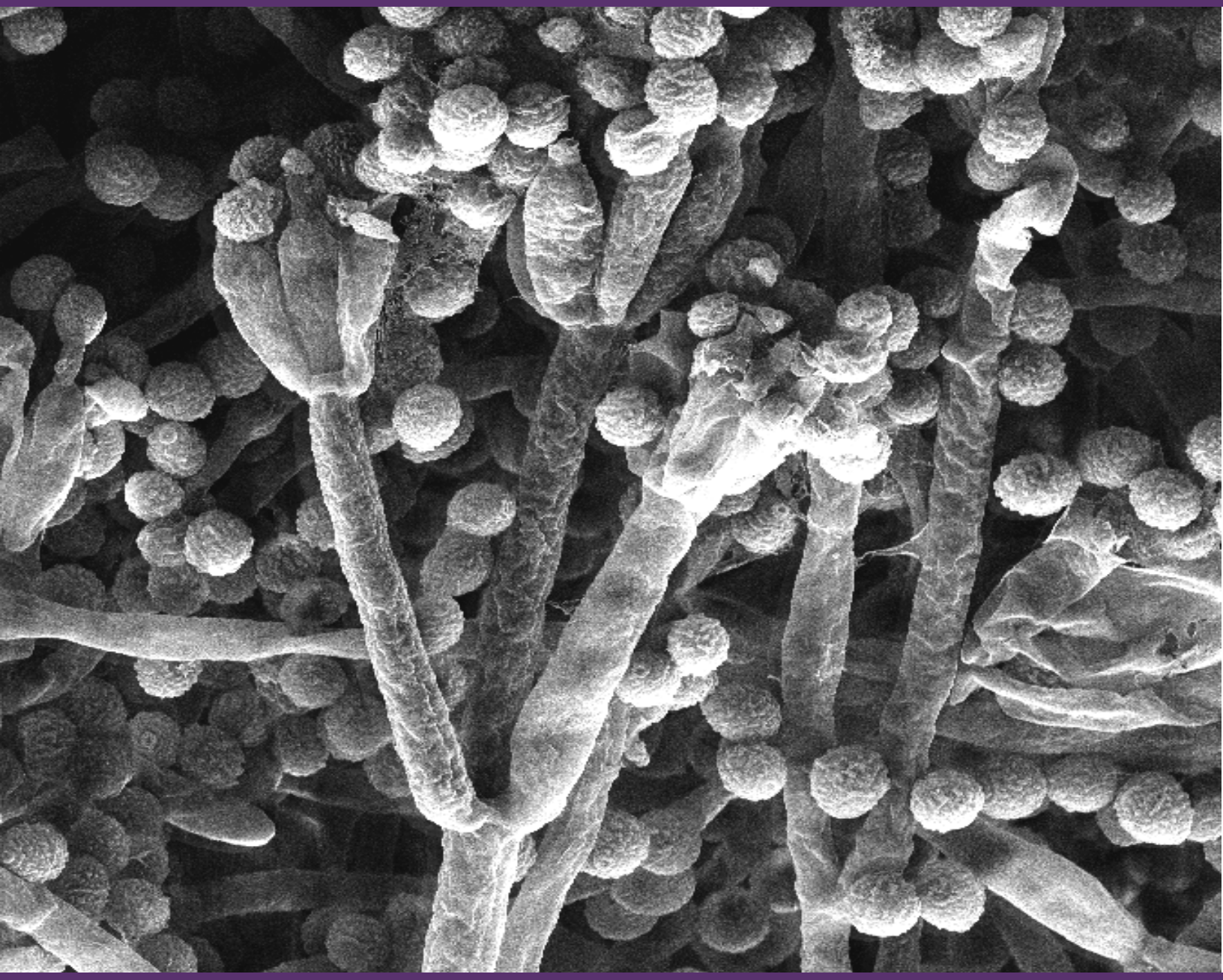
Mycology Proficiency Testing Program



Test Event Critique

October 2014

Wadsworth Center

New York State Department of Health

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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

Name	Responsibility	Phone	Email
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Mycology Proficiency Testing Program (PTP)

CATEGORY DESCRIPTION

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

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

> **Identification yeast only:** This category is for laboratories that isolate and identify pathogenic yeasts or yeast-like fungi to genus and species level (for detail, please see yeast master list). Laboratories holding this category may also perform susceptibility testing on yeasts. These laboratories are expected to refer mold specimens to another laboratory holding Mycology – Comprehensive permit.
>
> **Antigen detection:** This category is for laboratories that perform direct antigen detection methods.

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

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

Comprehensive

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

Restricted

Identification Yeast Only

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

Antigen Detection

- Antigen detection of *Cryptococcus neoformans**

TEST SPECIMENS& GRADING POLICY

Test Specimens

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

Grading Policy

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

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

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

Acceptable results for antifungal susceptibility testing are based on the consensus/all participating laboratories' MIC values within +/- 2 dilutions and then the interpretation per CLSI guidelines or related, peer-reviewed publications. Especially, when there is no interpretation, MIC values are the key judge points. One yeast species is to be tested against following drugs: amphotericin B, anidulafungin, caspofungin, flucytosine, fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. The participating laboratories are free to select any number of antifungal drugs from the test panel based upon test practices in their facilities. A maximum score of 100 is equally distributed to account for the drugs selected by an individual laboratory. If the result for any drug is incorrect then laboratory gets a score of zero for that particular test component or set.

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

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

TEST ANALYTE MASTER LISTS

Mold Master List

The mold master list is intended to provide guidance to the participating laboratories about the scope of the Mycology (Comprehensive) Proficiency Testing Program. The list includes most common pathogenic and non-pathogenic fungi likely to be encountered in the clinical laboratory. The list is compiled from published peer-reviewed reports as well as current practices in other proficiency testing programs. This list is meant to illustrate acceptable identifications used in grading of responses received after each test event. This list neither includes all molds that might be encountered in a clinical laboratory nor is it intended to be used for the competency assessment of the laboratory personnel in diagnostic mycology.

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

It is expected that major pathogenic fungi listed in the Master List will be completely identified to genus and species levels while those fungi either not listed (*Aspergillus lentulus*) or listed with genus name only (*Acremonium*) will be identified as *Aspergillus* species or *Acremonium* species. However, the laboratory can elect to provide only genus level identification if it reflects the standard operating procedures (SOP) for patient testing. Please use "group" or "species complex" where appropriate e.g. *Aspergillus glaucus* group or *Fusarium solani* species complex if it is consistent with current reporting format used by the laboratory.

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

Yeast Master List

The yeast master list is intended to provide guidance to the participating laboratories about the scope of the Mycology - Restricted to Yeasts Only Proficiency Testing Program. This list includes most common pathogenic and non-pathogenic yeasts likely to be encountered in the clinical laboratory. The list is compiled from published peer-reviewed reports as well as current practices in other proficiency testing programs. The list is meant to illustrate acceptable identifications used in grading of responses received after each test event. This list neither includes all yeasts that might be encountered in a clinical laboratory nor is intended to be used for the competency assessment of the laboratory personnel in diagnostic mycology.

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

It is expected that major pathogenic yeasts listed in the Master List will be completely identified to genus and species levels while those yeasts not listed in the master list will be identified to genus only (i.e. *Candida inconspicua* as *Candida* species). However, the laboratory can elect to provide only genus level identification if it reflects the standard operating procedures (SOP) for patient testing. Please use "species complex" where appropriate, e.g. *Candida parapsilosis* species complex if it is consistent with current reporting format used by the laboratory.

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

Summary of Laboratory Performance:

Mycology – Mold

	Specimen key	Validated specimen	Other acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
M-1	*Aspergillus nidulans*	*Aspergillus nidulans*	*Aspergillus nidulans* group *Aspergillus* species[1]	56/57 (98%)
M-2	*Penicillium* species	*Penicillium* species	*Penicillium janthinellum*	56/57 (98%)
M-3	*Scedosporium apiospermum*	*Scedosporium apiospermum*	*Pseudallescheria boydii* species complex *Scedosporium apiospermum* species complex *Scedosporium* species[2]	58/59 (99%)
M-4	*Paecilomyces lilacinus*	*Paecilomyces lilacinus*	*Paecilomyces* species[3]	55/57 (96%)
M-5	*Arthrographis* species	*Arthrographis* species	*Arthrographis kalrae*	55/57 (96%)

[1]Only if the laboratory does not speciate non-*fumigatus Aspergillus* for patient specimens routinely.
[2]Only if the laboratory does not speciate *Scedosporium* for patient specimens routinely.
[3]Only if the laboratory does not speciate *Paecilomyces* for patient specimens routinely.

Mycology – Yeast Only

	Specimen key	Validated specimen	Other acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
Y-1	*Cryptococcus neoformans*	*Cryptococcus neoformans*	*Cryptococcus neoformans-Cryptococcus gattii* species complex	51/51 (100%)
Y-2	*Candida guilliermondii*	*Candida guilliermondii*		52/53 (98%)
Y-3	*Blastoschizomyces capitatus*	*Blastoschizomyces capitatus*	*Geotrichum capitum Saprochaete capitata*	45/49 (92%)
Y-4	*Hansenula anomala*	*Hansenula anomala*	*Candida pelliculosa*	46/49 (94%)
Y-5	*Candida famata*	**Not validated**		29/49 (59%)

Mycology – Direct detection (*Cryptococcus* Antigen Test)

	Specimen key (Titer)	Validated specimen	Acceptable titer range	Correct responses / Total laboratories (% correct responses)	
				Qualitative	Quantitative
Cn-Ag-1	Negative	Negative		65/65 (100%)	NA
Cn-Ag-2	Positive (1:32)	Positive (1:32)	1:8 – 1:256	65/65 (100%)	65/65 (100%)
Cn-Ag-3	Positive (1:128)	Positive (1:128)	1:16 – 1:512	65/65 (100%)	65/65 (100%)
Cn-Ag-4	Negative	Negative		65/65 (100%)	NA
Cn-Ag-5	Negative	Negative		65/65 (100%)	NA

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

Drugs	Acceptable MIC (μg/ml) range	Interpretation	Laboratories with acceptable responses/ Total laboratories (% correct responses)
Amphotericin B	0.125 – 1.0	Susceptible / No interpretation	20/20 (100%)
Anidulafungin	<0.015 – 0.06	Susceptible	17/17 (100%)
Caspofungin	0.03 – 0.25	Susceptible / Intermediate	22/22 (100%)
Flucytosine (5-FC)	<0.03 – 0.125	Susceptible / No interpretation	23/23 (100%)
Fluconazole	16 – >256	Susceptible-dose dependent / Resistant	30/31 (97%)
Itraconazole	≥ 1.0	Resistant / No interpretation	22/25 (88%)
Ketoconazole	0.125 – 2.0	No interpretation	4/4 (100%)
Micafungin	≤0.016	Susceptible	17/17 (100%)
Posaconazole	2 – ≥ 8	No interpretation	14/16 (88%)
Voriconazole	1.0 – 8	No interpretation	20/23 (87%)

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	18
Dade Behring MicroScan Rapid Yeast Identification Panel	2
MALDI-TOF	1
Molecular Sequencing	1
Remel RapID Yeast Plus System	3
Vitek2	26
Antifungal Susceptibility*	
Disk diffusion	1
Etest	1
Vitek II	2
YeastOne– Mold	2
YeastOne –Yeast	23
CLSI Microbroth dilution method – Yeast	5
CLSI Microbroth dilution method – Mold	2
Cryptococcal antigen*	
Immuno-Mycologics Latex Cryptococcus Antigen Detection System	6
Immuno-Mycologics CrAg Lateral Flow Assay	12
Meridien BioScience Cryptococcal Antigen Latex Agglutination System (CALAS)	37
Immuno-Mycologics ALPHA Cryptococcal Antigen enzyme immunoassay(CrAg EIA)	1
Remel Cryptococcal Antigen Latex Test	9

*Include multiple systems used by some laboratories

MOLD DESCRIPTIONS

M-1 *Aspergillus nidulans*

Source: CSF / Sputum / Nose

Clinical Significance: Human infections of *Aspergillus nidulans* have been rarely reported. Most of these reports were from patients with chronic granulomatous disease involving skin, sinus, lungs etc.

Colony: At 25°C, colony on Sabouraud's dextrose agar is dark green with purplish peripheral pigment, powdery and rapid growing (Figure 1).

Microscopy: Lactophenol cotton blue mount shows septate hyphae with brown, wavy conidiophores. Conidiophore ended in vesicle, which is subglobose with its upper half-covered by two series of sterigmata (biseriate). Conidia, measuring 5 – 7 μm in diameter, are round and smooth- rough walled. Round hülle cells and reddish color cleistothecia are also seen. Hülle cells are specialized structures made up of loose network of hyphae, having globose, vesiculose cells with thick walls that occur in certain groups of *Aspergilli*. Their characteristic shape provides a valuable diagnostic tool. Cleistothecia are sexual structures i.e. network of hyphae where mating between a and α strains occur. Ascospores (sexual spores) produced within these cleistothecia, are purple in color, lens shaped with equatorial crests (Figure 1).

Differentiation from other *Aspergilli* – *Aspergillus nidulans* can be distinguished by its dark green colony with purple reverse; microscopically, brown conidiophores, biseriate phialides, round hülle cells, cleistothecia with lens shaped ascospores with equatorial crests are characteristics. Also, *A. nidulans* can be differentiated from A. versicolor by the absence of reduced conidiogenous structures, which are distinct feature of *A. versicolor*. Please refer to Table 1 for more details.

Molecular test: *Aspergillus nidulans* has a well-defined genetic system, which allows it to be used as a model organism in basic and applied research.

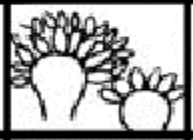
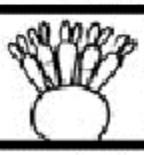
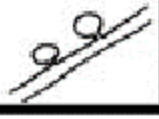
The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Aspergillus nidulans* UOA/HCPF 9011 (GenBank accession no. FJ878643).

Antifungal susceptibility: Susceptibility testing results indicate that most of the isolates are susceptible to amphotericin B, voriconazole, and variably susceptible to itraconazole.

Participant performance:

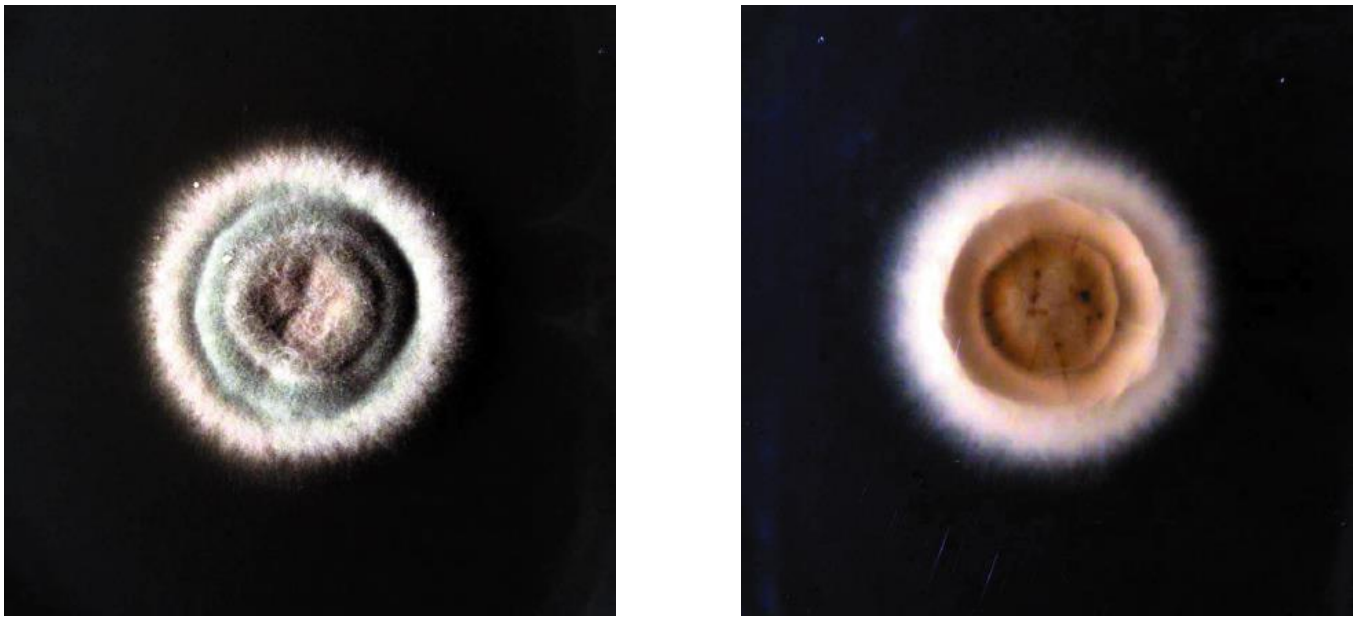
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	54
Laboratories with incorrect ID:	1
(*Aspergillus versicolor*)	(1)

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						

Illustrations:

Figure 1. Colony of *Aspergillus nidulans* with whitish to purplish edge on Sabouraud's dextrose agar (upper panel). Microscopic morphology of *Aspergillus nidulans* showing subglobose vesicle with biseriate, columnar head, cleistothecia with ascospores, and hülle cells (lower panel).



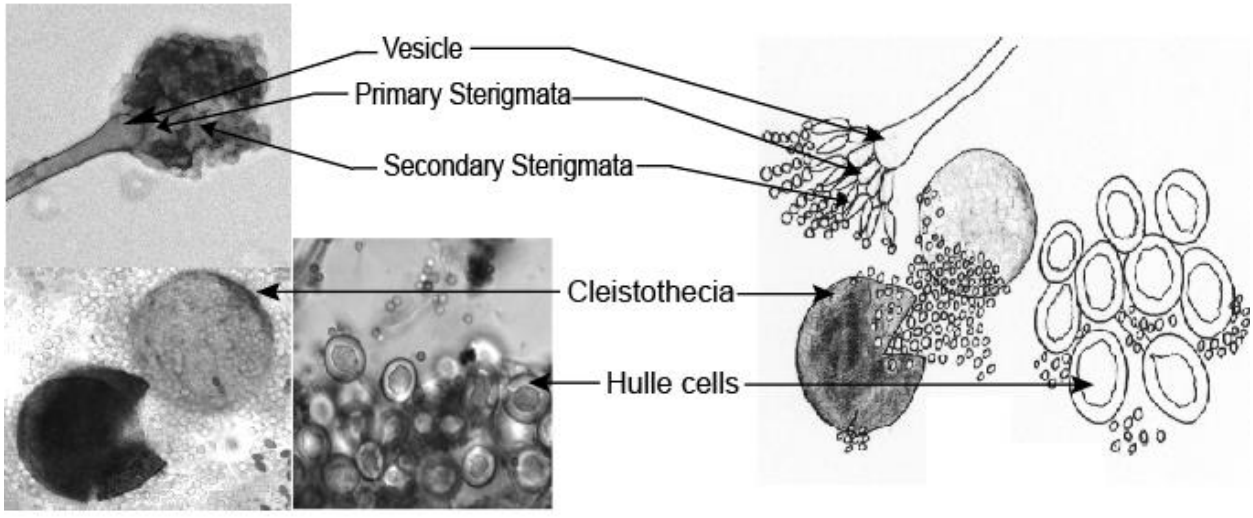
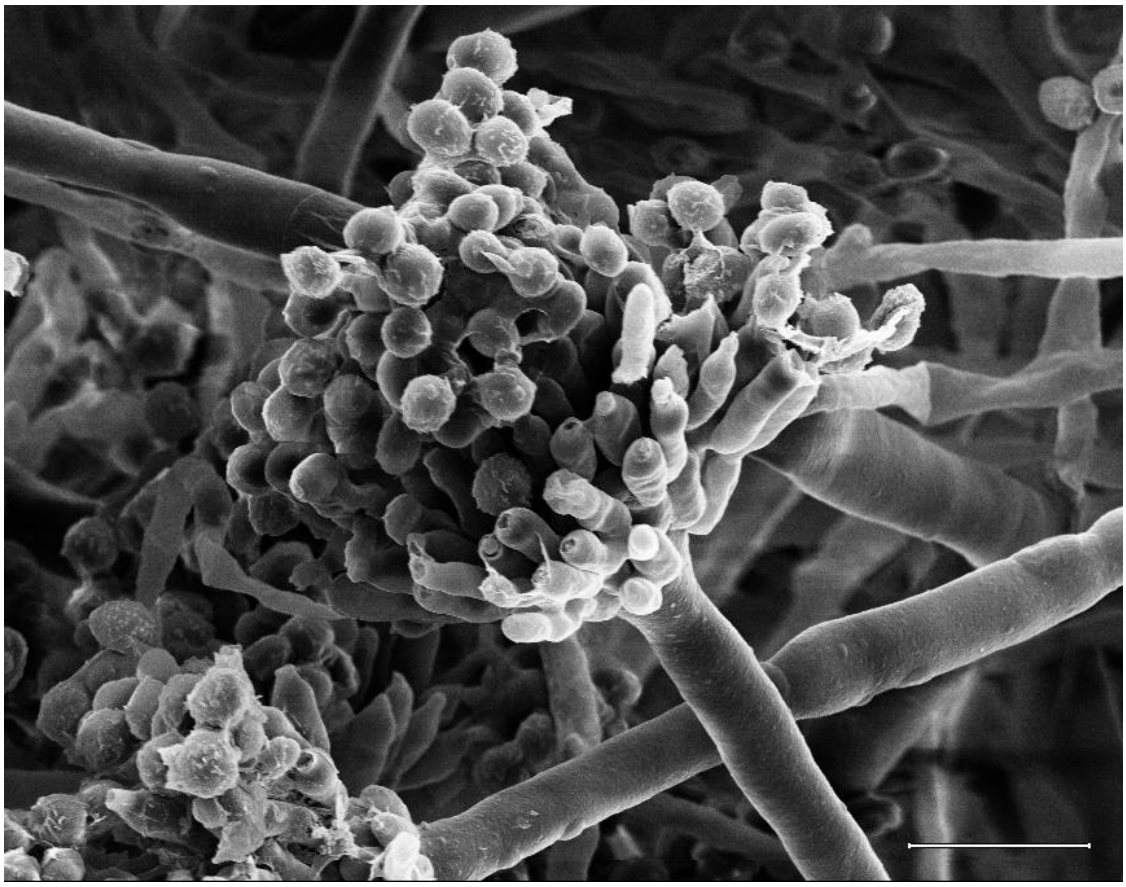
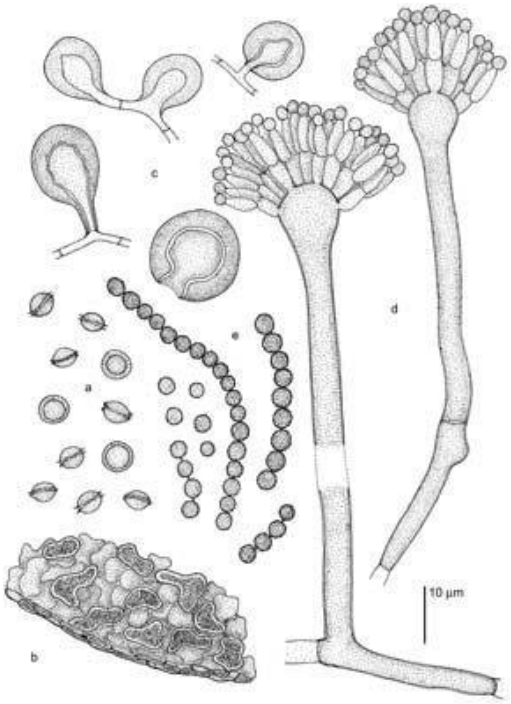


Figure 1A. Scanning electron micrograph of *Aspergillus nidulans* (bar = 10 μm, upper panel). Line drawings of *Aspergillus nidulans* (lower panel).





http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=3670

Further reading:

Bukhari E, Alrabiaah A. 2009. First case of extensive spinal cord infection with *Aspergillus nidulans* in a child with chronic granulomatous disease. *J Infect Dev Ctries.* 3: 321 - 323.

Dellepiane RM, Tortorano AM, Liotto N, Laicini E, Di Landro G, Carnelli V, Pietrogrande MC. 2008. Invasive *Aspergillus nidulans* infection in a patient with chronic granulomatous disease. *Mycoses*. 51: 458 - 460.

de Souza CC, Pellizzon CH, Hiraishi M, Goldman MH, Goldman GH. 1998. Isolation and characterisation of cycloheximide – sensitive mutants of *Aspergillus nidulans*. *Current Genetics.* 33: 60 - 69.

Henriet SS, Verweij PE, Warris A. 2012. *Aspergillus nidulans* and chronic granulomatous disease: a unique host-pathogen interaction. *J Infect Dis*. 206: 1128-1137.

Kim M, Shin JH, Suh SP, Ryang DW, Park CS, Kim C, Kook H, Kim J. 1997. *Aspergillus nidulans* infection in a patient with chronic granulomatous disease. *J Korean Medical Sci.* 12: 244 - 248.

Lucas GM, Tucker P, Merz WG. 1999. Primary cutaneous *Aspergillus nidulans* infection associated with a Hickman catheter in a patient with neutropenia. *Clin Infect Dis*. 29: 1594 - 1546.

Resen-Wolff A, Koch A, Friedrich W, Hahn G, Gahr M, Roesler J. 2004. Successful elimination of an invasive *Aspergillus nidulans* lung infection by voriconazole after failure of a combination of caspofungin and liposomal amphotericin b in a boy with chronic granulomatous disease. *Pediatric Infect. Dis J.* 23: 584 - 586.

Mizuki M, Chikuba K, Tanaka K. 1994. A case of chronic necrotizing pulmonary aspergillosis due to *Aspergillus nidulans. Mycopathologia*. 128: 75 - 79.

Ng KP, Saw TL, Madasamy M, Soo Hoo T. 1999. Onychomycosis in Malaysia. *Mycopathologia*. 147: 29 - 32.

Rösen-Wolff A, Koch A, Friedrich W, Hahn G, Gahr M, Roesler J. 2004. Successful elimination of an invasive *Aspergillus nidulans* lung infection by voriconazole after failure of a combination of caspofungin and liposomal amphotericin B in a boy with chronic granulomatous disease. *Pediatr Infect Dis J.* 23: 584 - 586.

Yano S, Kobayashi K, Shishido S, Nakano H. 1999. Intrabronchial *Aspergillus nidulans* infection in an immunocompetent man. *Internal Medicine.* 38: 372 - 375.

M-2 *Penicillium* species

Source: Foot / Eye / Lung

Clinical significance: *Penicillium* spp. other than *Penicillium marneffei* are commonly considered as laboratory contaminants but may cause infection in patients with immunocompromized status. *Penicillium* spp. have been isolated from patients with keratitis, endophtalmitis, otomycosis, necrotizing esophagitis, pneumonia, endocarditis, peritonitis, and urinary tract infections. Some species are known to produce mycotoxins, which are nephrotoxic and carcinogenic.

Colony: *Penicillium* sp. grows rapidly, velvety to powdery in texture. Generally, the colony is initially white and then becomes blue green, gray green, olive gray over time (Figure 2).

Microscopy: Lactophenol cotton blue or Calcofluor mounts shows septate hyaline hyphae, simple or branched conidiophores, and characteristic metulae, phialides. Metulae is the secondary branches that form on conidiophores. The brush-like clusters of phialides, referred to as "penicilli". The unicellular conidia are round, and form in chains at the tips of the phialides (Figure 2).

Differentiation: *Penicillium* sp. can be differentiated from *Paecilomyces* by flask-shaped phialides and globose to subglobose conidia; from *Gliocladium* by chains of conidia; and from *Scopulariopsis* by phialides. *Penicillium* species also can be differentiated from other fungi by their colony morphology.

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

The ribosomal ITS1 and ITS2 region of the test isolate showed 100% nucleotide identity with *Penicillium janthinellum* ATCC 4845 (GenBank accession no. AY373921).

Antifungal susceptibility: In general, *Penicillium* sp. is susceptible to amphotericin B, ketoconazole, itraconazole, and voriconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	56
Laboratories with incorrect ID:	1
(*Paecilomyces* species)	(1)

Illustrations:

Figure 2. White edge, blue green to olive green colony of *Penicillium* species on Sabouraud's dextrose agar (upper panel). Microscopic morphology of *Penicillium janthinellum* showing broom-shaped phialides and round conidia (lower panel).

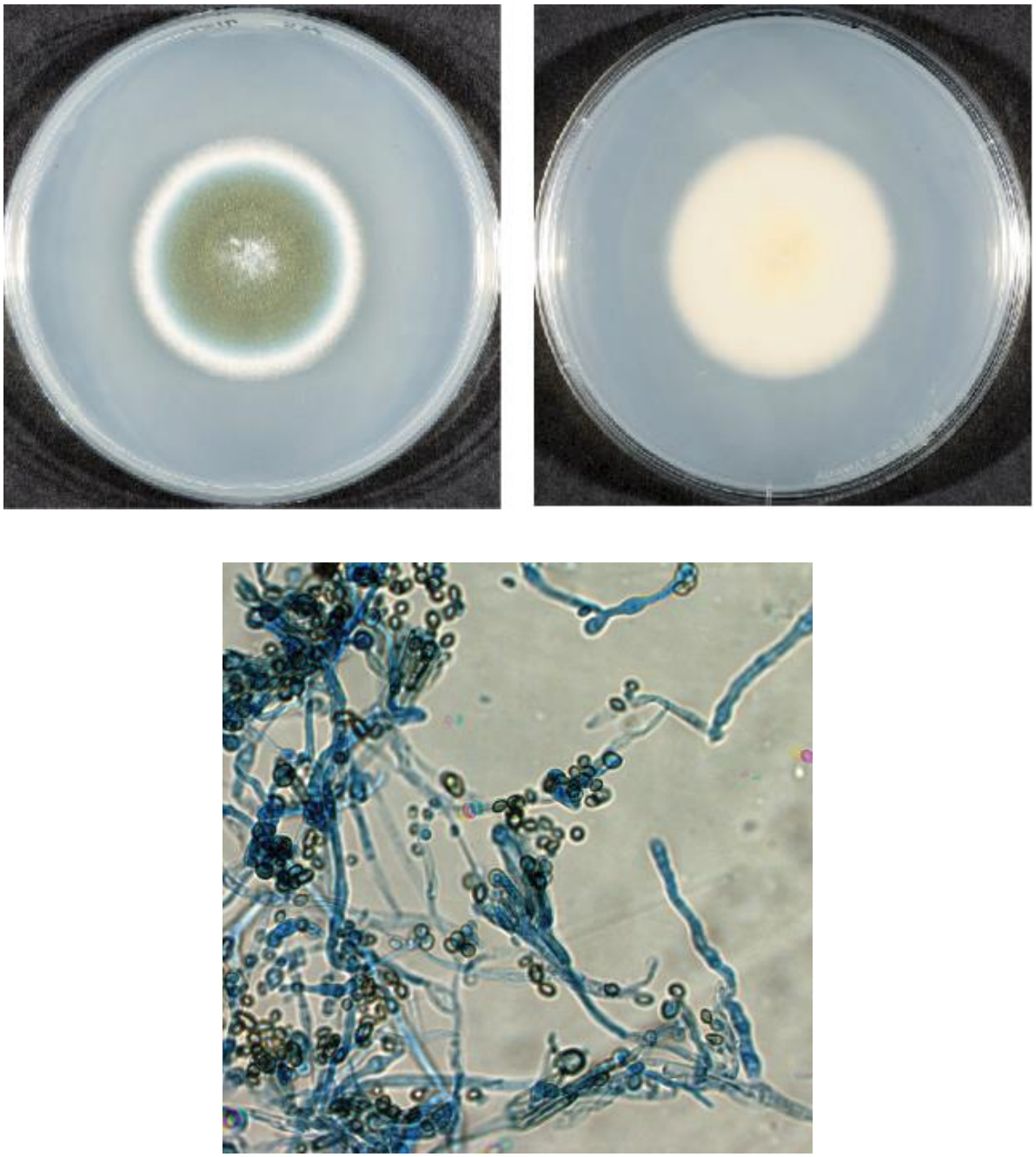
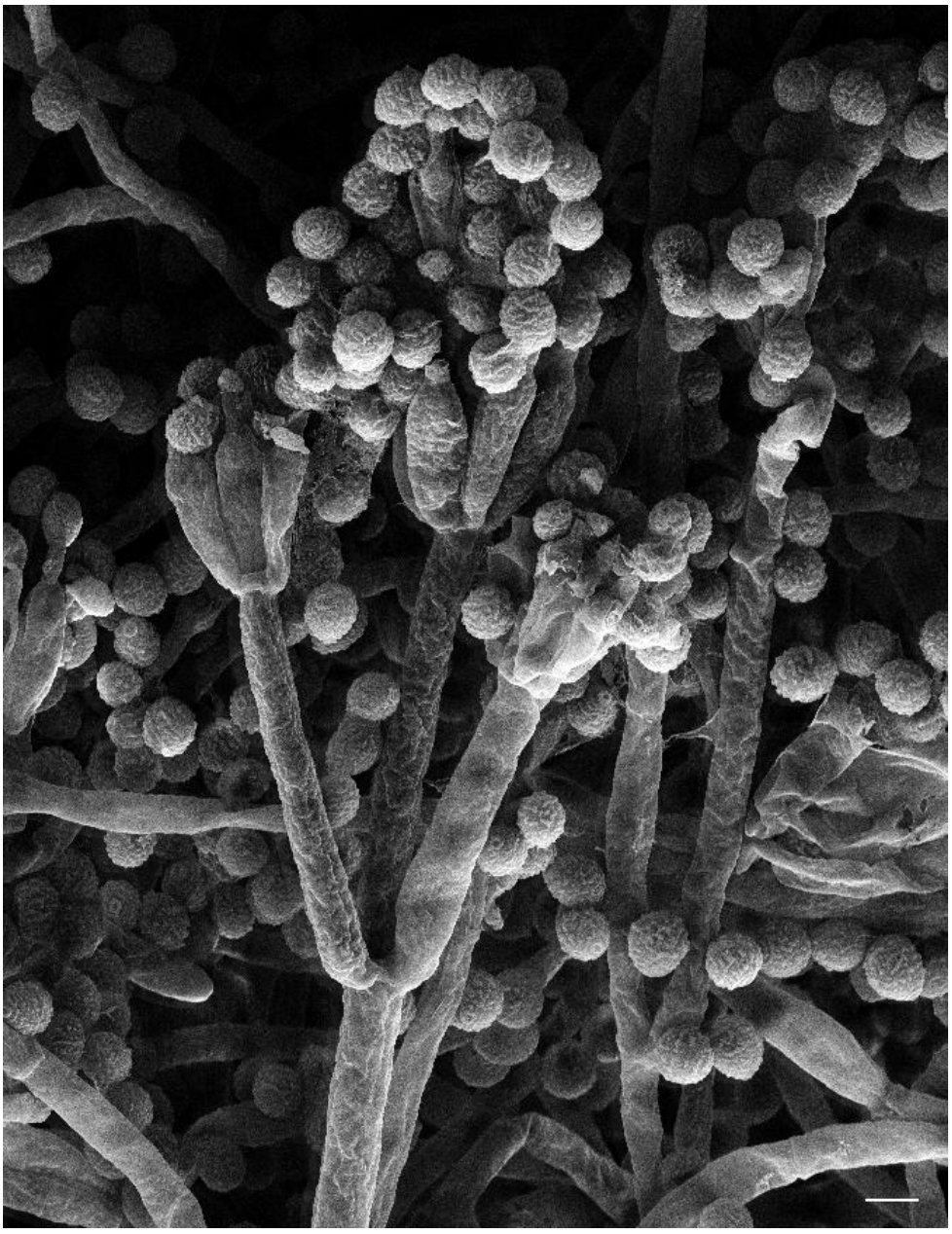


Figure 2A. Scanning electron micrograph of *Penicillium janthinellum* (bar = 2 μm, upper panel).



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M-3 *Scedosporium apiospermum*

Source: Toe / Sinus / Blood

Clinical significance: *Scedosporium apiospermum* is an emerging opportunistic pathogen and it can cause serious infections in patients with immunocompromized status. Besides mycetoma, *S. apiospermum* can cause cutaneous infections, sinusitis, keratitis, lymphadenitis, endophthalmitis, meningoencephalitis, brain abscess, endocarditis, pneumonia, lung abscess, pulmonary fungus ball, allergic bronchopulmonary fungal disease, bursitis, arthritis, osteomyelitis, and urethritis.

Colony: *S. apiospermum* grows rapidly at 25°C. The texture is wooly to cottony. The colony is initially white and later becomes dark gray or smoky brown (Figure 3).

Microscopy: Lactophenol cotton blue mount shows unicellular and oval conidia formed singly on simple conidiophores. Conidia, broadly club-shaped or clavate, and are typically truncate at the base (Figure 3).

Differentiation: Colonies of *S. apiospermum* are lighter compared to those of *Scedosporium prolificans*. The inflated conidiogenous cells (annelides) and slightly wider conidia of *S. prolificans*, and the inability of *S. prolificans* to assimilate ribitol, xylitol, and L-arabinitol helps in differentiation of the two species. Additionally, only *S. apiospermum* can convert to its sexual or perfect form termed *Pseudallescheria boydii*. *S. apiospermum* differs from *Blastomyces dermatitidis* and *Sporothrix schenckii* by not converting to a yeast phase at 37°C. It differs from *Petriella* by forming non-ostiolate cleistothecia when it produces the sexual reproductive structures.

Molecular test: Direct sequencing of an amplified portion of the genome encompassing the internal transcribed spacer 1 and 2 regions and sequence analysis was reported to be used for identification of *S. apiospermum*.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Scedosporium apiospermum* isolate 110-GT (Genebank accession number: KJ914785).

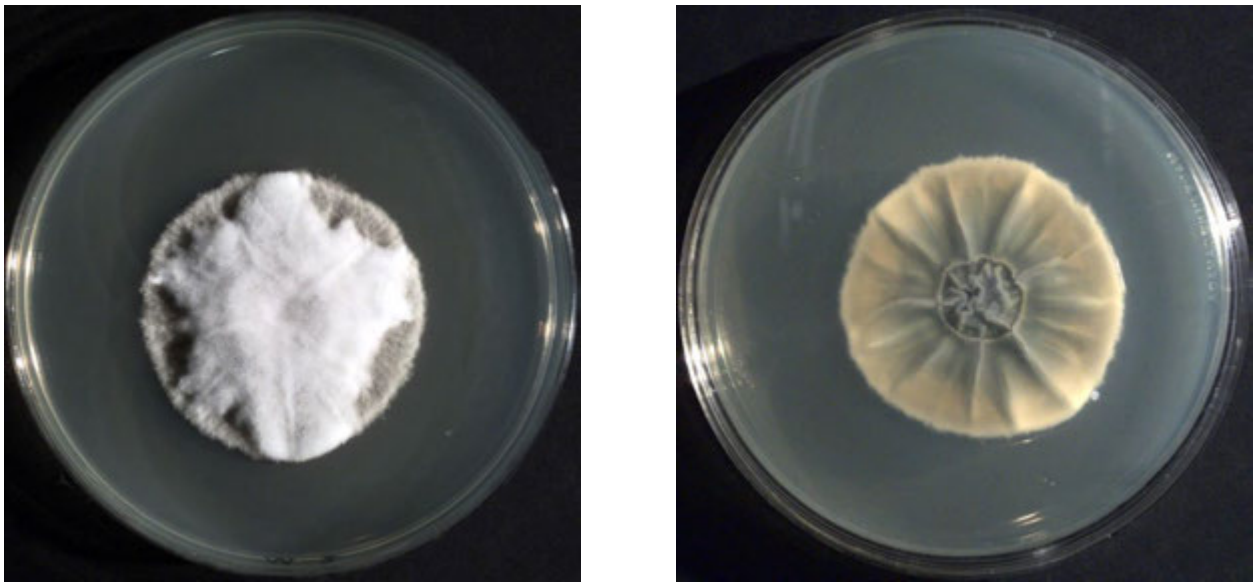
Antifungal susceptibility: *S. apiospermum* is susceptible to miconazole, itraconazole, ketoconazole voriconazole caspofungin, but resistant to amphotericin B. Terbinafine was found to be synergistic with azoles against this fungus.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	58
Laboratories with incorrect ID:	1
(*Scedosporium prolificans*)	(1)

Illustrations:

Figure 3. *Scedosporium apiospermum* colony is white to gray or dirty brown cottony texture on Sabouraud's dextrose agar (upper panel). Microscopic morphology of *S. apiospermum* showing single oval conidia on the simple conidiophores (lower panel).



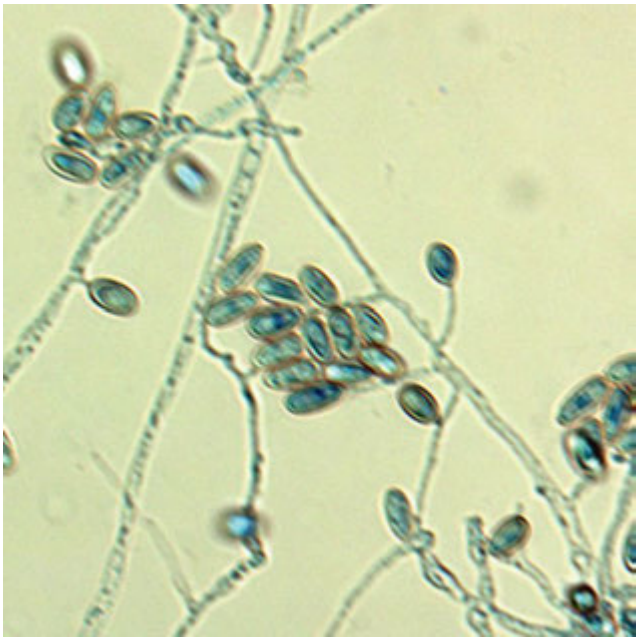
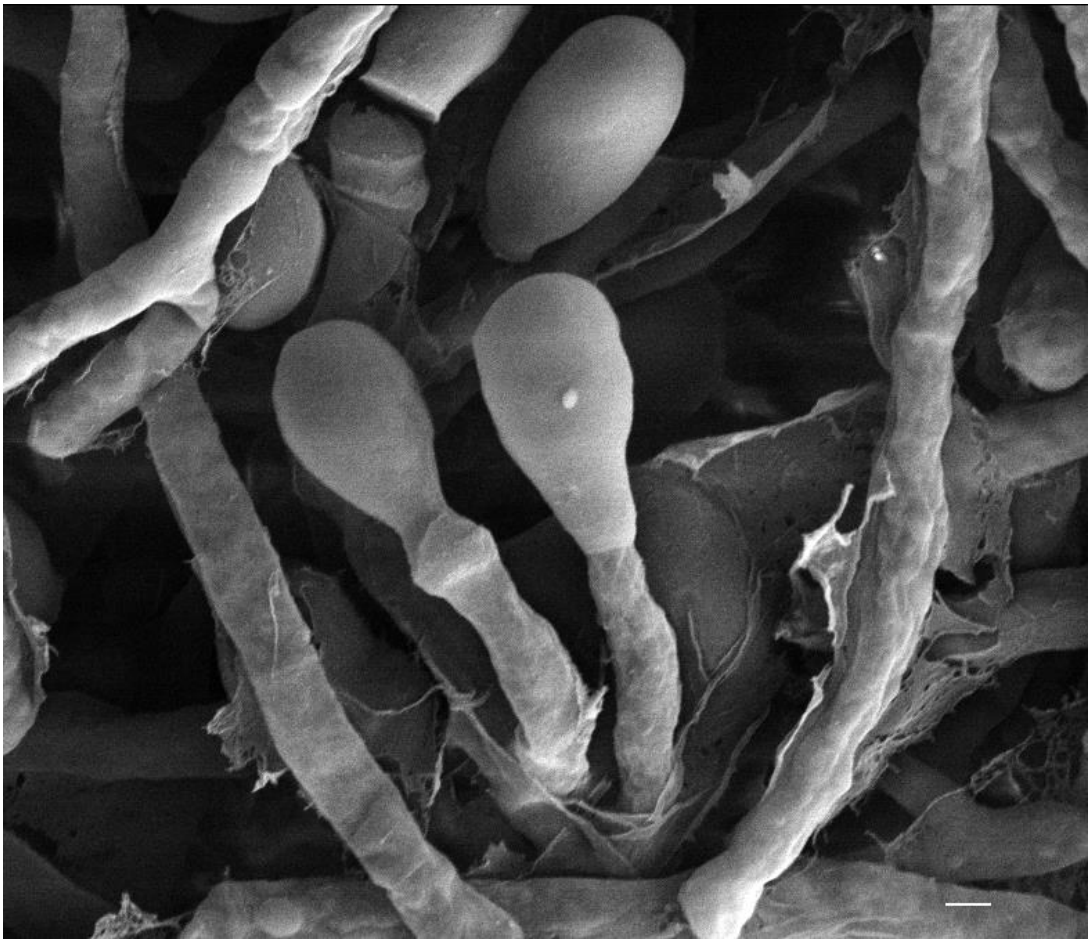


Figure 3A. Scanning electron micrograph of *Scedosporium apiospermum* (bar = 1 μm; upper panel).



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M-4 *Paecilomyces lilacinus*

Source: Bronchial wash / Nail

Clinical significance: *Paecilomyces lilacinus* is a less common pathogen, causing keratitis, endophthalmitis, cutaneous infections, and catheter-related fungemia.

Colony: *P. lilacinus* grows fast on Sabouraud's dextrose agar. Colony is pinkish, violet, cottony texture (Figure 4).

Microscopy: Lactophenol cotton blue shows branched conidiophores with thin and elongated phialides, brush shaped with spindal-shaped conidia in long chains (Figure 4).

Differentiation: *P. lilacinus* can be distinguished from related pathogen *P. variotii* as the latter has yellow-brown colony with sweet odor. *Paecilomyces* spp. may superficially resemble *Penicillium* spp., but the former has simpler conidiophores and no metulae.

Molecular test: PCR probes are available for molecular identification.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 98% nucleotide identity with *Paecilomyces lilacinus* strain 8-16p (Genebank accession number: KC790527).

Antifungal susceptibility: *P. lilacinus* is resistant to amphotericin B, itraconazole, and echinocandins, but susceptible to newer triazoles like posaconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	55
Laboratories with incorrect ID:	2
(*Acremonium* species)	(1)
(*Scopulariopsis* species)	(1)

Illustrations:

Figure 4. White to pinkish, violet colony of *Paecilomyces lilacinus* on Sabouraud's dextrose agar (upper panels). Microscopic morphology of *Paecilomyces lilacinus* showing phialides with thinly tapered tip and spindle-shapped conidia (lower panel).

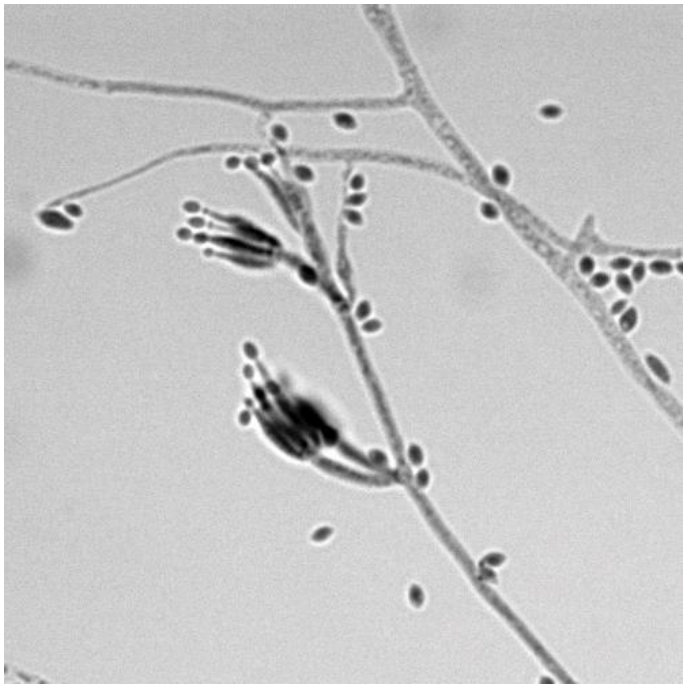
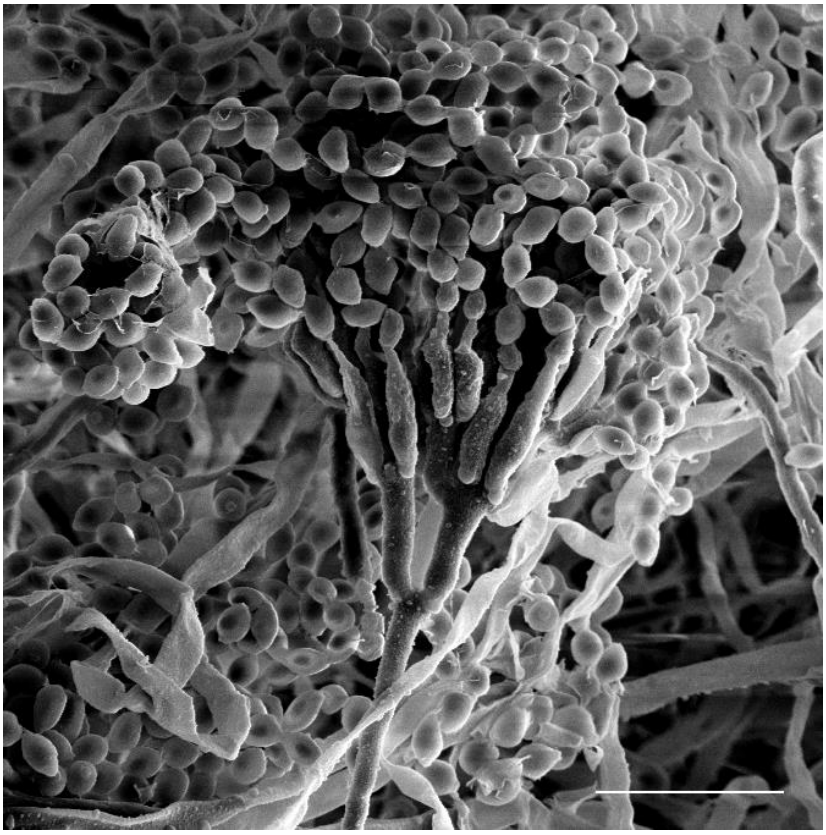
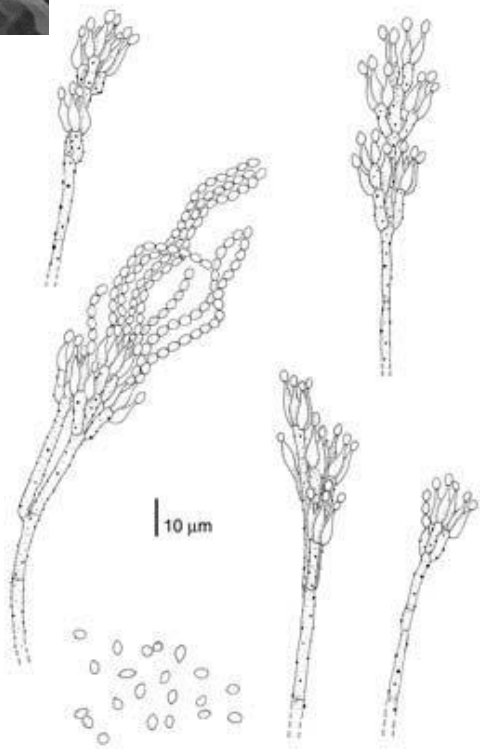


Figure 4A. Light microscopic and scanning electron micrograph of *Paecilomyces lilacinus* (bar = 10 μm; upper panel). Line drawing depicting details of *Paecilomyces lilacinus* (lower panel).





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M-5 *Arthrographis* species

Source: Nail / Sputum / Blood

Clinical significance: *Arthrographis* sp. occasionally causes mycetoma and keratitis. It is also the etiologic agent for sinusitis and meningitis in immunocompromised patients. Sinusitis and ophthalmitis in the healthy individual was also reported.

Colony: *Arthrographis* sp. growth is slow to rapid. Colony is white to pale yellow, powdery or velvety on its surface, on Sabouraud's dextrose agar at 25°C (Figure 5).

Microscopy: Lactophenol cotton blue mount shows septate hyphae and hyaline, simple or branched, short conidiophores. Arthroconidia are formed at the tips of conidiophores or intercalary in the hyphae (Figure 5).

Differentiation: *Arthrographis* sp. produces arthroconidia from conidiophores, but not *Malbranchea* species. Arthroconidia from *Malbranchea* are slightly curved too. *Arthrographis* sp. is distinguished from *Geotrichum* and *Scytalidium* by the presence of definite conidiophores. It is different from *Oidiodendron* by its conidiophores and conidia do not contain gray pigment. *Hormographiella* is characterized by its broad, erect conidiophores with whorls or tufts of arthroconidia at the apex, which cannot be seen in *Arthrographis* sp.

Molecular test:

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Arthrographis kalrae* strain UTHSC 08-1804 (Genebank accession number: HG004564).

Antifungal susceptibility: *Arthrographis kalrae* was reported to be more susceptible to terbinafine, and azoles, especially posaconazole. Amphotericin B had low activity whereas the echinocandins showed no antifungal activity.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	55
Laboratories with incorrect ID:	2
(*Malbranchea* species)	(1)
(*Trichophyton* species)	(1)

Illustrations:

Figure 5. White to pale yellow colony *Arthrographis kalrae* on Sabouraud's dextrose agar (upper panel). Microscopic morphology of *Arthrographis kalrae* showing the arthroconidia at the tips of conidiophores or intercalary in the hyphae (lower panel).

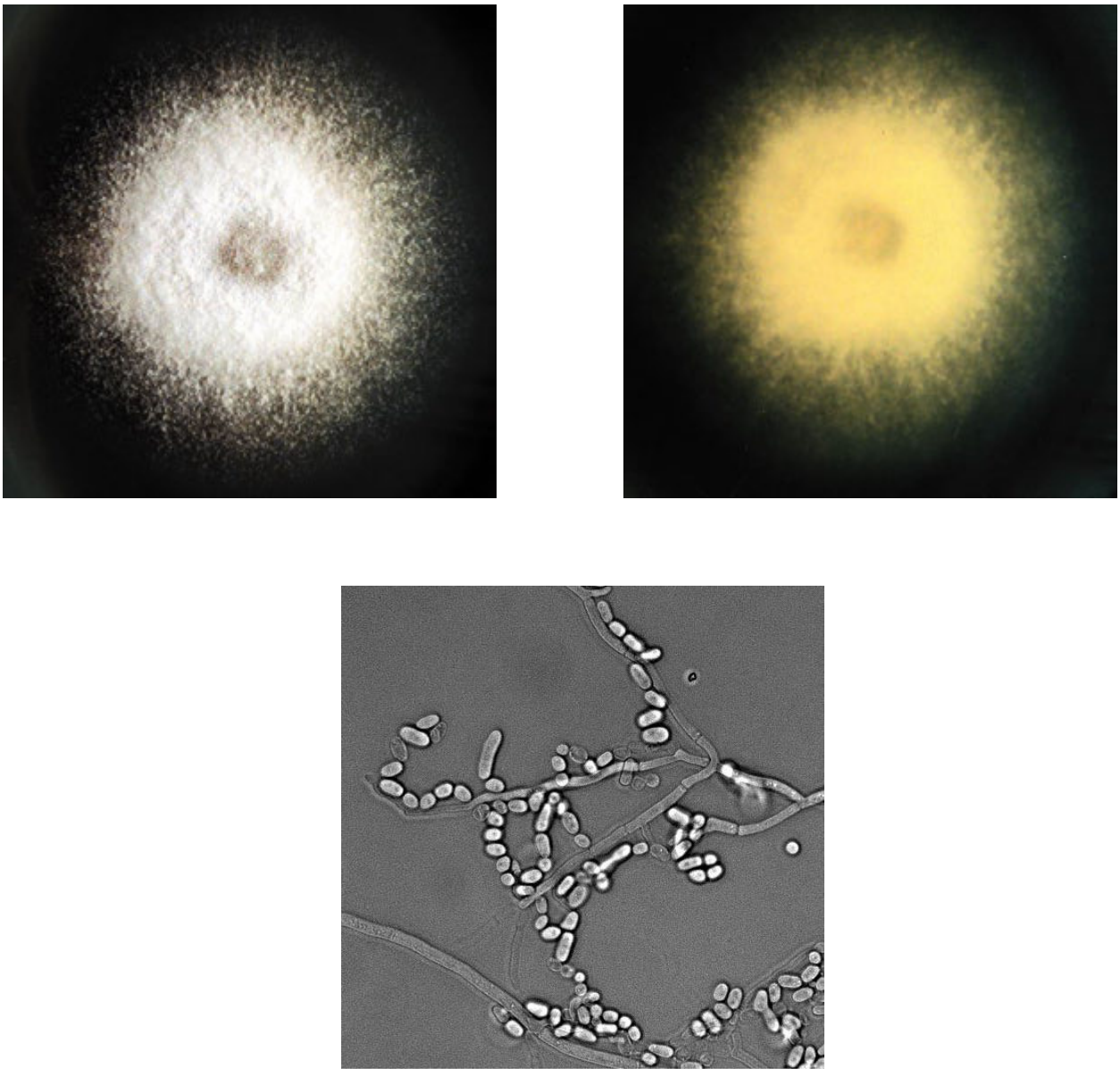
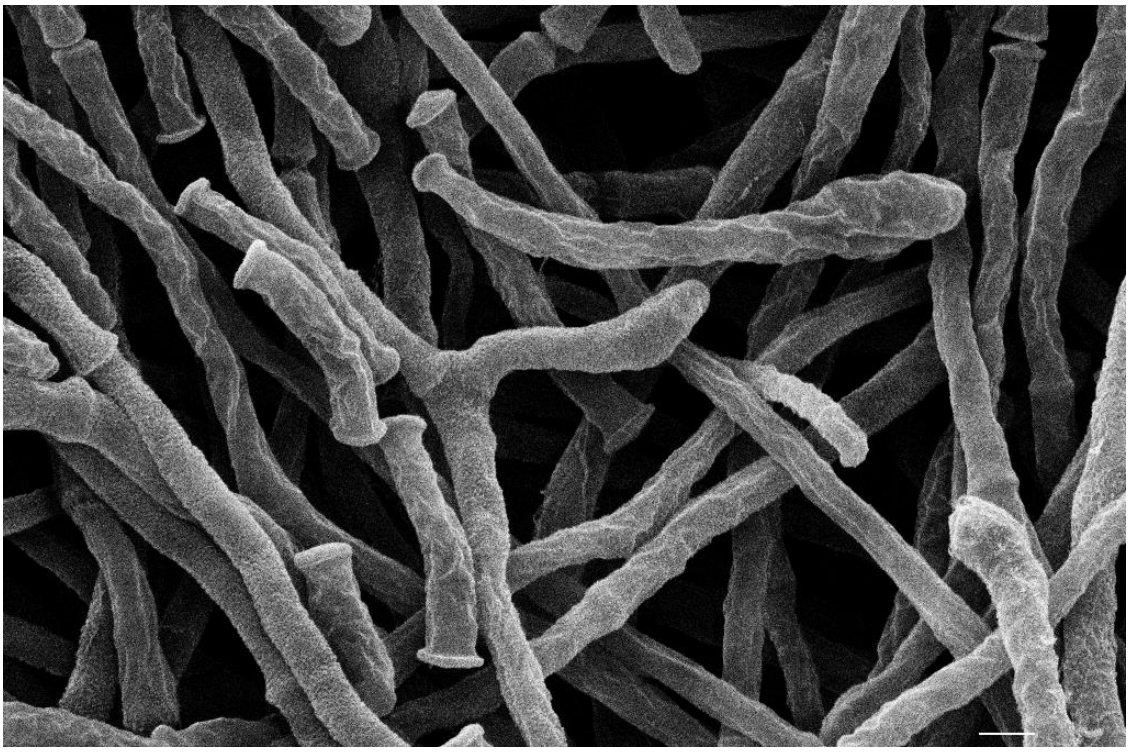
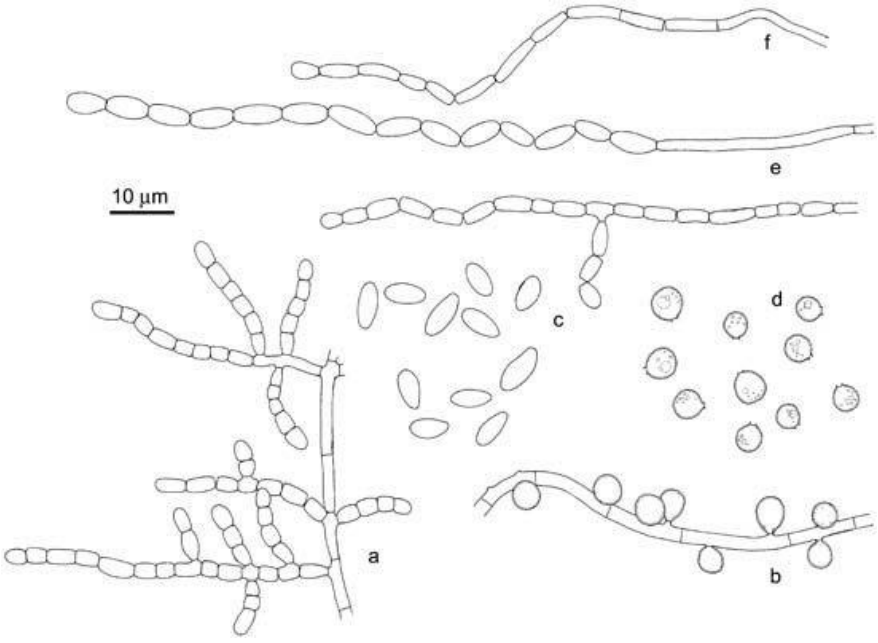


Figure 5A. Scanning electron micrograph of *Arthrographis kalrae* (bar = 1 µm, upper panel). Line drawing depicting details of *Arthrographis kalrae* (lower panel).





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YEAST DESCRIPTIONS

Y-1 *Cryptococcus neoformans*

Source: CSF / Blood / Sputum

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

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

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 6).

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:	52
Laboratories with incorrect ID:	0

Illustrations:

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

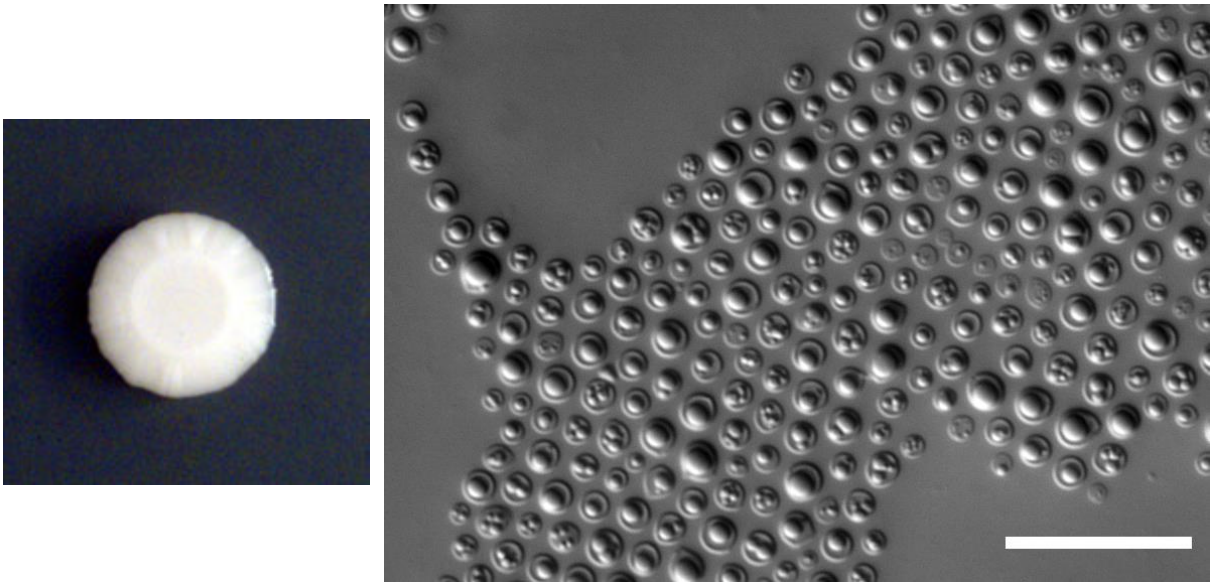
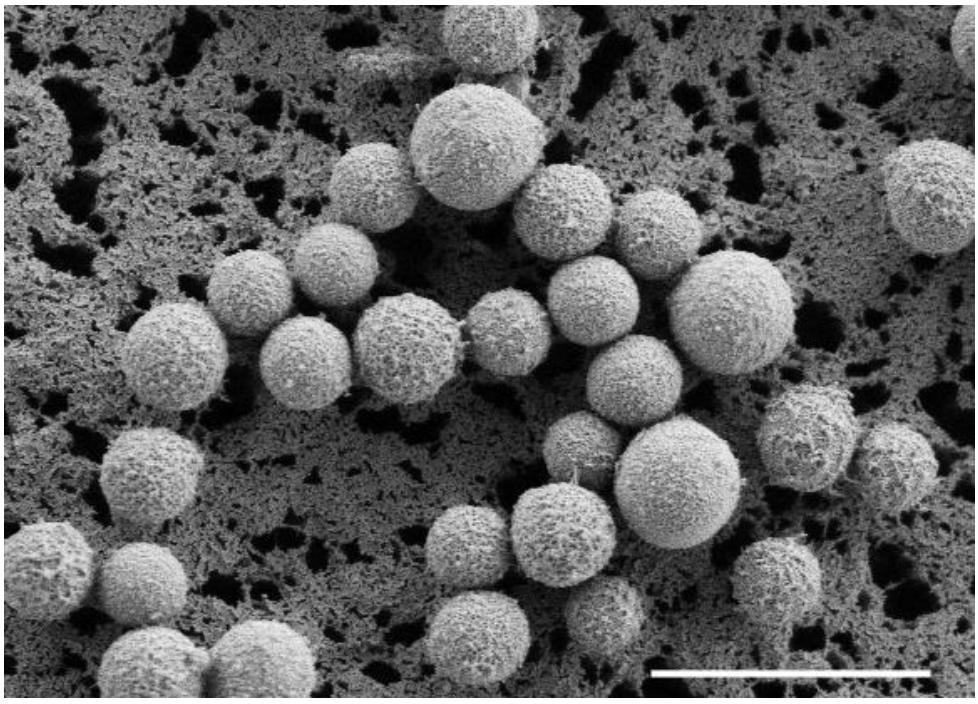


Figure 6A. Scanning electron micrograph with *Cryptococcus neoformans* (bar = 10 μm).



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Y-2 *Candida guilliermondii*

Source: Eye / Chest / Skin

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

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

Microscopy: *C. guilliermondii* shows few short pseudohyphae with clusters of blastoconidia on Corn meal agar with Tween 80 (Figure 7). Please check corn meal how it is written in book and change accordingly?

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

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

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

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

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	50
Laboratories with incorrect ID:	06
(*Candida famata*)	(1)

Illustrations:

Figure 7. *Candida guilliermondii,* flat, smooth, creamish colony on Sabouraud's dextrose agar, 5 days, 25°C. Microscopic morphology on corn meal agar with Tween 80, showing short pseudohyphae with clusters of blastoconidia (bar = 10 μm).

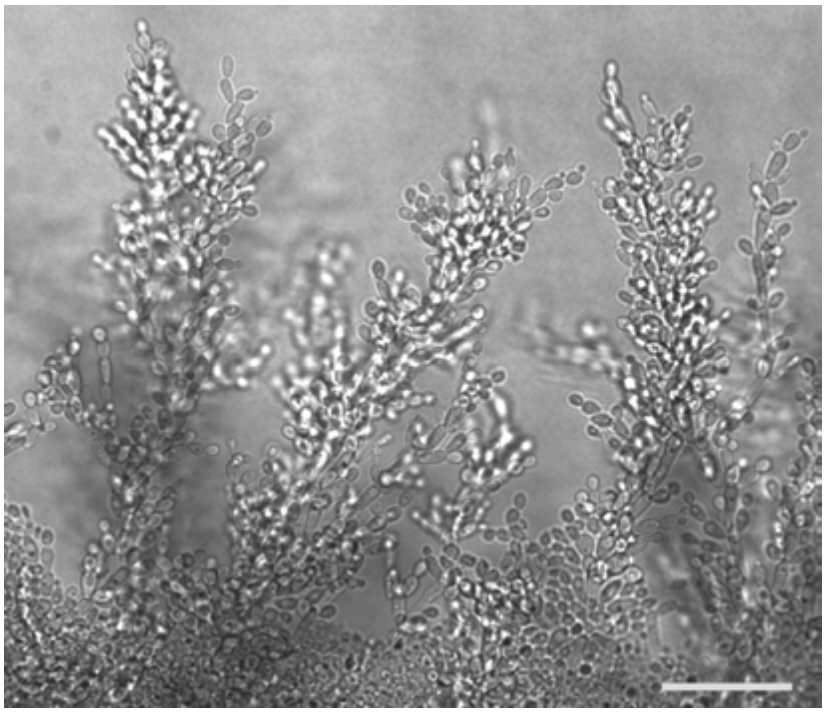
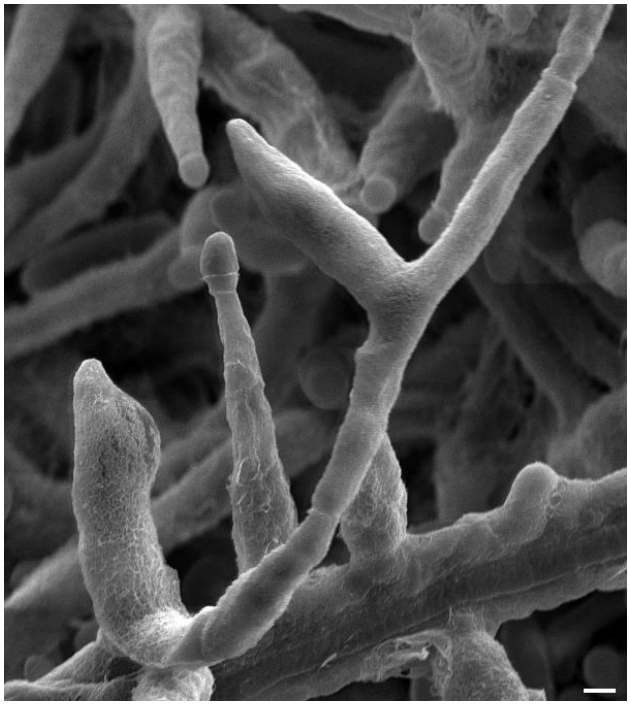


Figure 7A. Scanning electron micrograph of *Candida guilliermondii* (*Pichia guilliermondii*) illustrates pseudohyphae and blastoconidia (bar = 1 μm)



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Y-3 *Blastoschizomyces capitatus*

Source: Stool / Bone lesions / Urine

Clinical significance: *Blastoschizomyces capitatus* is an opportunistic pathogen in neutropenic patients.

Colony: *B. capitatus* colony is smooth to wrinkled, raised, and hyaline on Sabouraud's dextrose agar 7 days at 25°C (Figure 8).

Microscopy: On corn meal agar with Tween 80, it produces true hyphae. Annelloconidia emerged from the annellides. Annellides become longer and narrower with the production of each new conidium (Figure 8). The resulting conidia simulated the appearance of arthroconidia as seen in *Trichsporon* spp and *Geotrichum* spp.

Differentiation: *B. capitatus* can be differentiated from *G. candidum* by the lack of growth on a medium containing D-xylose as a carbon source. It can be differentiated from *T. beigelii* by urease negative and its growth at 45°C. *B. capitatus* is included in the database of commercial yeast identification systems.

Molecular test: Primers for large ribosomal subunit DNA sequences were used in PCR to differentiate between *C. famata* and *C. guilliermondii*. The amplification of 340 bp of the large rDNA led to rapid and specific identification of *C. famata*. RAPD-PCR analysis was applied to identify *C. famata* in dairy product.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Dipodascus capitatus* (*Geotrichum capitatus*) isolate wb410 (GenBank accession no. AF455443.1).

Antifungal susceptibility: *B. capitatus* is susceptible to amphotericin B; fluconazole resistant strains have been reported from cancer patients.

Participant performance:

Referee Laboratories with correct ID:	9
Laboratories with correct ID:	45
Laboratories with incorrect ID:	4
(*Candida krusei*)	(2)
(*Candida lipolytica*)	(1)
(*Geotrichum candidum*)	(1)

Illustrations:

Figure 8. White, smooth to slightly wrinkled, raised colony of *Blastoschizomyces captitatus* on Sabouraud's dextrose agar 7-day, 25°C. Microscopic morphology showing annelloconidia formed from true hyphae on Corn meal agar with Tween 80 (bar = 10 μm).

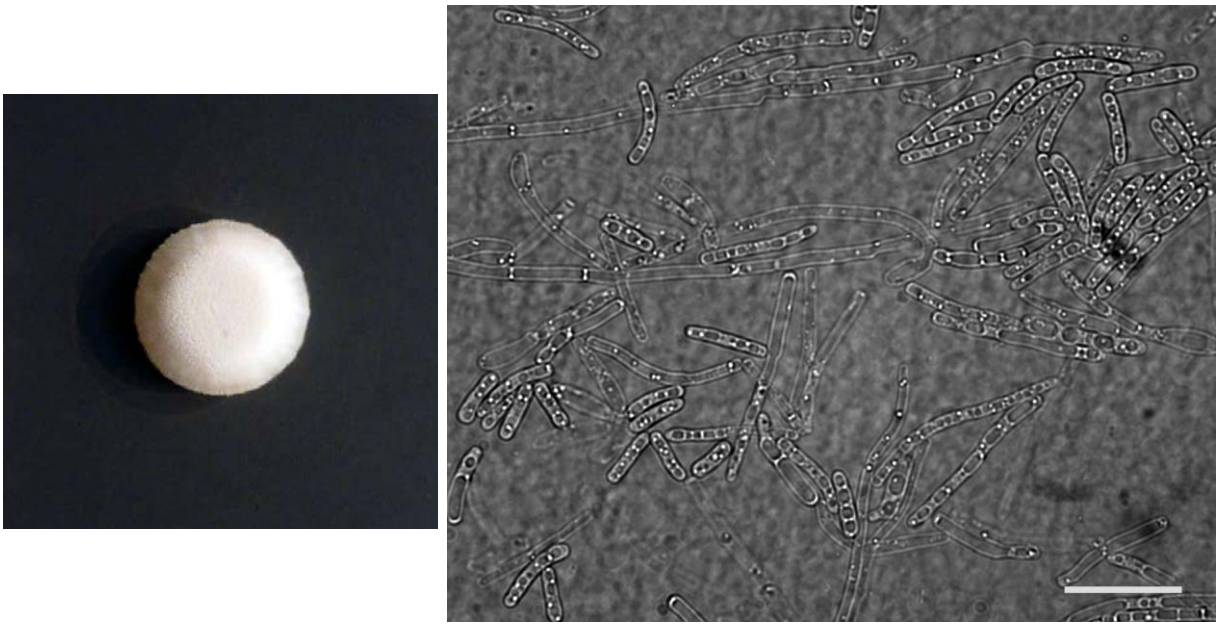
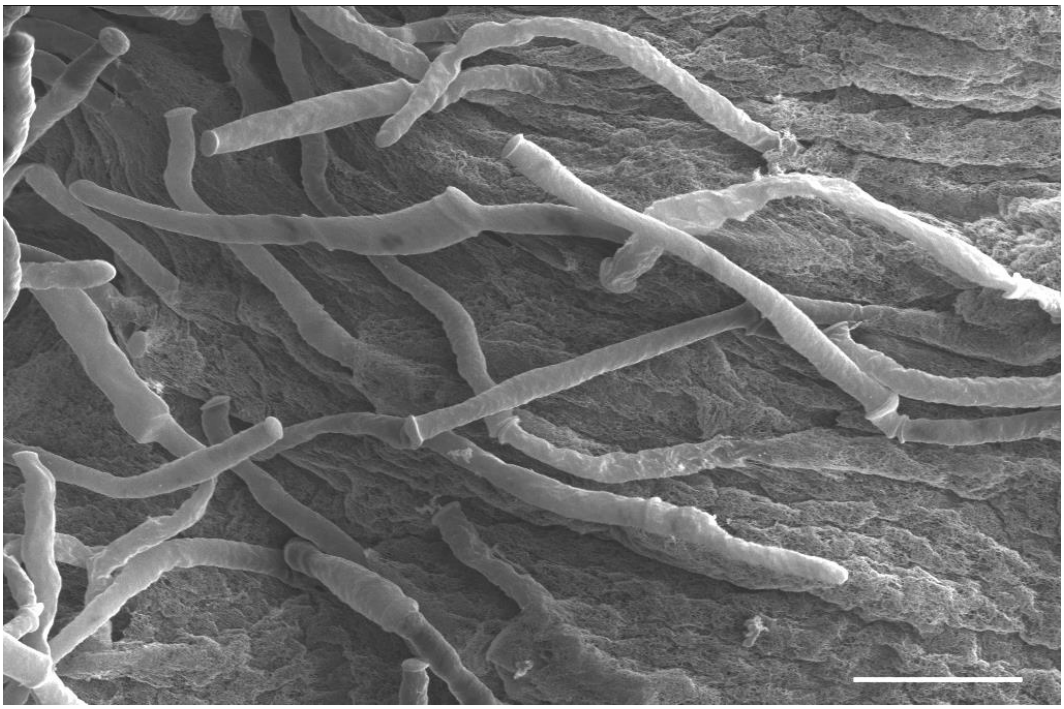


Figure 8A. Scanning electron micrograph illustrating true hyphae (bar = 10 μm).



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Y-4 *Hansenula anomala* (*Candida pelliculosa*)

Source: Blood / Lung / Nail

Clinical significance: *Candida pelliculosa* is an infrequently encountered pathogen causing nosocomial infections. Several cases of fungemia in neonates, and endocarditis in immunosuppressed patients, are reported in the literature.

Colony: *Candida pelliculosa* colony is smooth, creamy, and soft on Sabouraud's dextrose agar 5 days at 25°C (Figure 9).

Microscopy: *C. pelliculosa* showed blastoconidia and limited pseudohyphae on Corn meal agar with Tween 80 (Figure 9)

Differentiation: *Candida pelliculosa* is the anamorph (asexual form) of *Pichia anomala*. It does not grow on media containing cycloheximide, or at 42°C. It assimilates nitrate but is urease-negative.

Molecular test: PCR amplification of a specific fragment of 18S rDNA and heteroduplex mobility assays were performed to detect and distinguish *C. pelliculosa* from other clinically important yeasts. Phylogenetic analysis of domain sequences found four new species in the *C. pelliculosa* clade.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Candida pelliculosa* (*Pichia anomala*) isolate M10 (GenBank accession no. FJ865436.1).

Antifungal susceptibility: *C. pelliculosa* is susceptible to amphotericin B, 5-flucytosine, and azoles such as fluconazole, clotrimazole, and itraconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	55
Laboratories with incorrect ID:	02
(*Pichia ohmeri*)	(1)
(*Saccharomyces* sp.)	(1)

Illustrations:

Figure 9. *Candida pelliculosa*, smooth, creamy, soft colony on Sabouraud's dextrose agar, 4 days, 25°C. Microscopic morphology showing pseudohyphae on Corn meal agar with Tween 80 (BAR = 10 μm).

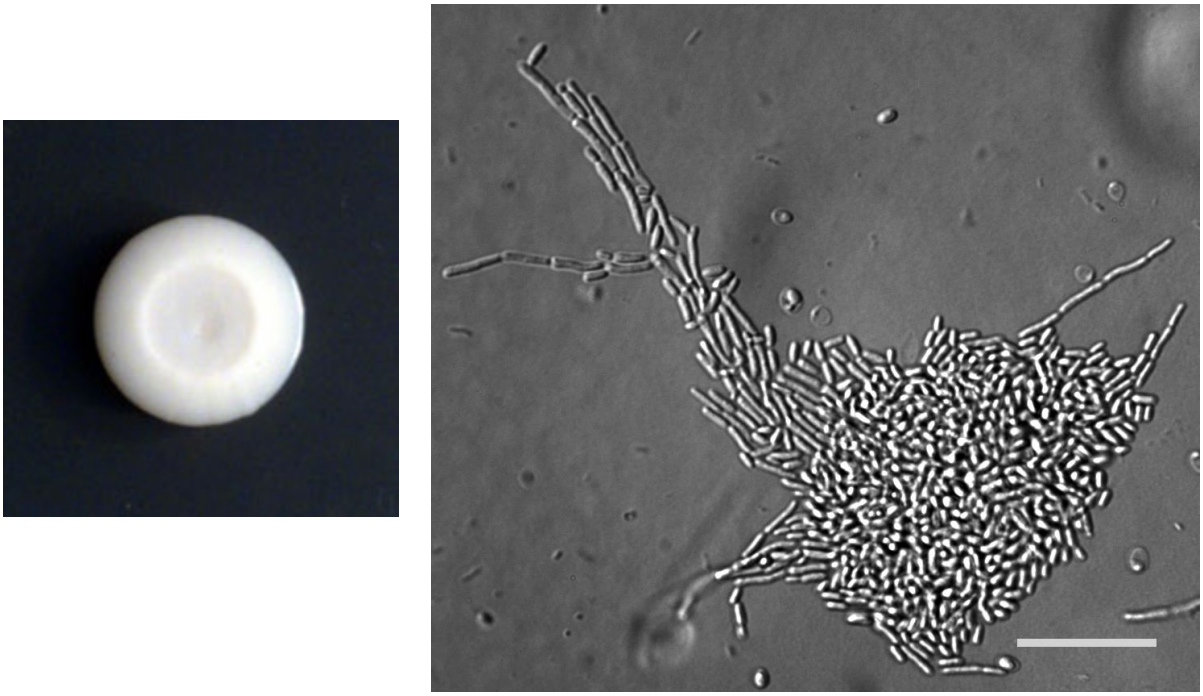
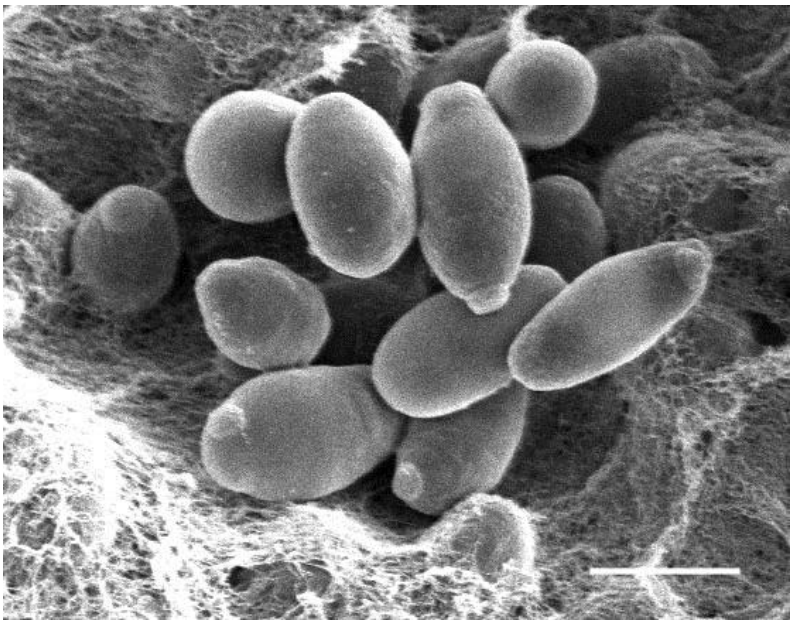


Figure 9A. Scanning electron micrograph illustrating pseudohyphae and blastoconidia (bar = 2 μm).



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Y-5 *Candida famata*

Source: Skin / Catheter / Blood

Clinical significance: *Candida famata* is an infrequent causal agent of nosocomial fungemia in immunosuppressed patients. Also, it is a rare causative agent of ocular infections, arthritis, and peritonitis.

Colony: *C. famata* colony is white to yellowish, soft, smooth to slightly wrinkled On Sabouraud's dextrose agar at 25°C (Figure 10).

Microscopy: On corn meal agar with Tween 80, *C. famata* shows round to oval blastoconidia with no or rudimentary pseudohyphae, but with longer incubation (more than a week) primitive or well-developed pseudohyphae are seen (Figure 10).

Differentiation: *C. famata* ferments glucose, sucrose, and trehalose, grows at 37°C. It forms primitive to well-developed pseudohyphae on corn meal agar or Dalmau plate when incubated longer, which differentiates it from *C. guilliermondii*. It does not produce true hyphae, which differentiates it from *C. ciferrii*. It does not grow at 45°C, differentiating it from *C. lusitaniae*. It assimilates sucrose and maltose, differentiating it from *C. zeylanoides*.

Molecular test: Primers for large ribosomal subunit DNA sequences were used in PCR to differentiate *C. famata* from *C. guilliermondii*. The amplification of 340 bp of the large rDNA led to rapid and specific identification of *C. famata*. RAPD-PCR analysis was applied to identify *C. famata* in dairy product.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Candida famata* strain SX1 (GenBank accession no. JN839959).

Antifungal susceptibility: Almost all clinical isolates are susceptible to amphotericin B, 5FC, and azoles such as fluconazole, itraconazole, ketoconazole, and voriconazole.

Participant performance:

Referee Laboratories with correct ID:	5
Laboratories with correct ID:	29
Laboratories with incorrect ID:	20
(*Candida zeylanoides*)	(16)
(*Candida glabrata*)	(1)
(*Candida guilliermondii*)	(1)
(*Cryptococcus laurentii*)	(1)
(*Prototheca wickerhamii*)	(1)

Illustrations:

Figure 10. *Candida famata*, white to yellowish, soft, smooth to slightly wrinkled colony on Sabouraud's dextrose agar, 25°C. *Candida famata* on corn meal agar with Tween 80 showing pseudohyphae with oval blastoconidia.

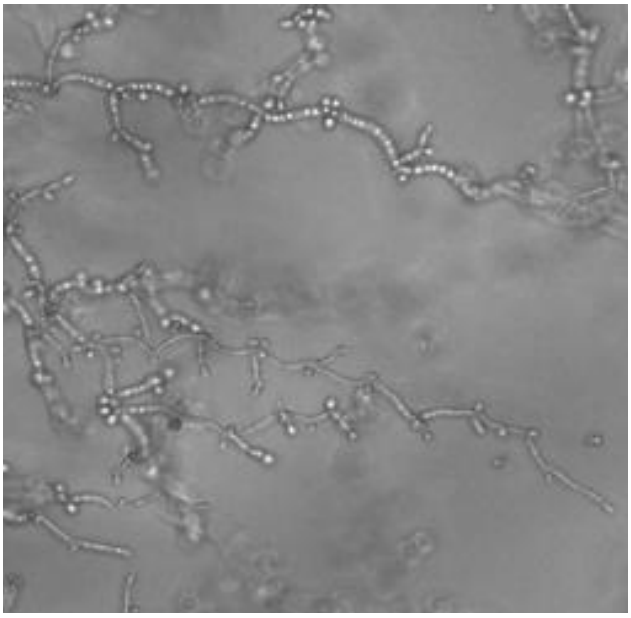
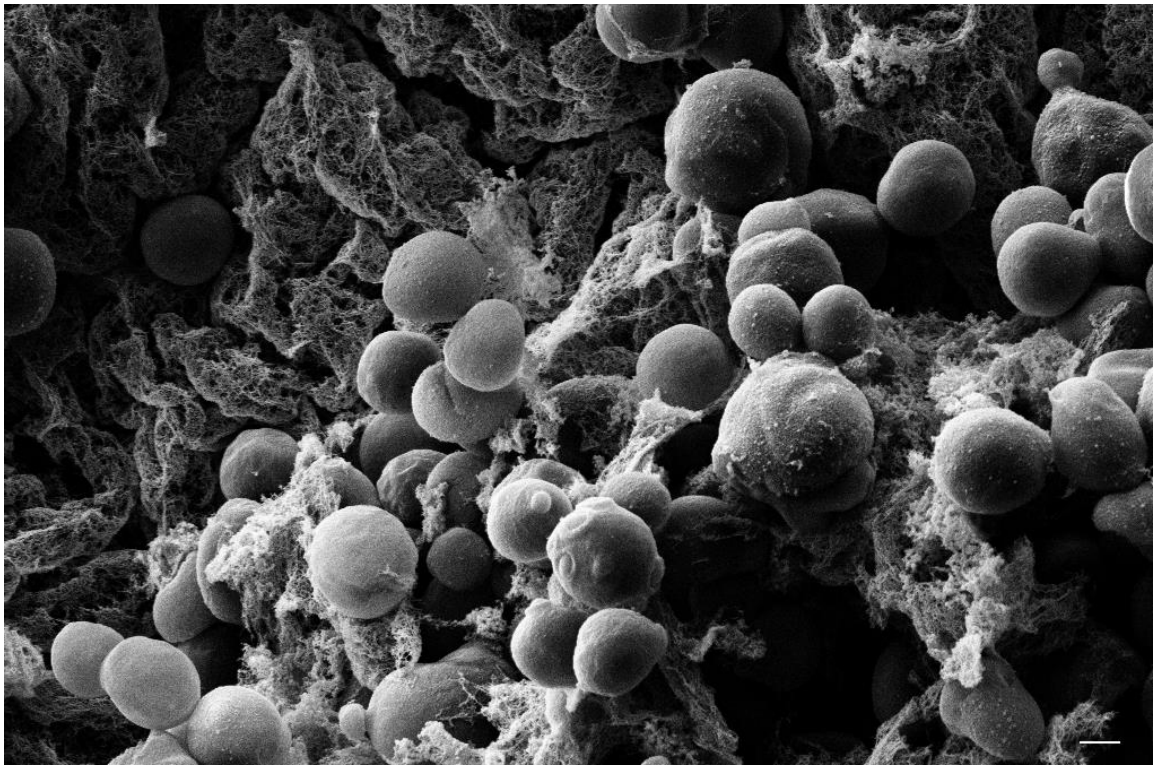


Figure 10A. Scanning electron micrograph illustrating blastoconidia (bar = 1 μm).



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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: Sixty-seven laboratories participated in the October 1, 2014 direct antigen detection test event. Three negative (Cn-Ag-1, Cn-Ag-4, and Cn-Ag-5) and two positive serum samples (Cn-Ag-2 and Cn-Ag-3) with the titer of 1:32 and 1:128, respectively for cryptococcal antigen were included.

Results: The consensus results for specimens Cn-Ag-1, Cn-Ag-4, and Cn-Ag-5 were negative, Cn-Ag-2 and Cn-Ag-3 were positive. The summary of laboratory performance for semi-quantitative detection of cryptococcal antigen is shown in Table 2. The acceptable titer ranges were 1:8 ~ 1:256 and 1:16 ~ 1:512 for Cn-Ag-2 and Cn-Ag-3 respectively. All the laboratories reported the titers within the range for both positive samples.

Table 2. Summary of laboratory performance for semi-quantitative detection of cryptococcal antigen.

Method		Cn-Ag-2 Titers										
No. laboratories		8	10	16	20	32	40	64	80	128	160	256
EIA	1						1					
Latex Agglutination	50	2		7	1	18		16	1	4		1
Immuno-Mycologics	5			2		1				2		
Meridien Diagnostic	37	2		5	1	14		13		1		1
Remel	8					3		3	1	1		
Lateral Flow Assay	9		2	1			3		2		1	
Total	60	2	2	8	1	18	4	16	3	4	1	1

Method		Cn-Ag-3 Titers									
No. laboratories		16	20	32	40	64	80	128	160	256	320
EIA	1					1					
Latex Agglutination	50	1		4	1	14		20	1	9	
Immuno-Mycologics	5			1		1		1		2	
Meridien Diagnostic	37	1		3	1	11		17		4	
Remel	8					2		2	1	3	
Lateral Flow Assay	9		2		1	1	1		2		2
Total	60	1	2	4	2	16	1	20	3	9	2

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ANTIFUNGAL SUSCEPTIBILITY TESTING FOR YEASTS

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

Materials: *Candida glabrata* (S-1) was the analyte in the October 1, 2014 antifungal proficiency testing event. The interpretation of MIC values for antifungal susceptibility testing of yeasts and molds is in a state of constant change. These changes are necessitated by new information emerging from clinical trials and laboratory susceptibility testing. NYSDOH Mycology Laboratory uses the consensus/all participating laboratories' MIC values within +/- 2 dilutions and then the interpretation per latest CLSI and EUCAST documents to score proficiency testing results. Especially, when there is no interpretation, MIC values are the key judge points. However, the participating laboratories are advised to regularly consult these organizations for the latest version of their standard documents.

Comments: Acceptable results were MICs +/-2 dilutions of the reference laboratory results for any single drug. Only 2 of the 31 laboratories participating in this test event tested all 10 antifungal drugs. The reported results were as follows: fluconazole (31 laboratories), itraconazole (25 laboratories), voriconasole (23 laboratories), caspofungin (22 laboratories), flucytosine (21 laboratories), amphotericin B (20 laboratories), anidulafungin (17 laboratories), micafungin (17 laboratories), posacoanazole (16 laboratories), and ketocoanzole (4 laboratories). CLSI document M27-S4 specifically stated that the current data are insufficient to demonstrate a correlation between *in vitro* susceptibility testing and clinical outcome for *C. glabrata* and voriconazole. So we strongly suggest laboratories follow the M27-S4 guideline.

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

S-1: *Candida glabrata* (M956)

Drug	No. labs	MIC (µg/ml)															
		0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
Amphotericin B	20					1	4	14	1								
Anidulafungin	17		13	3	1												
Caspofungin	22			7	10	1	4										
Flucytosine (5-FC)	23			3	17	1											
Fluconazole	31*											1	1	5	6	9	8
Itraconazole	25*						1	2	4	5			12				
Ketoconazole	4*					1				2							
Micafungin	17	2	15														
Posaconazole	16							1	1	4	1	9					
Voriconazole	23						2	1	5	5	8	2					

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

Colors represent the testing method used:

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

Table 4. Antifungal Susceptibility Interpretations Reported by the Participating Laboratories

S-1: *Candida glabrata* (M956)

Drug	No. laboratories	Susceptible	Susceptible-dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	20	3					17
Anidulafungin	17	17					
Caspofungin	22	21		1			
Flucytosine	23	14					7
Fluconazole	31		6	2	23		
Itraconazole	25		1		16		8
Ketoconazole	4		1				3
Micafungin	17	17					
Posaconazole	16		1		5		10
Voriconazole	23	2	2	2	5		12

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR MOLDS (EDUCATIONAL)

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

Materials: *Aspergillus fumigatus* M2036 was used as a test analyte; it was obtained from a reference laboratory. Participating laboratories volunteered to perform the test and they were free to choose any number of drugs and a test method. Two laboratories used CLSI broth microdilution method while the remaining two used TREK YeastOne Colorimetric method.

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

Table 5. MIC (µg/ml) Values of Mold Antifungal Susceptibility: *Aspergillus fumigatus* M2036

Drugs (µg/ml)	Total # of labs	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1.0	2.0	4.0	64	128	256
Amphotericin B	4							1	2	1				
Anidulafungin	4		2	1	1									
Caspofungin	4	1	1		1	1								
Fluconazole	3											1	1	1
Itraconazole	4			1			2	1						
Ketoconazole	1										1			
Micafungin	4	2	1		1									
Posaconazole	4		1			2		1						
Voriconazole	4				1		1	2						

CLSI microbroth dilution method
YeastOne Colorimetric method
Both CLSI microdilution and YeastOne Colorimetric methods

Further Reading:

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