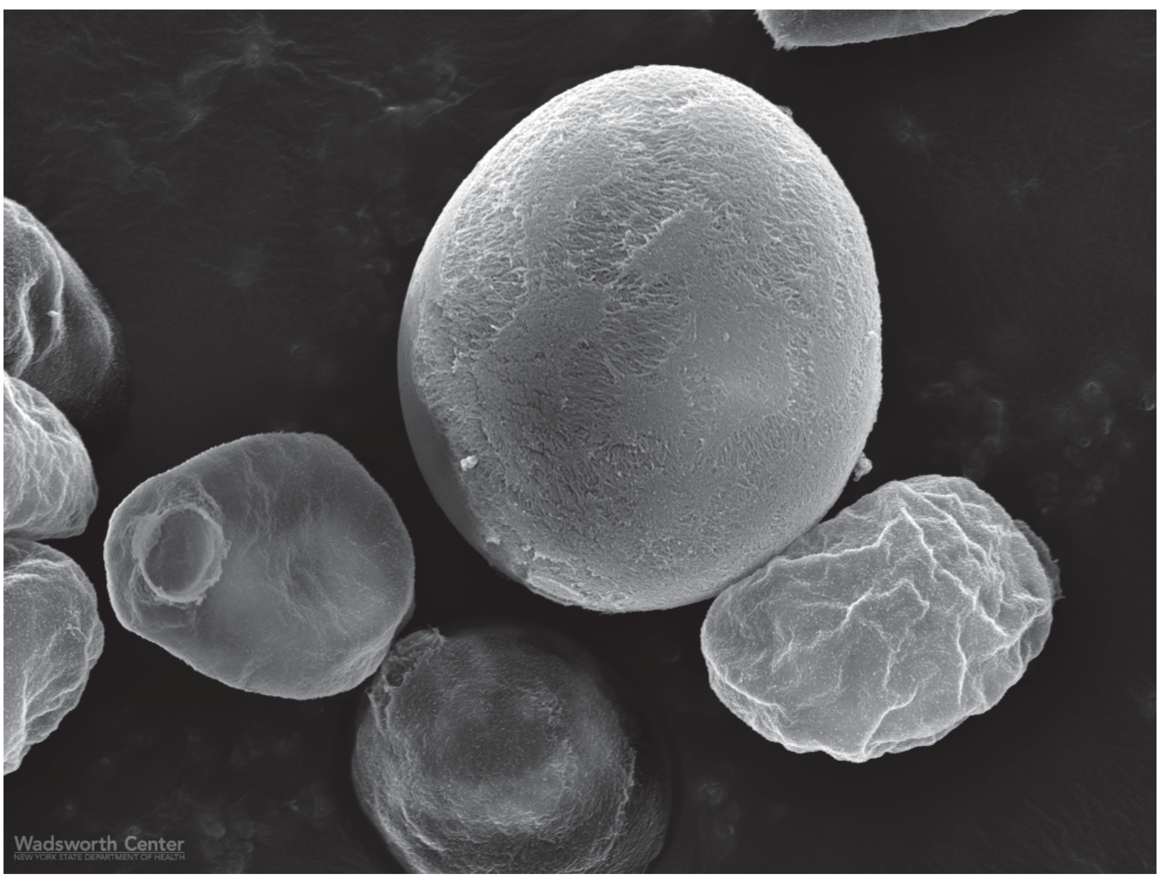


Figure 9A. Scanning electron micrograph illustrating blastoconidia.



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Y-5 *Cryptococcus neoformans*

Source: Blood / Sputum / Urine

Clinical significance: *Cryptococcus neoformans* is a major pathogen of humans and animals. It is differentiated from its sibling pathogenic species *Cr. gattii* by biochemical and genetic features and host predilection. The incidence of cryptococcosis due to *Cr. neoformans* increased with the spread of AIDS and other immunosuppressive conditions. *Cr. neoformans* var. *grubii* and var. *neoformans* mainly cause meningoencephalitis in patients with AIDS or other underlying immune dysfunctions. *Cr. neoformans* var. *neoformans* infections are more likely to have cutaneous involvement, and to infect older patients, than are infections caused by *Cr. grubii*. *Cr. gattiii* causes pulmonary cryptococcosis and systemic cryptococcosis in normal and immunocompromised hosts.

Colony: *Cr. neoformans* colony is cream to tan in color, smooth, moist, and soft on Sabouraud's dextrose agar at 25°C (Figure 10).

Microscopy: *Cr. neoformans* yeast cells are large and round, with no pseudohyphae or true hyphae on corn meal agar with Tween 80. In India-ink preparation, encapsulated yeasts are seen (Figure 10).

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

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

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

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

Participant performance:

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

<u>Illustrations</u>:

Figure 10. *Cryptococcus neoformans* colony cream to tan colored, smooth, moist, and soft colony of on Sabouraud's dextrose agar, 25°C. Microscopic morphology of *Cryptococcus neoformans* showing round, large blastoconidia on Corn meal agar with Tween 80 (bar = 25 μm).

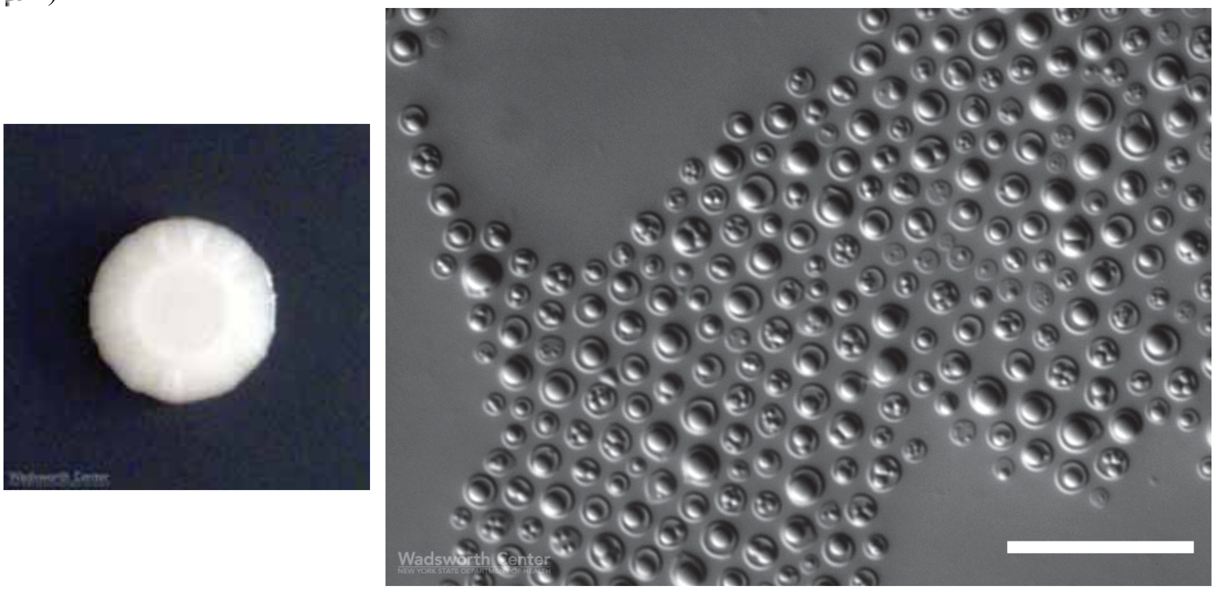
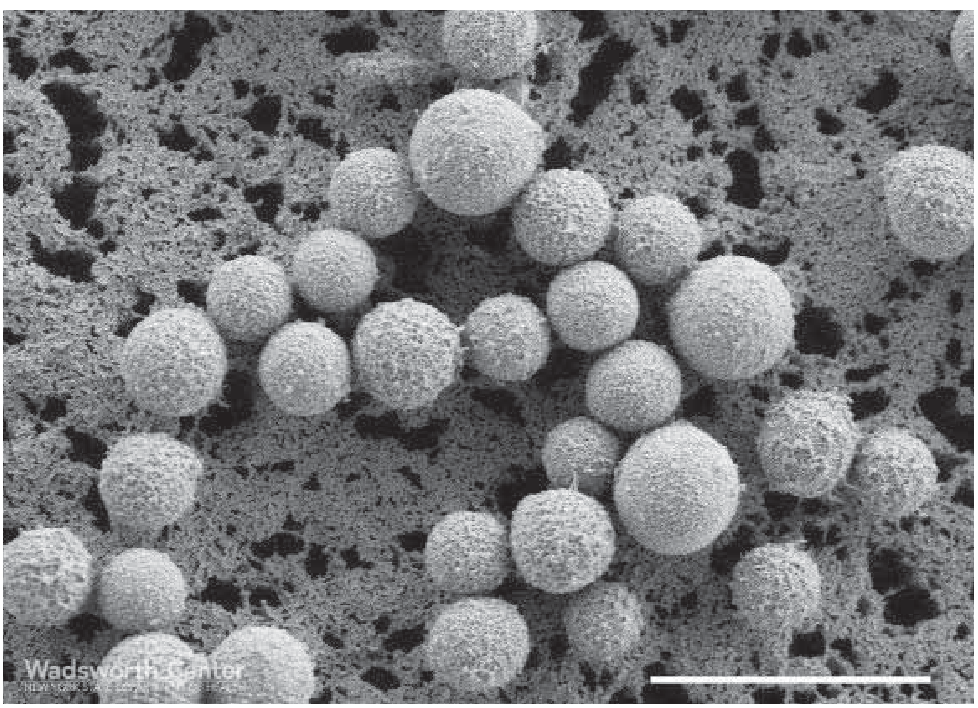


Figure 10A. Scanning electron micrograph with *Cryptococcus neoformans* (bar = 10 μm).



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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. 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 for point-of-care testing of cryptococcosis from serum.

Materials & Methods: Sixty-four laboratories participated in the September 26, 2012 direct detection antigen test event. Two positive serum samples (Cn-Ag-1 and Cn-Ag-2) with the titer of 1:16/1:32 and 1:64/1:128, respectively for cryptococcal antigen were 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 except one. The consensus results for specimens Cn-Ag-3, Cn-Ag-4, and Cn-Ag-5 were negative as expected. Cn-Ag-1 was reported positive by all the participating laboratories with the acceptable titer ranges 1:4 – 1:128 except two laboratories. Cn-Ag-2 was reported positive by all the participating laboratories with the acceptable titer ranges 1:16 – 1:512 except one laboratory. Two laboratories reported higher titer than the acceptable range for specimen Cn-Ag-2 (Table 3).

Table 3. Summary of laboratory performance for semi-quantitative detection of cryptococcal antigen

Method		Cn-Ag-1 Titers						
No. laboratories		4	8	16	20	32	64	128
EIA	2			2				
Latex Agglutination	59	1	7	19	2	20	9	1
Immuno-Mycologics	7		1	5		1		
Meridien Diagnostic	43	1	4	13	2	17	5	1
Remel	9		2	1		2	4	
Total	61	1	7	21	2	20	9	1

Method		Cn-Ag-2 Titers									
No. laboratories		16	32	40	64	100	128	256	512	1024	2048
EIA	2				1		1				
Latex Agglutination	60	1	4	1	18	1	19	8	6	1	1
Immuno-Mycologics	8		1		5		2				
Meridien Diagnostic	43	1	3	1	9	1	16	6	4	1	1
Remel	9				4		1	2	2		
Total	62	1	4	1	19	1	20	8	6	1	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 of 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 practices in their facilities.

Materials: *Candida tropicalis* (S-1) was the analyte in the September 26, 2012 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 (Table 4 and EUCAST data). However, the participating laboratories are advised to regularly consult these organizations for the latest version of their standard documents.

Adapted from M-27S3 Vol. 28 No. 15, February 2010

Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Third Informational Supplement

Table 4. Interpretative Guidelines for *In Vitro* Susceptibility Testing of *Candida* spp.

Antifungal Agent	Susceptible (**S**)	Susceptible- dose dependent (**S-DD**)	Intermediate (**I**)	Resistant (**R**)	Nonsusceptible (**NS**)
Anidulafungin	**≤2**	-	-	-	**>2**
Caspofungin	**≤2**	-	-	-	**>2**
Fluconazole	**≤8**	**16-32**	-	**≥64**	-
Flucytosine	**≤4**	-	**8-16**	**≥32**	-
Itraconazole	**≤0.125**	**0.25-0.5**	-	**≥1**	-
Micafungin	**≤2**	-	-	-	**>2**
Voriconazole	**≤1**	**2**	-	**≥4**	-

Note: Please consult relevant CLSI publications for further details about these guidelines. No recommended guideline is currently available for the interpretation of MIC values for ketocoanzole and posaconazole.

***Candida* spp. EUCAST Antifungal Clinical Breakpoint Table v. 4.1, valid from 2012-03-05**

Antifungal agent	MIC breakpoint (mg/L)													
	C. albicans		*C. glabrata*		*C. krusei*		*C. parapsilosis*		*C. tropicalis*		*C. guillermondii*		Non-species related breakpoints[1]	
	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >
Amphotericin B	1	1	1	1	1	1	1	1	1	1	IE	IE	IE	IE
Anidulafungin	0.03	0.03	0.06	0.06	0.06	0.06	-	-	0.06	0.06	IE[2]	IE[2]	IE	IE
Caspofungin	Note[3]	Note[3]	Note[3]	Note[3]	Note[3]	Note[3]	-	-	Note[3]	Note[3]	IE[2]	IE[2]	IE	IE
Fluconazole	2	4	IE[2]	IE[2]	-	-	2	4	2	4	IE[2]	IE[2]	2	4
Itraconazole	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP
Micafungin	IP	IP	IP	IP	IP	IP	-	-	IP	IP	IE[2]	IE[2]	IP	IP
Posaconazole	0.06	0.06	IE[2]	IE[2]	IE[2]	IE[2]	0.06	0.06	0.06	0.06	IE[2]	IE[2]	IE	IE
Voriconazole	0.12[4]	0.12[4]	IE	IE	IE	IE	0.12[4]	0.12[4]	0.12[4]	0.12[4]	IE[2]	IE[2]	IE	IE

IE Insufficient Evidence   IP In preparation

Notes

1. Non-species related breakpoints have been determined mainly on the basis of PK/PD data and are independent of MIC distributions of specific species. They are for use only for organisms that do not have specific breakpoints.

2. The ECOFFs for these species are in general higher than for *C. albicans*.

3. Due to significant inter-laboratory variation in MIC ranges for caspofungin, EUCAST breakpoints have not yet been established.

4. Strains with MIC values above the S/I breakpoint are rare or not yet reported. The identification and antimicrobial susceptibility tests on any such isolate must be repeated and if the result is confirmed the isolate sent to a reference laboratory. Until there is evidence regarding clinical response for confirmed isolates with MIC above the current resistant breakpoint (in italics) they should be reported resistant.

Comments: Acceptable results were MICs +/-2 dilutions of the reference laboratory results for any single drug. Only 3 of the 29 laboratories participating in this test event tested all 10 antifungal drugs. The reported results were as follows: itraconazole (28 laboratories), flucytosine (24 laboratories), amphotericin B (20 laboratories), caspofungin (21 laboratories), posacoanazole (15 laboratories), anidulafungin (16 laboratories), and micafungin (16 laboratories), ketocoanzole (5 laboratories). Fluconazole was the only drug tested by all 29 laboratories. Only one laboratory reported high MIC value with the interpretation of 'resistant' for voriconazole. Seven laboratories did not report any interpretation for amphotericin B and six laboratories had no interpretation for posaconazole MIC.

Table 5. Laboratory Performance

S- 1: *Candida tropicalis* (M2698)

Drug	Laboratories with acceptable responses / Total Laboratories (% acceptable responses)
Amphotericin B	20/20 (100%)
Anidulafungin	16/16 (100%)
Caspofungin	21/21 (100%)
Flucytosine (5-FC)	24/24 (100%)
Fluconazole	29/29 (100%)
Itraconazole	28/28 (100%)
Ketoconzole	5/5 (100%)
Micafungin	16/16 (100%)
Posaconazole	15/15 (94%)
Voriconazole	23/24 (96%)

Table 6. Antifungal MICs (µg/ml) Reported by the Participating Laboratories

S-1: *Candida tropicalis* (M2698)

Drug	No. labs	MIC (µg/ml)															
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256
Amphotericin B	20				1		6	12	1								
Anidulafungin	16		2	2	7	5											
Caspofungin	21			1	9	7	4										
Flucytosine (5-FC)	24			2	18	3	1										
Fluconazole	28*						1	2	20	3	2						
Itraconazole	27*				4	12	11										
Ketoconazole	4*				2	2											
Micafungin	16	1	5	10													
Posaconazole	15				3	9	3										
Voriconazole	24			4	12	6	1						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
- CLSI microdilution, YeastOne Colorimetric, and Etest methods

Table 7. Antifungal Susceptibility Interpretations Reported by the Participating Laboratories

S-1: *Candida tropicalis* (M2698)

Drug	No. laboratories	Susceptible	Susceptible-dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	20	13					7
Anidulafungin	16	16					
Caspofungin	21	21					
Flucytosine	24	24					
Fluconazole	29	29					
Itraconazole	28	18	10				
Ketoconazole	5	2					3
Micafungin	16	16					
Posaconazole	15	9					6
Voriconazole	24	23			1		

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* M2036 was used as 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. Three laboratories used CLSI Microdilution method while the remaining three used YeastOne Colorimetric method.

Comments: Five out of twenty-nine laboratories, which hold antifungal susceptibility testing for yeasts permit, voluntarily participated in this test event for molds. Please refer to Table 8 and 9 for summary of performances. Since too few laboratories have participated in this test, no consensus data can be generated.

Table 8. Mold Antifungal Susceptibility: *Aspergillus fumigatus* M2036.

Drugs	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	0.5	0.5 – 1.0	0.5 – 1.0
Anidulafungin	0.015	0.015 – 0.06	<0.015 – <0.06
Caspofungin	0.5	0.015 – 8.0	<0.008 – 0.5
Fluconazole	64	64 – 256	>64 – 256
Itraconazole	0.5	0.12 – 0.5	<0.015 – 0.5
Ketoconzole	1.0	1.0 – 8.0	4.0 – 8.0
Micafungin	0.015	0.008 – 0.06	<0.008 – <0.06
Posaconazole	0.25	0.06 – 0.25	<0.008 – 0.25
Voriconazole	0.5	0.12 – 1.0	0.12 – 0.5

Table 9. MIC (µg/ml) Values of Mold Antifungal Susceptibility: *Aspergillus fumigatus* M2036

Drugs (µg/ml)	Total # of labs	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1.0	2.0	4.0	8.0	16	32	64	256
Amphotericin B	5							2	3							
Anidulafungin	4		3		1											
Caspofungin	4	1	1		1			1								
Fluconazole	4														2	2
Itraconazole	5		1				2	2								
Ketoconazole	2										1	1				
Micafungin	4	2	1		1											
Posaconazole	4	1			2		1									
Voriconazole	4					2		2								

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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