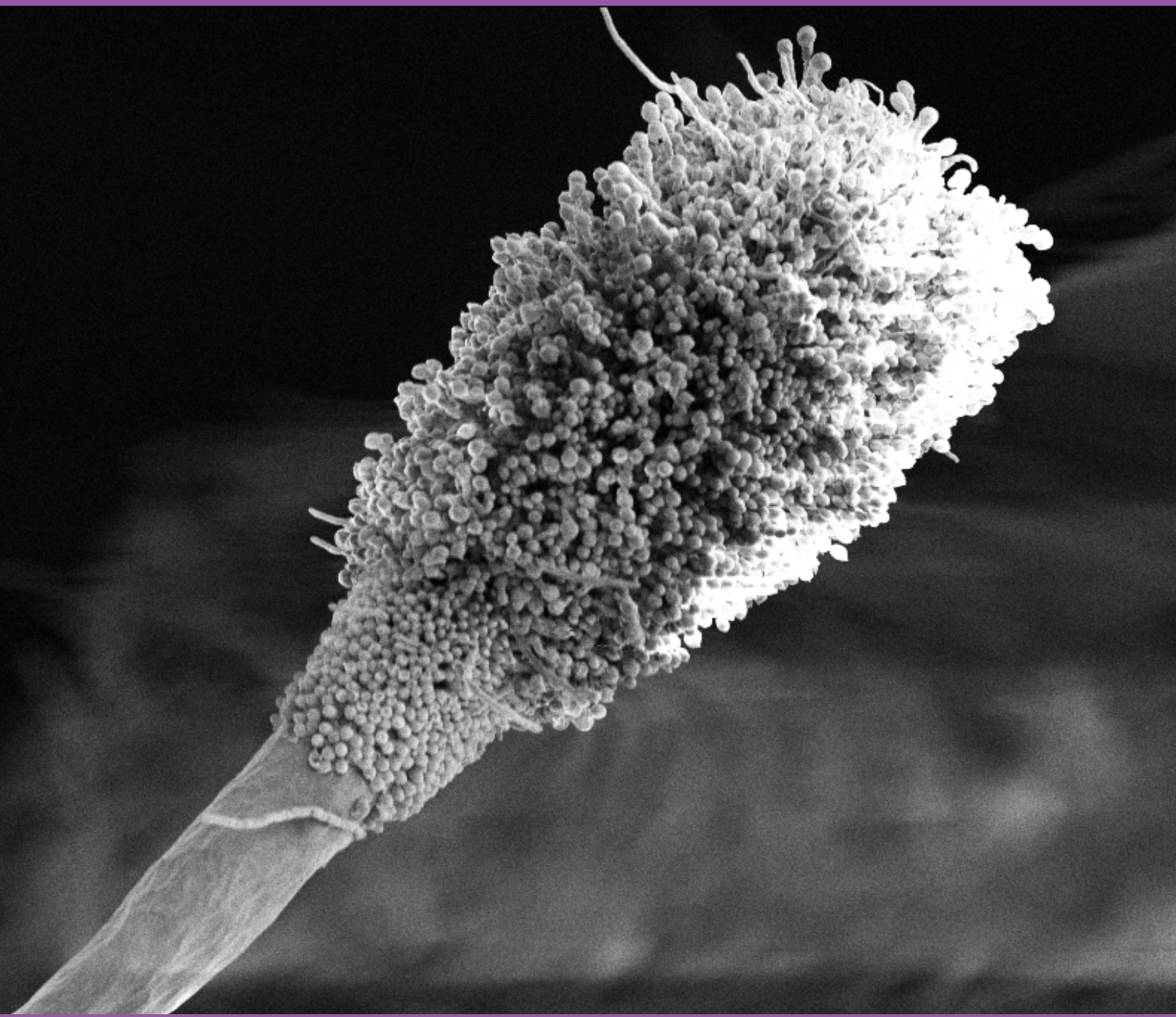
Mycology Proficiency Testing Program



Test Event Critique
January 2014

Wadsworth Center
NEW YORK STATE DEPARTMENT OF HEALTH

Table of Contents

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 on the next page.

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

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

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

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

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

TEST ANALYTE MASTER LISTS

Mold Master List

The mold master list is intended to provide guidance to the participating laboratories about the scope of the Mycology (Comprehensive) Proficiency Testing Program. The list includes most common pathogenic and non-pathogenic fungi likely to be encountered in the 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	*Stachybotrys chartarum*	*Stachybotrys chartarum*	*Stachybotrys atra* *Stachybotrys* species	57/59 (97%)
M-2	*Aspergillus clavatus*	*Aspergillus clavatus*		57/58 (98%)
M-3	*Microsporum gypseum*	*Microsporum gypseum*	*Microsporum gypseum* species complex *Microsporum* species*	58/59 (99%)
M-4	*Scopulariopsis* species	*Scopulariopsis* species	*Scopulariopsis brevicaulis*	53/59 (90%)
M-5	*Sporothrix schenckii* species complex	*Sporothrix schenckii* species complex	*Sporothrix schenckii* *Sporothrix* species	49/58 (84%)

*Only if the laboratory does not speciate *Microsporum* 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 uniguttulatus*	*Cryptococcus uniguttulatus*		52/53 (98%)
Y-2	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*		52/53 (98%)
Y-3	*Candida dubliniensis*	*Candida dubliniensis*		44/53 (83%)
Y-4	*Candida lipolytica*	*Candida lipolytica*		50/53 (94%)
Y-5	*Cryptococcus laurentii*	*Cryptococcus laurentii*		52/53 (98%)

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	Positive (1:32)	Positive (1:32)	1:8 – 1:128	67/67 (100%)	60/62 (97%)
Cn-Ag-2	Negative	Negative		67/67 (100%)	NA
Cn-Ag-3	Negative	Negative		67/67 (100%)	NA
Cn-Ag-4	Negative	Negative		67/67 (100%)	NA
Cn-Ag-5	Positive (1:8)	Positive (1:8)	1:2 – 1:64	67/67 (100%)	62/62 (100%)

Antifungal Susceptibility Testing for Yeast (S-1: *Candida krusei* M2559)

Drugs	Acceptable MIC (μg/ml) range	Acceptable interpretation	Laboratories with acceptable responses/ Total laboratories (% correct responses)
Amphotericin B	0.5 – 2	Susceptible / No interpretation	21/21 (100%)
Anidulafungin	0.015 – 0.25	Susceptible	16/16 (100%)
Caspofungin	0.125 – 0.25	Susceptible	20/20 (100%)
Flucytosine (5-FC)	4 – 8	Susceptible / No interpretation	23/23 (100%)
Fluconazole	16 – 256	Resistant	26/27 (96%)
Itraconazole	0.125 – 0.5	Susceptible / No interpretation	27/27 (100%)
Ketoconazole	0.25 – 0.5	Susceptible / No interpretation	5/5 (100%)
Micafungin	0.06–0.25	Susceptible	16/16 (100%)
Posaconazole	0.125 – 0.25	Susceptible / No interpretation	15/15 (100%)
Voriconazole	0.06 – 0.25	Susceptible	26/26 (100%)

Commercial Device Usage Statistics:

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

Device	**No. laboratories**
Yeast Identification*	
AMS Vitek	1
API 20C AUX	25
Bruker MicroFlex LT Biotyper	1
Dade Behring MicroScan Rapid Yeast Identification Panel	3
Remel RapID Yeast Plus System	4
Vitek2	27
Antifungal Susceptibility*	
Disk diffusion	1
Etest	1
Vitek II	1
YeastOne– Mold	2
YeastOne –Yeast	25
CLSI Microbroth dilution method – Yeast	4
CLSI Microbroth dilution method – Mold	3
Cryptococcal antigen*	
Immuno-Mycologics Latex Cryptococcus Antigen Detection System	8
Immuno-Mycologics CrAg Lateral Flow Assay	6
Meridien BioScience Cryptococcal Antigen Latex Agglutination System (CALAS)	41
Immuno-Mycologics ALPHA Cryptococcal Antigen enzyme immunoassay(CrAg EIA)	2
Remel Cryptococcal Antigen Latex Test	10

*Include multiple systems used by some laboratories

MOLD DESCRIPTIONS

M-1 *Stachybotrys chartarum*

Source: Pleural fluid / Arm / Nail

Clinical Significance: *Stachybotrys chartarum* is an environmental contaminant. It produces trichothecene mycotoxins, which are potent inhibitor of DNA, RNA, and protein synthesis. Toxin upon ingestion or inhalation can cause mucous membrane inflammation, bleeding disorders, diarrhea, and upper and lower respiratory tract disorders.

Colony: *Stachybotrys* grows rapidly, white initially and turns to black by ageing on Sabouraud's dextrose agar at 25°C (Figure 1).

Microscopy: Lactophenol cotton blue mount shows septate hyphae, branched conidiophores, cylindrical phialides bearing the pigmented conidia clustered at the tip (Figure 1).

Differentiation:

Molecular test: PCR based amplification of ITS1 and ITS 2 region sequences are available in GenBank.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Stachybotrys chartarum* strain CECT 20565 (GenBank accession no. AM180510.1).

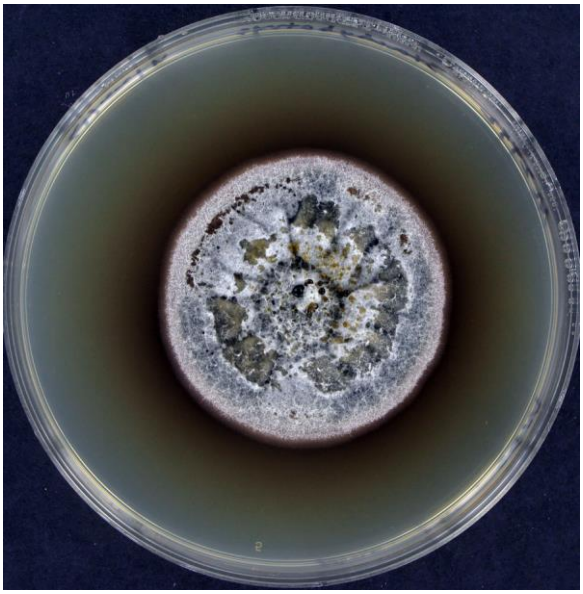
Antifungal susceptibility: No information available.

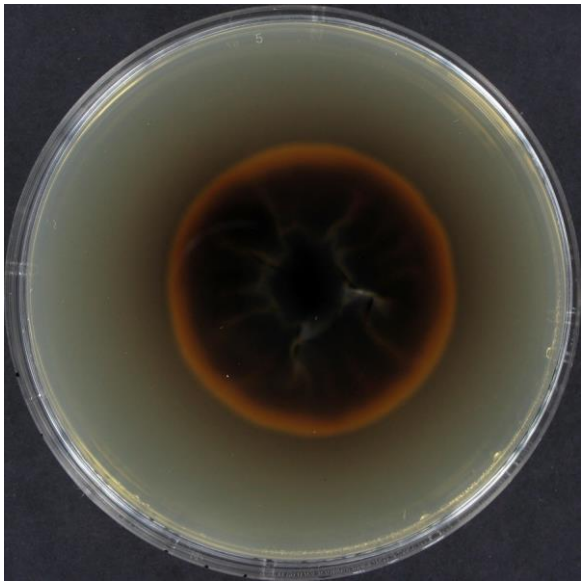
Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	57
Laboratories with incorrect ID:	2
(*Gliocladium* species)	(1)
(*Sporothrix schenckii* species complex)	(1)

Illustrations:

Figure 1. Colony of *Stachybotrys chartarum* on Sabouraud's dextrose agar (upper panel). Microscopic morphology of *Stachybotrys chartarum* showing conidiophores, phialides and clustered conidia (bar = 10 µm, lower panel).





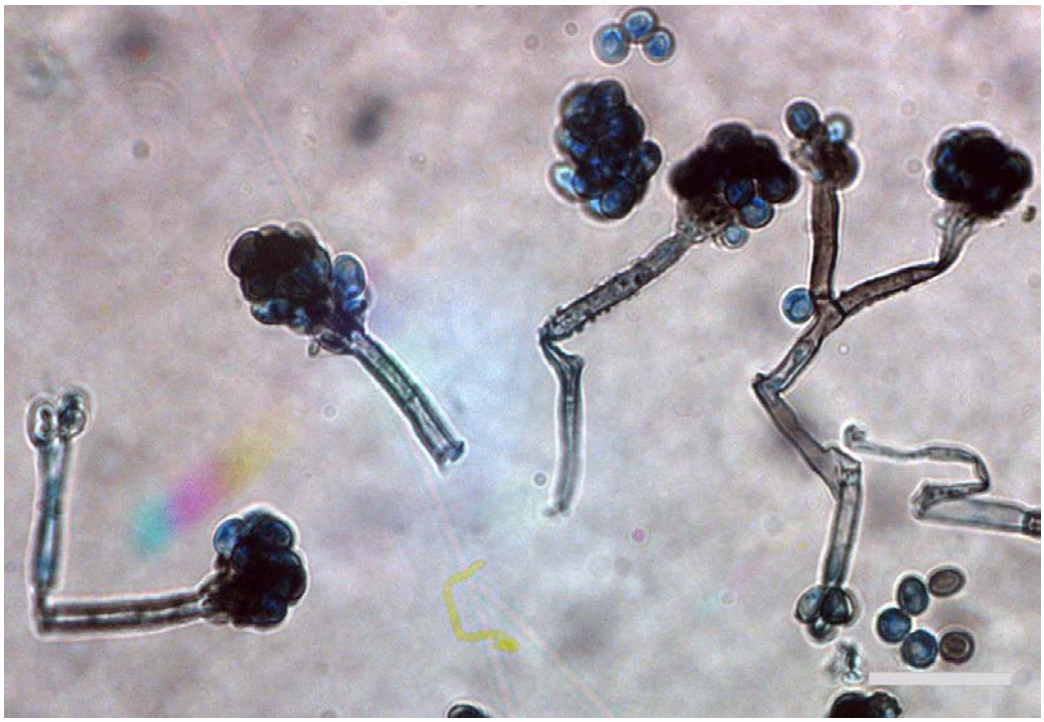
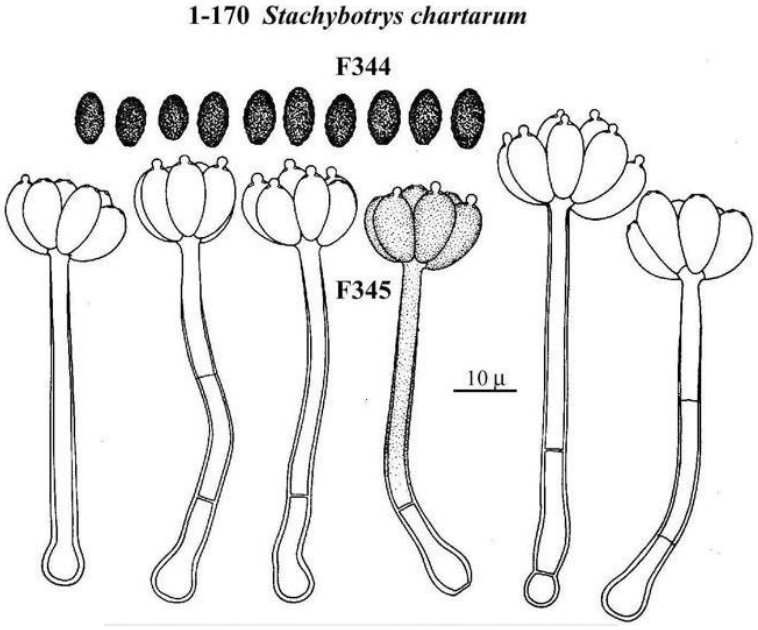


Figure 1A. Scanning electron micrograph of *Stachybotrys chartarum* (bar = 2 μm, upper panel). Line drawings of *Stachybotrys chartarum* (lower panel).



http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=11488

Further reading:

Hodgson MJ, Morey P, Leung WY, Morrow L, Miller D, Jarvis BB, Robbins H, Halsey JF, Storey E. 1998. Building-associated pulmonary disease from exposure to *Stachybotrys chartarum* and *Aspergillus versicolor*. *J Occup Environ Med.* 40: 241-249.

Hossain MA, Ahmed MS, Ghannoum MA. 2004. Attributes of *Stachybotrys chartarum* and its association with human disease. *J Allergy Clin Immunol.* 113: 200-208.

Miller JD, Rand TG, Jarvis BB. 2003. *Stachybotrys chartarum*: cause of human disease or media darling? *Med Mycol.* 41: 271-291.

Sudakin DL. 2000. *Stachybotrys chartarum*: current knowledge of its role in disease. *MedGenMed.* 2: E11.

Terr AI. 2001. *Stachybotrys:* relevance to human disease. *Ann Allergy Asthma Immunol*. 87: 57-63.

Pestka JJ, Yike I, Dearborn DG, Ward MD, Harkema JR. 2007. *Stachybotrys chartarum*, trichothecene mycotoxins, and damp building-related illness: new insights into a public health enigma. *Toxicol Sci*. 104: 4-26.

Pieckova E, Hurbankova M, Cerna S, Pivovarova Z, Kovacikova Z. 2006. Pulmonary cytotoxicity of secondary metabolites of *Stachybotrys chartarum* (Ehrenb.) Hughes. *Ann Agric Environ Med.* 13: 259-262.

M-2 *Aspergillus clavatus*

Source: Bone marrow / Nail

Clinical significance: *Aspergillus clavatus* is the occasional cause of pulmonary and ear infections. It produces toxin patulin in certain foodstuff like cereal that can cause disease in both humans and animals. It is also one of the causal agents of occupational hypersensitivity pneumonitis known as Malt Worker's Lung.

Colony: *A. clavatus* grows very fast. Colonies are bluish-green, powdery on Sabouraud's dextrose agar (Figure 2).

Microscopy: Lactophenol cotton blue mount shows septate hyphae with colorless conidiophores. Conidiophores terminates in vesicle, which is clavate shaped and the entire surface of this vesicle is covered (radiating) with one series of sterigmata (uniseriate). Conidia are smooth, ellipsoidal (Figure 2).

Differentiation: *A. clavatus* is differentiated from other *Aspergilli* by distinctive large, club shaped vesicle. Conidial head is radiate and uniseriate.

Molecular test: A PCR based amplification of ITS1 and ITS 2 regions has been used.

The ribosomal ITS1 and ITS2 region of the test isolate showed 100% nucleotide identity with *Aspergillus clavatus* strain USMO08 (GenBank accession no. KF669482.1).

Antifungal susceptibility: *A. clavatus* resistance to itraconazole has been documented.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	57
Laboratories with incorrect ID:	1
(*Aspergillus flavus*)	(1)

Illustrations:

Figure 2. Green-bluish colony of *Aspergillus clavatus* on Sabouraud's dextrose agar (upper panels). Microscopic morphology of *Aspergillus clavatus* depicting typical radiate heads with clavate vesicle, uniserate sterigmata, and round conidia (bar = 20 µm; lower panel).

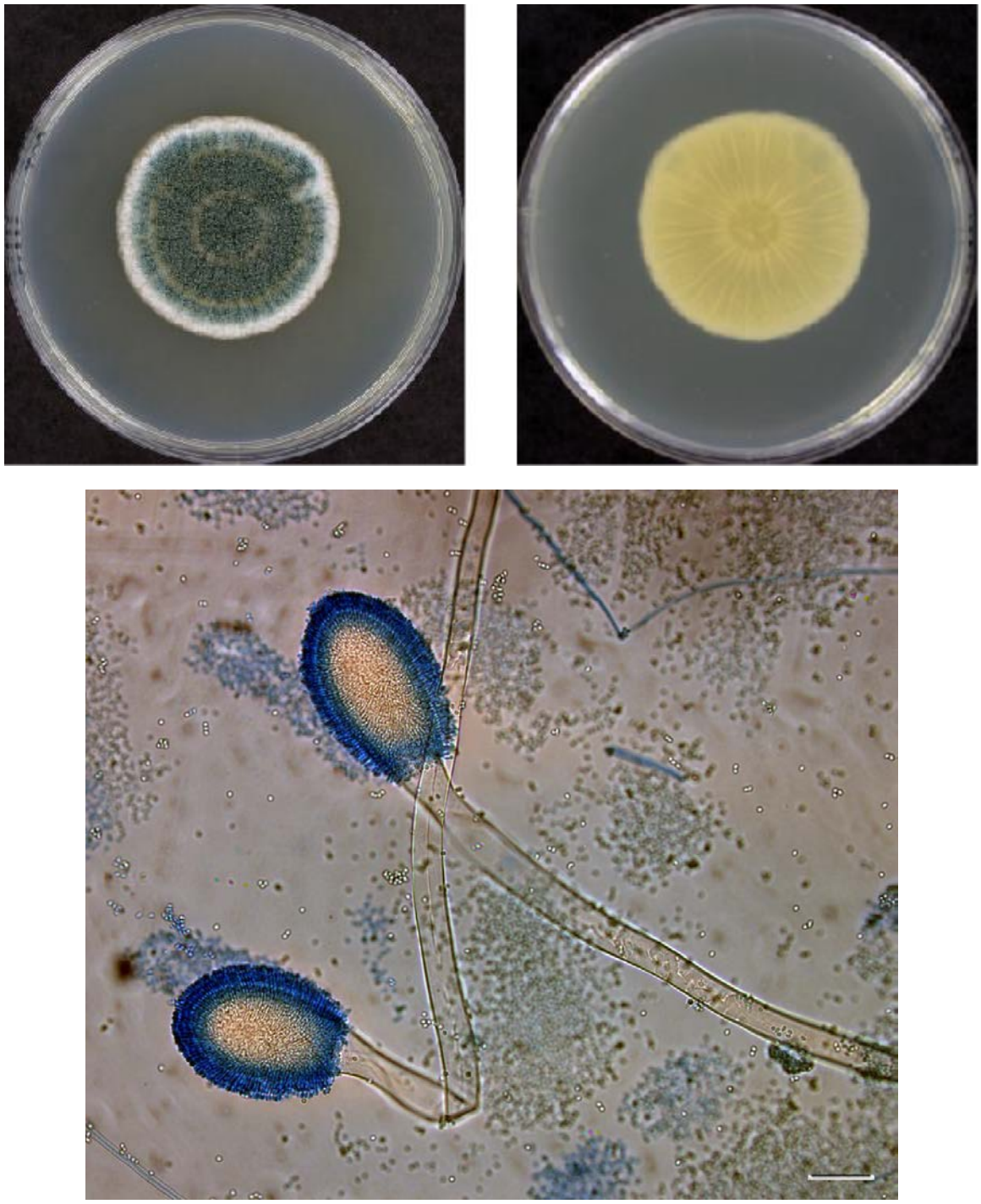
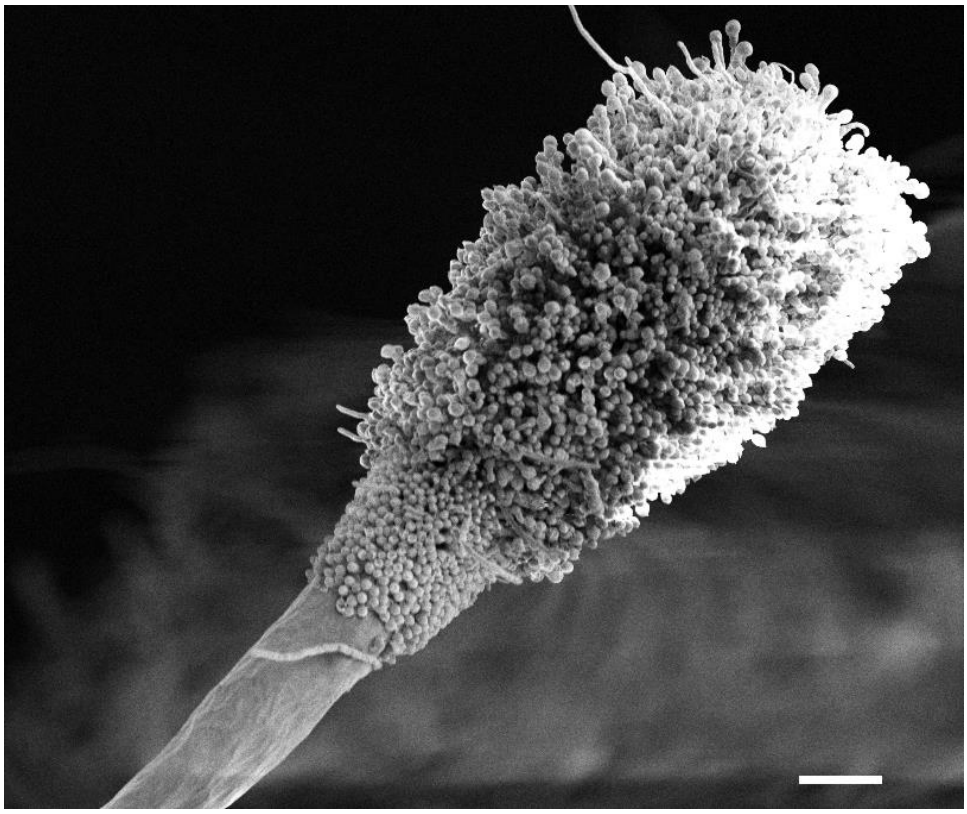
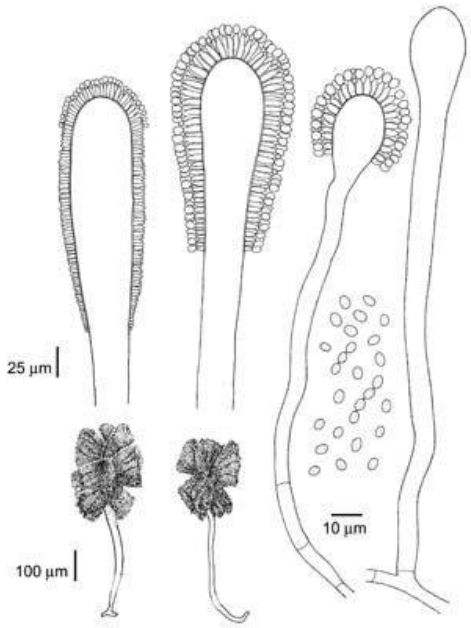


Figure 2A. Scanning electron micrograph of *Aspergillus clavatus* (bar = 20 µm, upper panel). Line drawings of *Aspergillus clavatus* (lower panel).





http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=3650

Further reading:

Gilmour JS, Inglis DM, Robb J, Maclean M. 1989. A fodder mycotoxicosis of ruminants caused by contamination of a distillery by-product with *Aspergillus clavatus*. *Vet Rec.* 124: 133-135.

Kellerman TS, Newsholme SJ, Coetzer JA, Van der Westhuizen GC. 1984. A tremorgenic mycotoxicosis of cattle caused by maize sprouts infested with *Aspergillus clavatus*. *Onderstepoort J Vet Res*. 51: 271-274.

Loretti, A.P., Colodel, E.M., Driemeier, D., Corrêa, A.M., Bangel, J.J. Jr, and Ferreiro, L. 2003. Neurological disorder in dairy cattle associated with consumption of beer residues contaminated with *Aspergillus clavatus*. *J Vet Diagn Invest*. 15: 123-132.

Piecková, E. and Jesenská, Z. 1999. Occurrence of itraconazole-tolerant micromycetes in the soil and food products. *Folia Microbiol (Praha)*. 44: 677-682.

Shlosberg, A., Zadikov, I., Perl, S., Yakobson, B., Varod, Y., Elad, D., Rapoport, E., and Handji, V. 1991. *Aspergillus clavatus* as the probable cause of a lethal mass neurotoxicosis in sheep. *Mycopathologia.* 114: 35-39.

Tremasov MIa, Smetov PK, Nazypov MN, Sergeĭchev AI. 1993. Tremorogenic mycotoxicosis caused by the fungi *Aspergillus clavatus*, clinical features, pathomorphology, and isolation of metabolites. *Prikl Biokhim Mikrobiol.* 29: 56-59.

van der Werff, P.J. 1951. Fungus affections of the lungs. *Ned Tijdschr Geneeskd.* 95: 1682-1690.
Yasin, A., Maher, A., and Moawad, M.H. 1978. Otomycosis: a survey in the eastern province of Saudi Arabia. *J. Laringol. Otol.* 92: 869-876.

M-3 *Microsporum gypseum*

Source: Scalp / Skin

Clinical significance: *Microsporum gypseum* is a well-known dermatophyte, which commonly infects hair and skin.

Colony: Generally *M. gypseum* grows relatively rapidly on Sabouraud's dextrose agar, powdery to granular with beige to cinnamon brown color (Figure 3).

Microscopy: Lactophenol cotton blue mount shows septate hyphae with both macroconidia and microconidia. Macroconidia are abundant, fusiform and symmetrical in shape with rounded ends. The walls of macroconidia are thin and rough and they contain 3-6 cells. Microconidia are moderately numerous in number, club-shaped and located along the hyphae (Figure 3).

Differentiation: *Microsporum* differs from *Trichophyton* and *Epidermophyton* by its spindle-shaped macroconidia with echinulate to rough walls.

Molecular test: PCR and PCR-restriction fragment length polymorphism (RFLP) methods targeting the DNA topoisomerase II genes was reported for identification of dermatophytes.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Microsporum gypseum* isolate UTHSCSA R-4017 (Genebank accession number: EU151494.1).

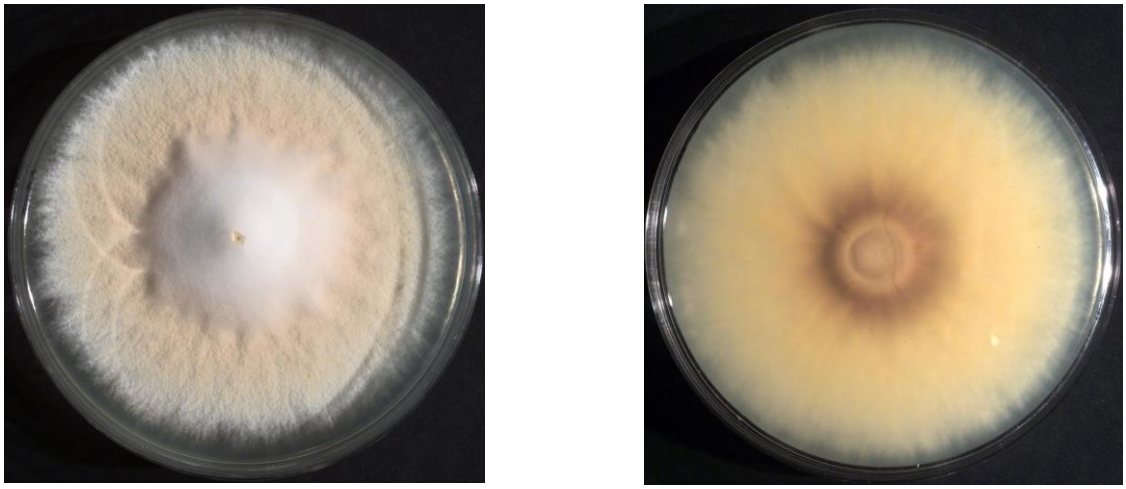
Antifungal susceptibility: Limited information available indicates that *M. gypseum* is susceptible to both terbinafine and itraconazole.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	58
Laboratories with incorrect ID:	1
(*Trichophyton mentagrophytes* species complex)	(1)

Illustrations:

Figure 3. *Microsporum gypseum* colony is powdery to granular, beige to cinnamon brown on Sabouraud's dextrose agar (upper panel). Microscopic morphology of *Microsporum gypseum* showing 3-6 celled macroconidia with thin and rough wall and club-shaped microconidia (bar = 20 μm; lower panel).



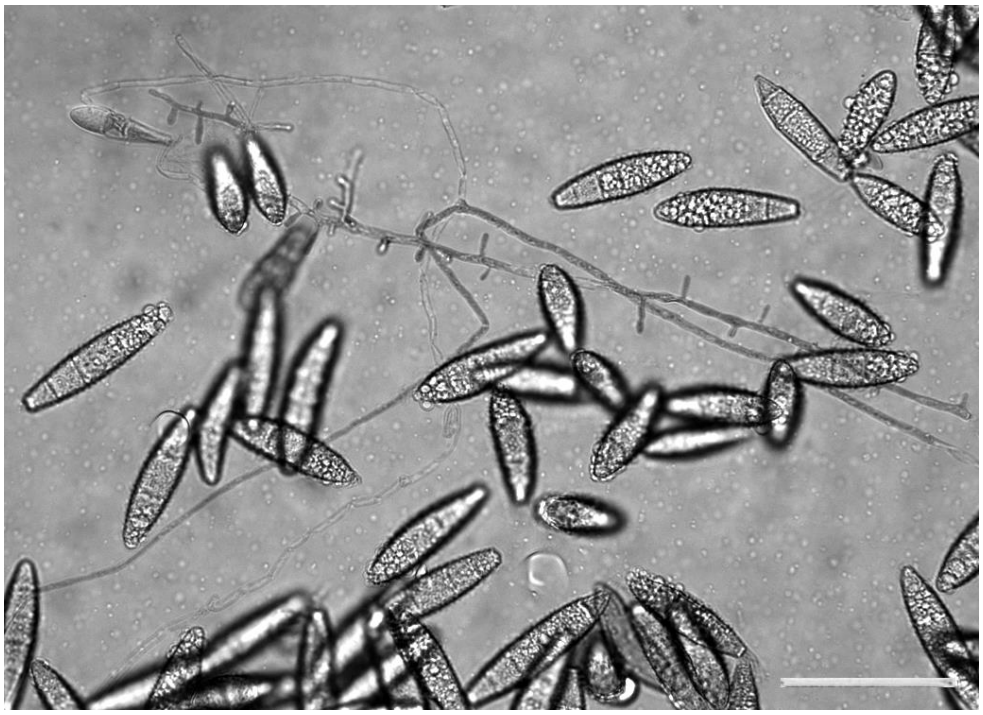
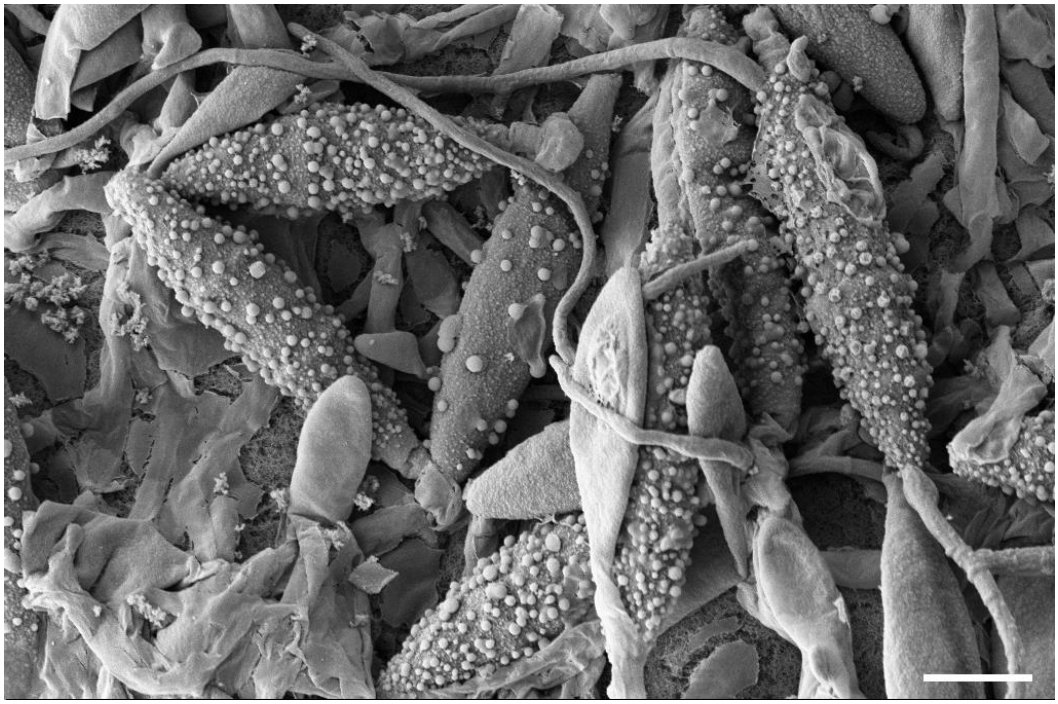
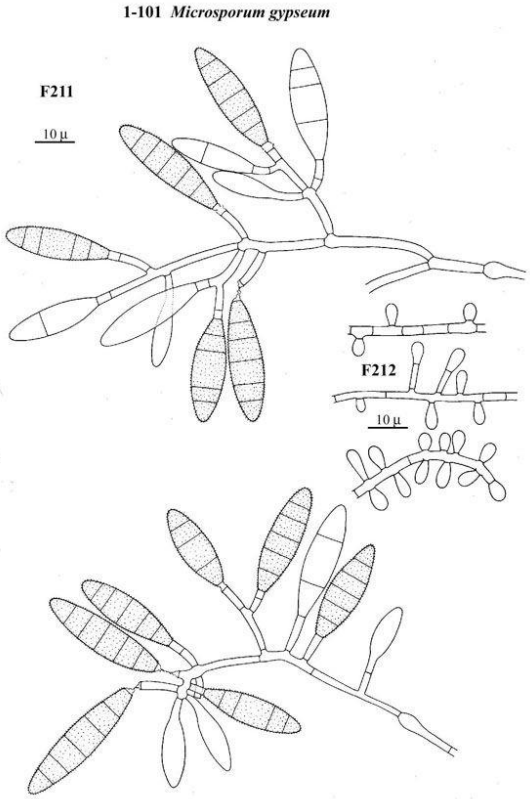


Figure 3A. Scanning electron micrograph of *Microsporum gypseum* (bar = 10 µm; upper panel). Line drawing depicting details of *Microsporum gypseum* (lower panel).





http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=11376

Further reading:

Bhagra S, Ganju SA, Sood A, Guleria RC, Kanga AK. 2013. *Microsporum gypseum* dermatophytosis in a patient of acquired immunodeficiency syndrome: a rare case report. *Indian J Med Microbiol.* 31(3):295-8.

Feng J, Liu F, Wu F, De Deng Q, Zeng HM, Kong TQ, Chen J, Sang H. 2013. Tinea infection with scutula-like lesions caused by *Microsporum gypseum* in a SLE patient: case report and literature review.
Mycopathologia. 176: 255-258.

Galhardo, M.C., Wanke, B., Reis, R.S., Oliveira, L.A., and Valle, A.C.2004. Disseminated dermatophytosis caused by *Microsporum gypseum* in an AIDS patient: response to terbinafine and amorolfine. *Mycoses.* 47: 238-241.

Hubka V, Dobiašova S, Dobiaš R, Kolarík M. 2014. *Microsporum aenigmaticum* sp. nov. from *M. gypseum* complex, isolated as a cause of tinea corporis. *Med Mycol.* [Epub ahead of print]

Iwasawa M, Yorifuji K, Sano A, Takahashi Y, Nishimura K. 2009. Case of kerion celsi caused by *Microsporum gypseum* (*Arthroderma gypseum*) in a child. *Nippon Ishinkin Gakkai Zasshi.* 50: 155-160.

Kamiya, A., Kikuchi, A., Tomita, Y., and Kanbe, T. 2004. PCR and PCR-RFLP techniques targeting the DNA topoisomerase II gene for rapid clinical diagnosis of the etiologic agent of dermatophytosis. *J Dermatol Sci.* 34: 35-48.

Machado, A.P., Hirata, S.H., Ogawa, M.M., Tomimori-Yamashita, J., and Fischman, O. 2005. Dermatophytosis on the eyelid caused by *Microsporum gypseum*. *Mycoses*. 48: 73-75.

Nenoff P, Gräser Y, Kibuka-Serunkuma L, Muylowa GK. 2007. Tinea circinata manus due to *Microsporum gypseum* in a HIV-positive boy in Uganda, east Africa. *Mycoses.* 50: 153-155.

Polilli E, Fazii P, Ursini T, Fantini F, Di Masi F, Tontodonati M, Sozio F, Parruti G. 2011. Tinea incognito Caused by *Microsporum gypseum* in a Patient with Advanced HIV Infection: A Case Report. *Case Rep Dermatol*. 3: 55-9.

Romano C, Massai L, Gallo A, Fimiani M. 2009. *Microsporum gypseum* infection in the Siena area in 2005-2006. *Mycoses.* 52: 67-71.

Skerlev M, Miklić P. 2010. The changing face of *Microsporum* spp. infections. *Clin Dermatol*. 28: 146-50.

M-4 *Scopulariopsis* species

Source: Lesion / Bronchi alveolar lavage / Nail

Clinical significance: *Scopulariopsis* sp. may infect various body parts including toenails, superficial skin tissue, deep skin tissues, nose, eyes, lungs, heart, and brain. Invasive *Scopulariopsis* infections are seen mainly in immunocompromised hosts, such as bone marrow transplant recipients, cancer patients, etc.

Colony: *Scopulariopsis* species grow moderately rapidly. On Sabouraud's dextrose agar, it is granular to powdery in texture. Colony is white initially and becomes light brown or buff tan with prolong incubation (Figure 4).

Microscopy: Lactophenol cotton blue mount shows septate hyphae, conidiophores, annelides, and conidia. Conidiophores are hyphae-like and simple or branched. Annellides are solitary, in clusters, or formed a penicillus (conidiophores that branch in multiple layers, providing a bushlike appearance); they are cylindrical and slightly swollen. Conidia are globose to pyriform, truncate, and forming basipetal chains (Figure 4).

Differentiation: *Scopulariopsis* differs from *Penicillium* by forming annellides.

Molecular test: The nuclear small-subunit (18S) ribosomal DNA and domains D1 and D2 of the nuclear large-subunit (28S) ribosomal DNA was used to investigate phylogenetic relationships among representative species of Zygomycetes.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 98% nucleotide identity with *Scopulariopsis brevicaulis* strain LPSC 1189 (Genebank accession number: KF753948.1).

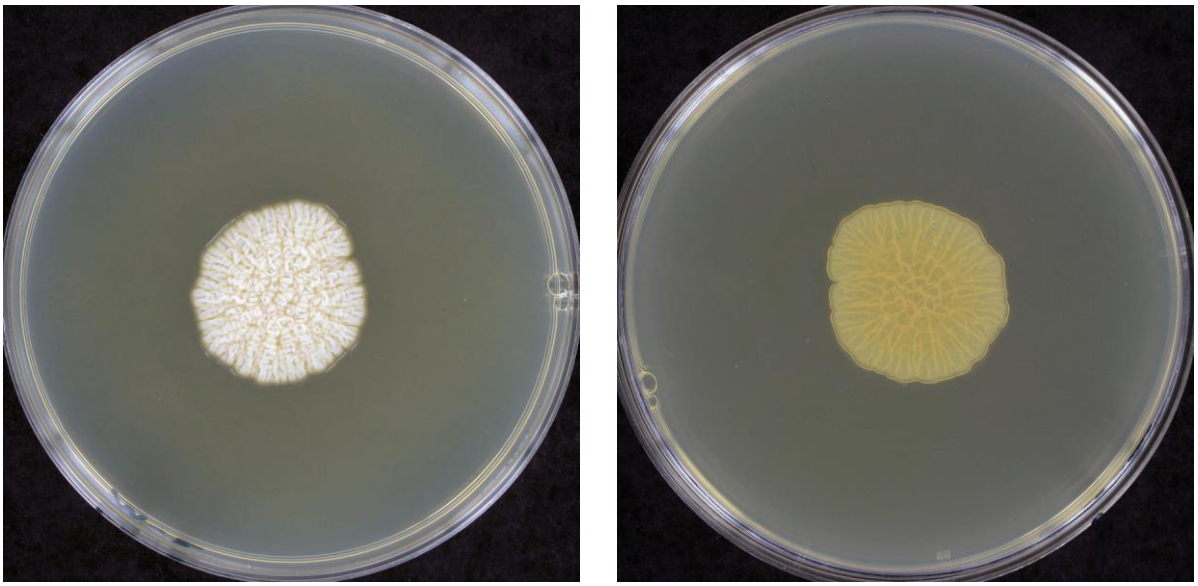
Antifungal susceptibility: In general, *Scopulariopsis* sp. is resistant to the fluconazole, itraconazole but susceptible to amphotericin B, ketoconazole and voriconazole. Terbinafine appears synergistic with azoles against *Scopulariopsis brevicaulis* isolates.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	53
Laboratories with incorrect ID:	6
(*Chrysosporium* species)	(5)
(*Trichoderma* species)	(1)

Illustrations:

Figure 4. White to buff tan colony of *Scopulariopsis* species on Sabouraud's dextrose agar. (upper panel). Microscopic morphology of *Scopulariopsis* species showing septate hyphae, conidiophores, solitary annelides, and globose to pyriform, truncate rough-walled conidia in basipetal chains (bar = 20 µm; lower panel).



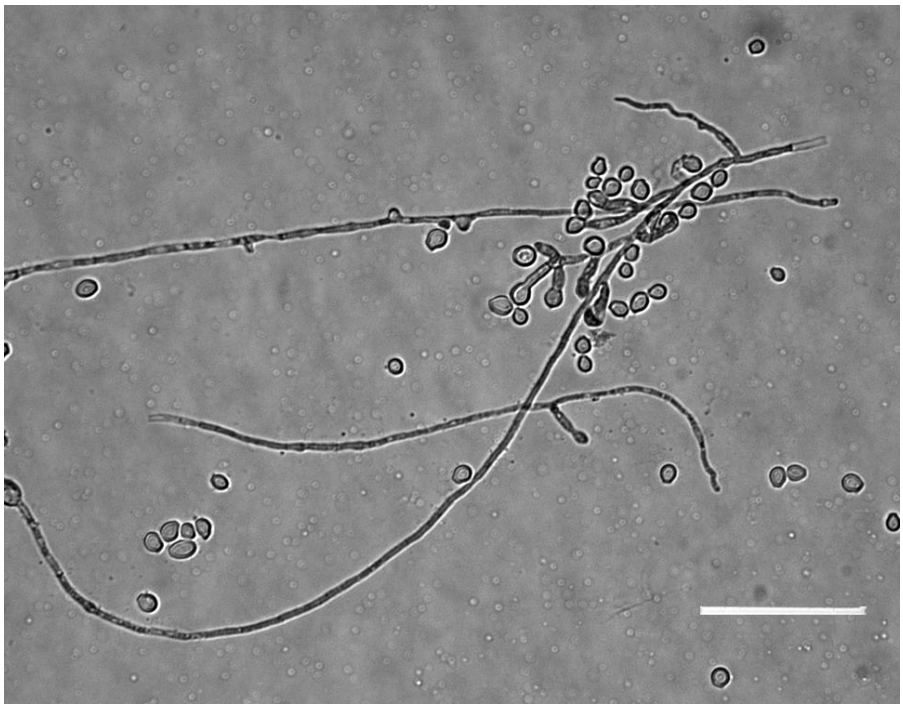
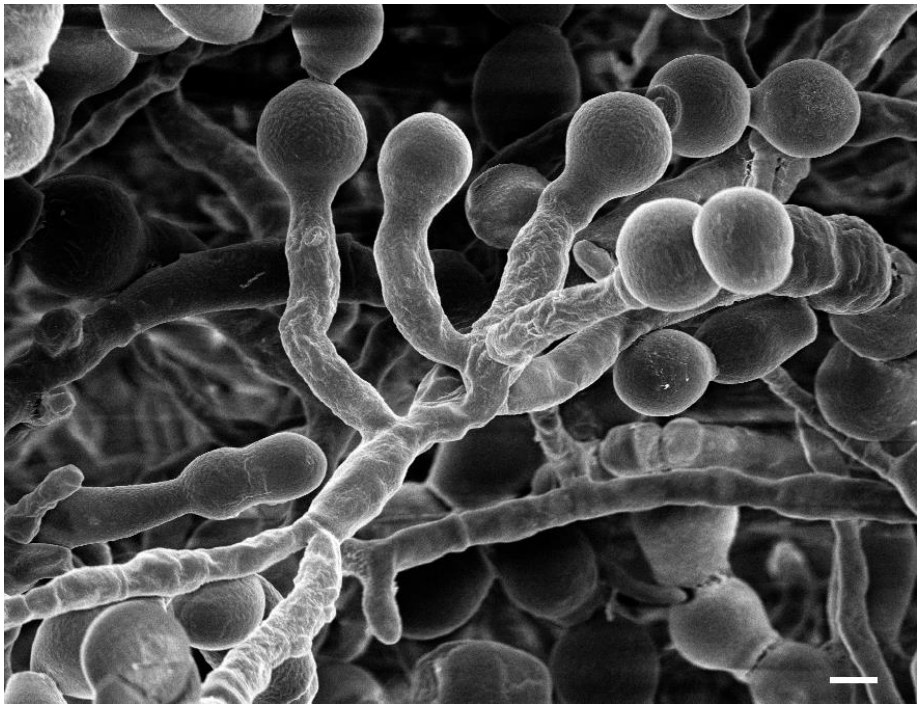
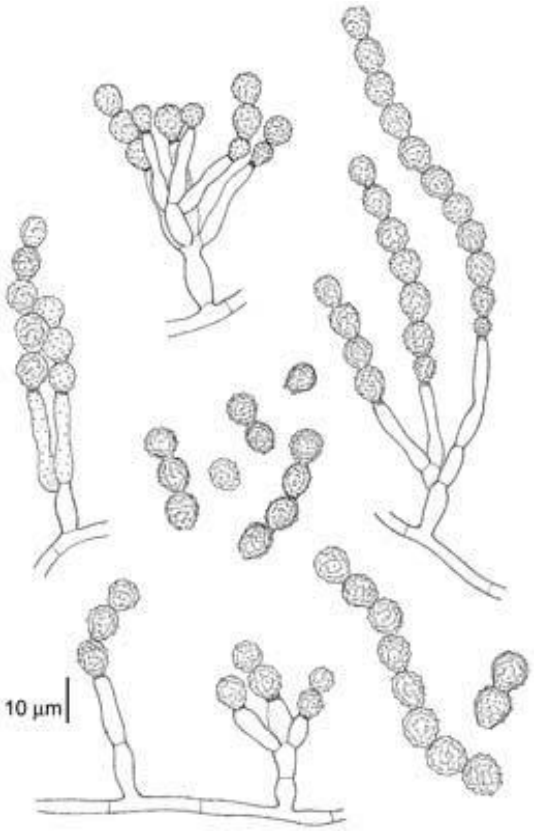


Figure 4A. Light microscopic and scanning electron micrograph of *Scopulariopsis* spceies (bar = 2 μm; upper panel). Line drawing depicting details of *Scopulariopsis* species (lower panel).





http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=3988

Further reading:

Aydin S, Ertugrul B, Gultekin B, Uyar G, Kir E. 2007. Treatment of two postoperative endophthalmitis cases due to *Aspergillus flavus* and *Scopulariopsis* spp. with local and systemic antifungal therapy. *BMC Infect Dis.* 7: 87.

Carrillo-Muñoz AJ, Giusiano G, Cárdenes D, Hernández-Molina JM, Eraso E, Quindós G, Guardia C, del Valle O, Tur-Tur C, Guarro J. 2008. Terbinafine susceptibility patterns for onychomycosis-causative dermatophytes and *Scopulariopsis brevicaulis*. *Int J Antimicrob Agents.* 31(6): 540-543.

Carrillo-Muñoz AJ, Giusiano G, Guarro J, Quindós G, Guardia C, del Valle O, Rodríguez V, Estivill D, Cárdenes CD. 2007. In vitro activity of voriconazole against dermatophytes, *Scopulariopsis brevicaulis* and other opportunistic fungi as agents of onychomycosis. *Int J Antimicrob Agents*. 30: 157-161.

Cuenca-Estrella M, Gomez-Lopez A, Buitrago MJ, Mellado E, Garcia-Effron G, Rodriguez-Tudela JL. 2006. In vitro activities of 10 combinations of antifungal agents against the multiresistant pathogen *Scopulariopsis brevicaulis*. *Antimicrob Agents Chemother.* 50: 2248-2250.

Mohammedi, I., Piens, M.A., Audigier-Valette, C., Gantier, J.C., Argaud, L., Martin, O., and Robert, D. 2004. Fatal *Microascus trigonosporus* (anamorph *Scopulariopsis*) pneumonia in a bone marrow transplant recipient. *Eur J Clin Microbiol Infect Dis.* 23: 215-217.

Steinbach, W.J., Schell, W.A., Miller, J.L., Perfect, J.R., and Martin, P.L. 2004 Fatal *Scopulariopsis brevicaulis* infection in a paediatric stem-cell transplant patient treated with voriconazole and caspofungin and a review of *Scopulariopsis* infections in immunocompromised patients. *J Infect.* 48: 112-116.

Wu CY, Lee CH, Lin HL, Wu CS. 2009. Cutaneous granulomatous infection caused by *Scopulariopsis brevicaulis*. *Acta Derm Venereol*. 89: 103-104.

M-5 *Sporothrix schenckii* species complex

Source: Tissue / Bronchial wash / Skin

Clinical significance: *Sporothrix schenckii* is the causal agent of sporotrichosis, a subcutaneous infection caused by implanatation of the fungus. The infection spreads in the body via lymphatic system. It is often called as "rose handler's disease" because of the increased frequency of occurrence in this group of individuals. Pulmonary and disseminated sporotrichosis are infrequently reported. Laboratory acquired infections have also been documented.

Colony: *Sporothrix schenckii* is a thermal dimorphic fungus. At 25°C, on Sabouraud's dextrose agar, colonies are initially white, becoming pinkish tan on the surface (Figure 5). At 37°C, on enriched media like blood agar, brain heart infusion agar, it converts to yeast form, which are cream to buff color.

Microscopy: Lactophenol cotton blue mount shows thin, hyaline septate hyphae; conidiophores are slender and tapering. At the end of conidiophores, ovoid, hyaline conidia are formed sympodially (rosette formation) (Figure 5). At 37°C, ovoid, single or multiple budding yeast cells were seen.

Differentiation: *S. schenckii* is differentiated from other fungi by its slow growth, initially white colonies turning black, and ovoid conidia produced sympodially (rosette formation). Nonpathogenic *Sporothrix* species do not convert to yeast phase at 37°C on enriched media. *Ophiostoma stenoceras*, a nonpathogenic fungus microscopically resembling *Sporothrix*, produces long necked perithecia after 2-3 weeks. *Exophiala* species produce annelloconidia, while *Phialophora* species produce phialoconidia, thus differentiating it from *S. schenckii*

Molecular test: Karyotyping by pulse field gel electrophoresis (PFGE) of clinical isolates demonstrated 6-8 chromosomes and genome size of 28 Mbp. Mitrochondrial DNA analysis of *Sporothrix schenckii* for epidemiology purposes has been done.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 99% nucleotide identity with *Sporothrix schenckii* strain CMW7617 (Genebank accession number: AF484471.1).

Antifungal susceptibility: Susceptibility testing results indicate that *S. schenckii* isolates are susceptible to amphotericin B, itraconazole, and ketoconazole, but less susceptible to fluconazole

Participant performance:

Referee Laboratories with correct ID:	9
Laboratories with correct ID:	49
Laboratories with incorrect ID:	9
(*Acremonium* species)	(6)
(*Pseudallescheria boydii* species complex)	(1)
(*Scedosporium* species)	(1)
(*Stachybotrys* species)	(1)

Illustrations:

Figure 5. White to cream colony *Sporothrix schenckii* on Sabouraud's dextrose agar (upper panel). Microscopic morphology of *Sporothrix schenckii* showing the hyphae and slender conidiophores with ovoid conidia formed sympodially (rosette formation) (bar = 10 μm; lower panel).

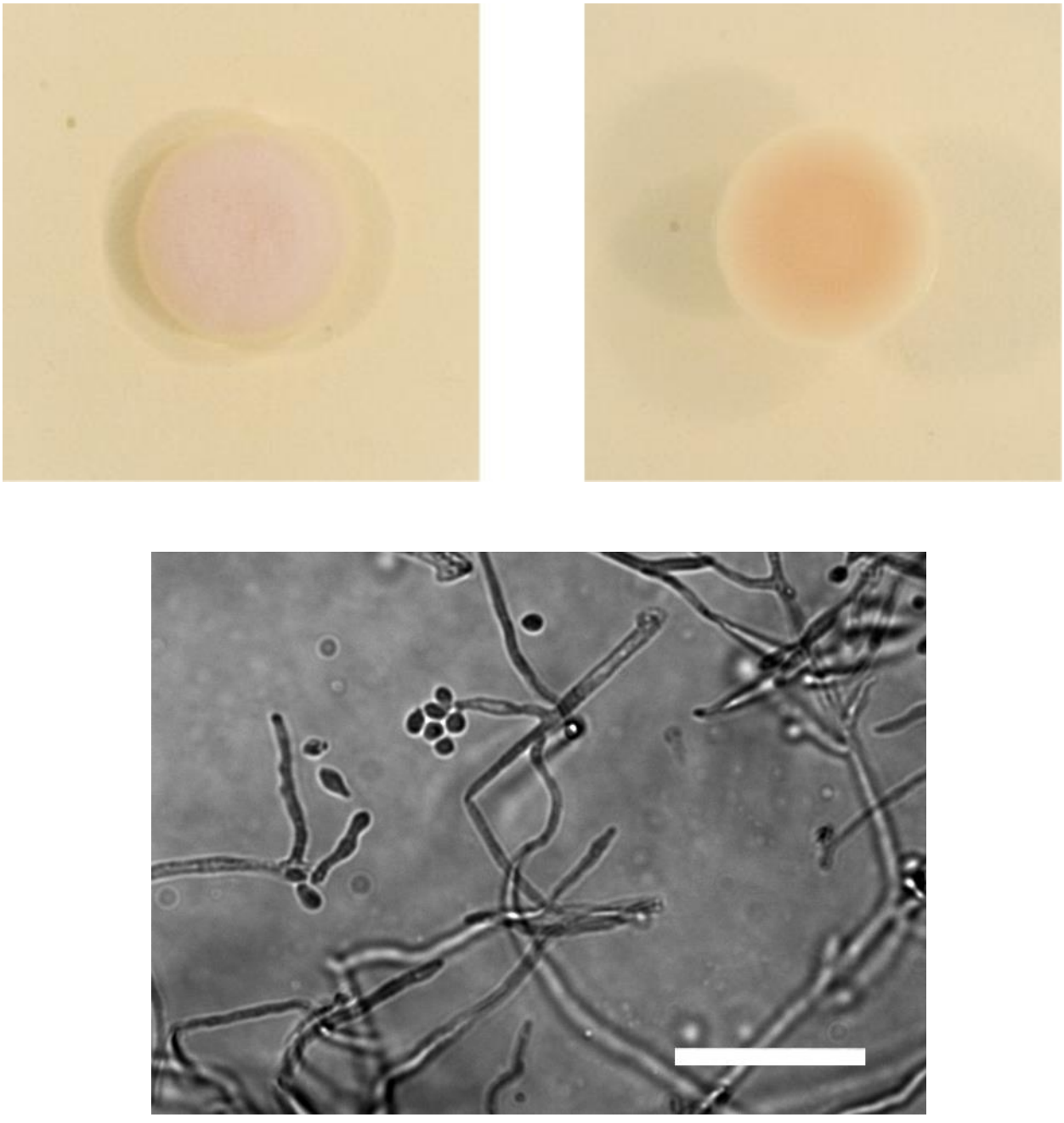
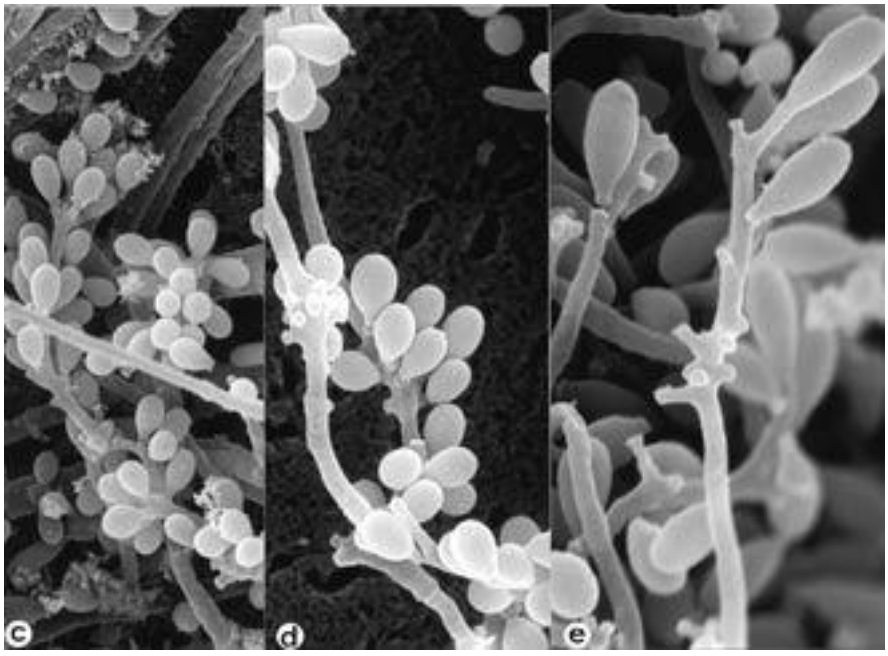


Figure 5A. Scanning electron micrograph of *Sporothrix schenckii* (upper panel). Line drawing depicting details of *Sporothrix schenckii* (lower panel).



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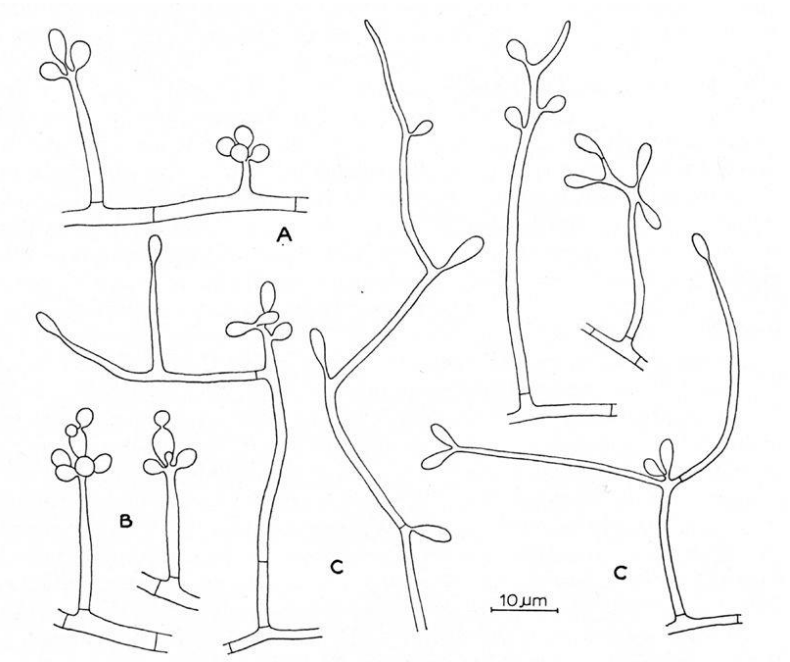


http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=2111

Further reading:

Appenzeller, S., Amaral, T.N., Amstalden, E.M., Bertolo, M.B., Neto, J.F., Samara, A.M., Fernandes, S.R. 2006. *Sporothrix schenckii* infection presented as monoarthritis: report of two cases and review of the literature. *Clin Rheumatol.* 25: 926-928.

Arenas R, Miller D, Campos-Macias P. 2007. Epidemiological data and molecular characterization (mtDNA) of *Sporothrix schenckii* in 13 cases from Mexico. *Int J Dermatol.* 46: 177-179.

Baroni, A., Palla, M., Iovene, M.R., Faccenda, F., Aiello, F.S., Puca, R.V., Satriano, R.A. 2007. Sporotrichosis: success of itraconazole treatment. *Skinmed.* 6: 41-44.

Barros MB, de Almeida Paes R, Schubach AO. 2011. *Sporothrix schenckii* and Sporotrichosis. *Clin Microbiol Rev.* 24: 633-654.

Callens, S.F., Kitetele, F., Lukun, P., Lelo, P., Van Rie, A., Behets, F., Colebunders, R. 2006. Pulmonary *Sporothrix schenckii* infection in a HIV positive child. *J Trop Pediatr*. 52: 144-146.

Galhardo MC, Silva MT, Lima MA, Nunes EP, Schettini LE, de Freitas RF, Paes Rde A, Neves Ede S, do Valle AC. 2010. *Sporothrix schenckii* meningitis in AIDS during immune reconstitution syndrome. *J Neurol Neurosurg Psychiatry*. 81: 696-699.

Howe, W.R., Wisco, O.J., Sartori, C. 2006. Fixed cutaneous sporotrichosis in an adolescent boy: a case report. *Cutis.* 78: 337-340.

Iyengar SS, Khan JA, Brusco M, FitzSimmons CJ. 2010. Cutaneous *Sporothrix schenckii* of the human eyelid. *Ophthal Plast Reconstr Surg.* 26: 305-306.

Kohler, L.M., Soares, B.M., de Assis Santos, D., Da Silva Barros, M.E., Hamdan, J.S. 2006. In vitro susceptibility of isolates of *Sporothrix schenckii* to amphotericin B, itraconazole, and terbinafine: comparison of yeast and mycelial forms. *Can J Microbiol.* 52: 843-847.

Lopes-Bezerra, L.M., Schubach, A., Costa, R.O. 2006. *Sporothrix schenckii* and sporotrichosis. *An Acad Bras Cienc*. 78: 293-308.

López-Romero E, Reyes-Montes Mdel R, Pérez-Torres A, Ruiz-Baca E, Villagómez-Castro JC, Mora-Montes HM, Flores-Carreón A, Toriello C. 2011. *Sporothrix schenckii* complex and sporotrichosis, an emerging health problem. *Future Microbiol.* 6: 85-102.

Sivagnanam S, Bannan AM, Chen SC, Ralph AP. 2012. Sporotrichosis (*Sporothrix schenckii* infection) in the New South Wales mid-north coast, 2000-2010. *Med J Aust.* 196: 588-590.

Yelverton, C.B., Stetson, C.L., Bang, R.H., Clark, J.W., Butler, D.F. 2006. Fatal sporotrichosis. *Cutis.* 78: 253-256.

YEAST DESCRIPTIONS

Y-1 *Cryptococcus uniguttulatus*

Source: Urine / Catheter

Clinical significance: *Cryptococcus uniguttulatus* ventriculitis was documented in a case report in 2001.

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

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

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

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

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

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

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	52
Laboratories with incorrect ID:	1
(*Rhodotorula glutins*)	(1)

Illustrations:

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

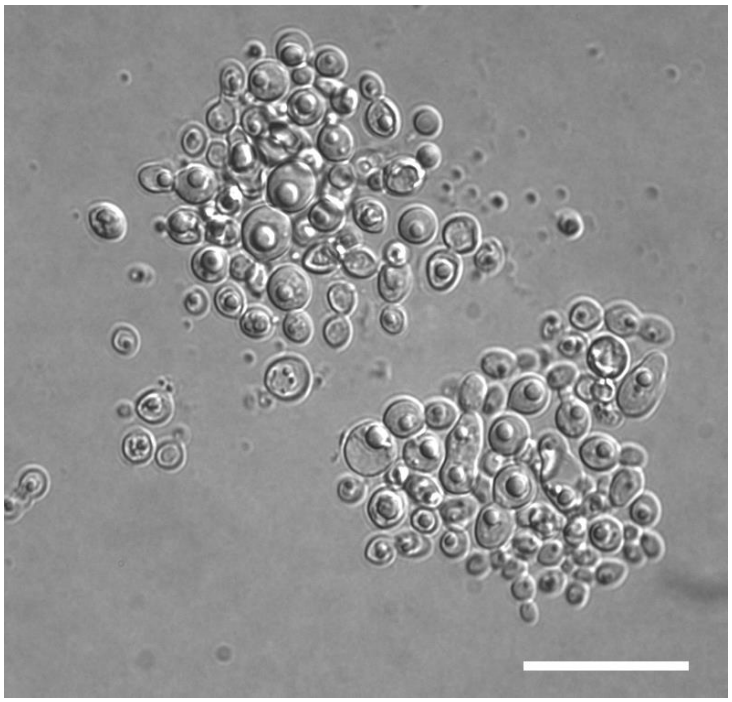
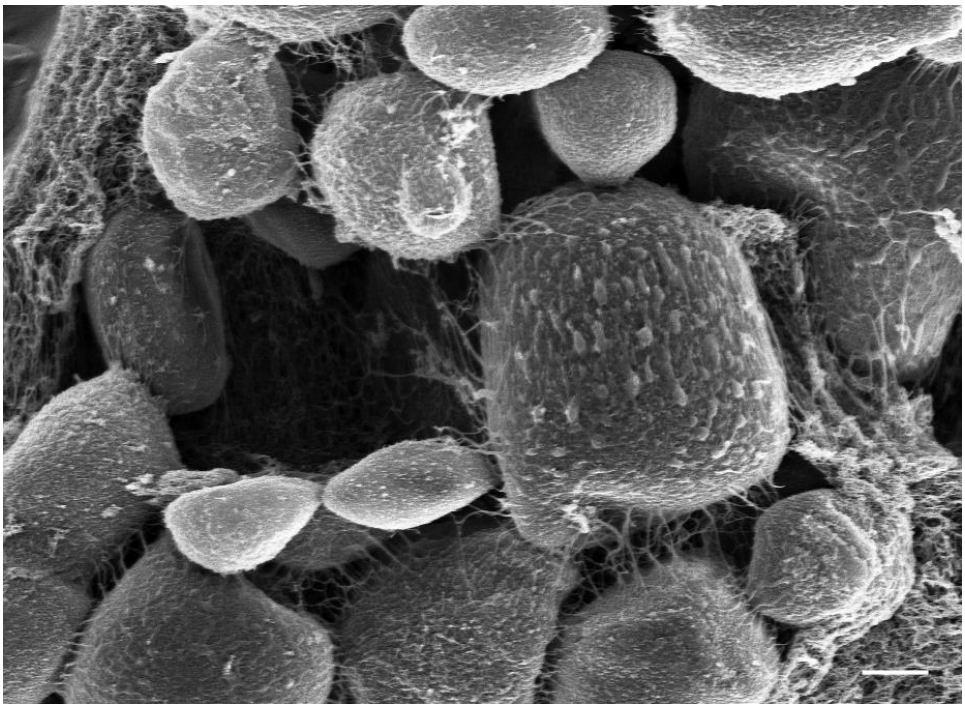


Figure 6A. Scanning electron micrograph illustrates blastoconidia (bar = 1 μm).



Further reading:

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

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

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

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

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

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

Y-2 *Sacchromyces cerevisiae*

Source: Sputum / Urine / Stool

Clinical significance: *Saccharomyces cerevisiae,* the baker's yeast, causes disseminated infections in immunocompromised hosts.

Colony: *Sacharomyces cerevisiae* colonies appear creamy, smooth, dull, or buttery texture after 3 – 5 days of incubation on Sabouraud's dextrose agar at 25°C (Figure 7).

Microscopy: *Sacharomyces cerevisiae* are round to oval yeast cells with no pseudohyphae or rudimentary pseudohyphae on Corn meal agar with Tween 80, characteristic ascospores encased in asci are seen (Figure 7).

Differentiation: *Saccharomyces cerevisiae* ferments glucose, maltose and sucrose, does not grow on the media containing cycloheximide, and grows at 37°C. On the API 20C AUX, a specific assimilation biocode is obtained for identification of this organism.

Molecular test: *Saccharomyces cerevisiae* is the most intensely studied model organism also being the first eukaryote to have its entire genome sequenced and mapped.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Saccharomyces cerevisiae* isolate D3C (GenBank accession no. JF715201.1).

Antifungal susceptibility: Most isolates are susceptible to amphotericin B, 5-FC, and to azoles like fluconazole, miconazole, voriconazole, etc.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	52
Laboratories with incorrect ID:	1
(*Candida parapsilosis* species complex)	(1)

Illustrations:

Figure 7. *Sacchromycetes cerevisiae*, creamy, smooth, dull butyrous colony on Sabouraud's dextrose agar, 5-day, 25°C. Microscopic morphology showing round to oval blastoconidia on Corn meal agar with Tween 80 (bar = 10 μm).

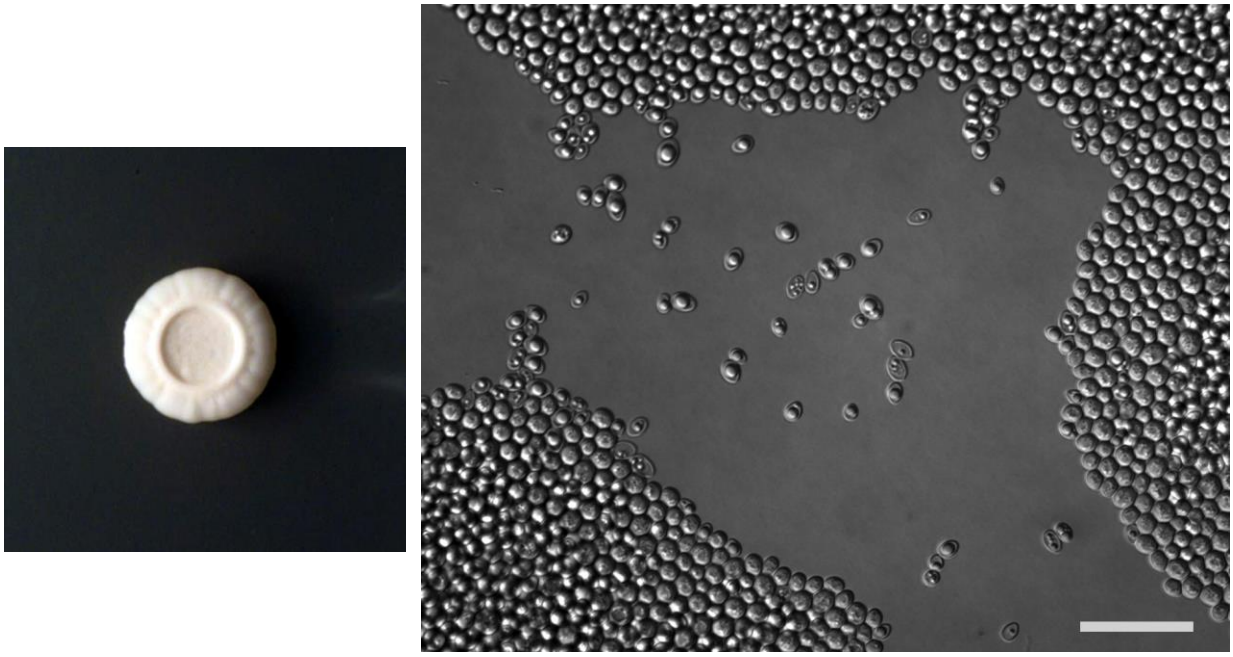
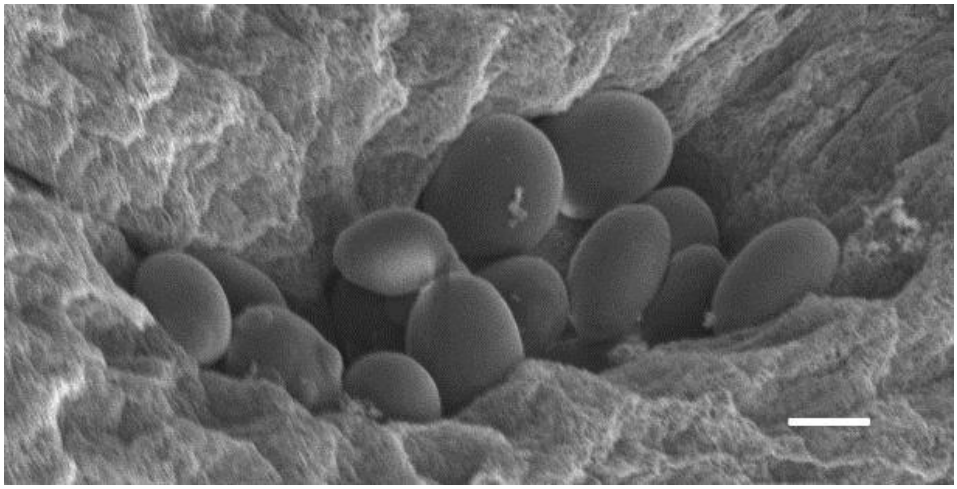


Figure 7A. Scanning electron micrograph illustrating oval blastoconidia (bar = 2 μm).



Further reading:

Buchta V, Vejsova M, Vale-Silva LA. 2008. Comparison of disk diffusion test and Etest for voriconazole and fluconazole susceptibility testing. *Folia Microbiol (Praha).* 53: 153-160.

Cherifi S, Robberecht J, Miendje Y. 2004. *Saccharomyces cerevisiae* fungemia in an elderly patient with *Clostridium difficile* colitis. *Acta Clin Belg.* 59: 223-224.

Enache-Angoulvant A, Hennequin C. 2005. Invasive *Saccharomyces* infection: a comprehensive review. *Clin Infect Dis.* 41: 1559-1568.

Fiore NF, Conway JH, West KW, Kleiman MB. 1998. *Saccharomyces cerevisiae* infections in children. *Pediatric Infectious Disease J.* 17: 1177 –1179.

Henry S, D'Hondt L, Andre M, Holemans X, Canon JL. 2004 *Saccharomyces cerevisiae* fungemia in a head and neck cancer patient: a case report and review of the literature. *Acta Clin Belg.* 59: 220-222.

Hamoud S, Keidar Z, Hayek T. 2011. Recurrent *Saccharomyces cerevisiae* fungemia in an otherwise healthy patient. Isr Med Assoc J. 13:575-6.

Konecny P, Drummond FM, Tish KN, Tapsall JW. 1999. *Saccharomyces cerevisiae* oesophagitis in an HIV – Infected patient. *International J STD & AIDS.* 10: 821 –822.

Ren P, Sridhar S, Chaturvedi V. 2004. Use of paraffin-embedded tissue for identification of *Saccharomyces cerevisiae* in a baker's lung nodule by fungal PCR and nucleotide sequencing. *J Clin Microbiol.* 42: 2840 -2842.

Munoz P, Bouza E, Cuenca-Estrella M, Eiros JM, Perez MJ, Sanchez-Somolinos M, Rincon C, Hortal J, Pelaez T. 2005. *Saccharomyces cerevisiae* fungemia: an emerging infectious disease. *Clin Infect Dis.* 40: 1625-1634.

Savini V, Catavitello C, Manna A, Talia M, Febbo F, Balbinot A, D'Antonio F, Di Bonaventura G, Celentano C, Liberati M, Piccolomini R, D'Antonio D. 2008. Two cases of vaginitis caused by itraconazole-resistant *Saccharomyces cerevisiae* and a review of recently published studies. *Mycopathologia*. 166: 47-50.

Y-3 *Candida dubliniensis*

Source: Urine / Blood / Bronchial wash

Clinical significance: *Candida dubliniensis* was initially recovered from the oral cavities of HIV infected individuals and AIDS patients causing erythematous and/or pseudomembranous oral candidiasis or angular cheilitis. *C. dubliniensis* has also been isolated from other body sites including lungs, vagina, blood, and feces.

Colony: *C. dubliniensis* colony is white to cream, smooth, and soft on Sabouraud's dextrose agar after 7 days of incubation at 25°C (Figure 8). *C. dubliniensis* does not grow at 45°C.

Microscopy: *C. dubliniensis* shows abundant, branched pseudohyphae and true hyphae with blastoconidia. Chlamydospores are single, or in pairs, or in chains, or clusters on Corn meal agar with Tween 80 (Figure 8).

Differentiation: *C. dubliniensis* is practically indistinguishable from *C. albicans* on the basis of many common phenotypic tests. One physiologic feature that does appear to be fairly stable is that *C. dubliniensis* grows poorly at 42°C or does not at all at 45°C while *C. albicans* grows well at both of these temperatures. In addition, *C. dubliniensis* is able to assimilate glycerol, but not xylose or trehalose as opposed to observations in *C. albicans*. Some commercial yeast identification kits such as the API 20C AUX, VITEK2, or the ID 32C have biocodes for *C. dubliniensis* included in the databases. These two closely related yeasts can also be distinguished by molecular methods.

Molecular test: Genetically, *C. dubliniensis* has been found to be distinct from *C. albicans* in DNA fingerprinting studies even- though the two species are closely related phylogenetically. Several *C. dubliniensis* molecular probes are available in reference laboratories.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100 % nucleotide identity with *Candida dubliniensis* isolate CD36 (GenBank accession no. FM992695.1).

Antifungal susceptibility: Several isolates of *C. dubliniensis* have been found to have higher resistance to fluconazole than other pathogenic species of *Candida*, and the resistance to fluconazole may be induced in some originally sensitive strains. This fact may have serious implications for immunocompromised individuals prescribed fluconazole for prolonged periods.

Participant performance:

Referee Laboratories with correct ID:	9
Laboratories with correct ID:	44
Laboratories with incorrect ID:	9
(*Candida albicans*)	(9)

Illustrations:

Figure 8. *Candida dubliniensis,* white, glossy, and smooth colony on Sabouraud's dextrose agar, 4 days, 25°C. Microscopic morphology on corn meal agar with Tween 80, showing abundant branched pseudohyphae and true hyphae with blastoconidia (bar = 10 μm).

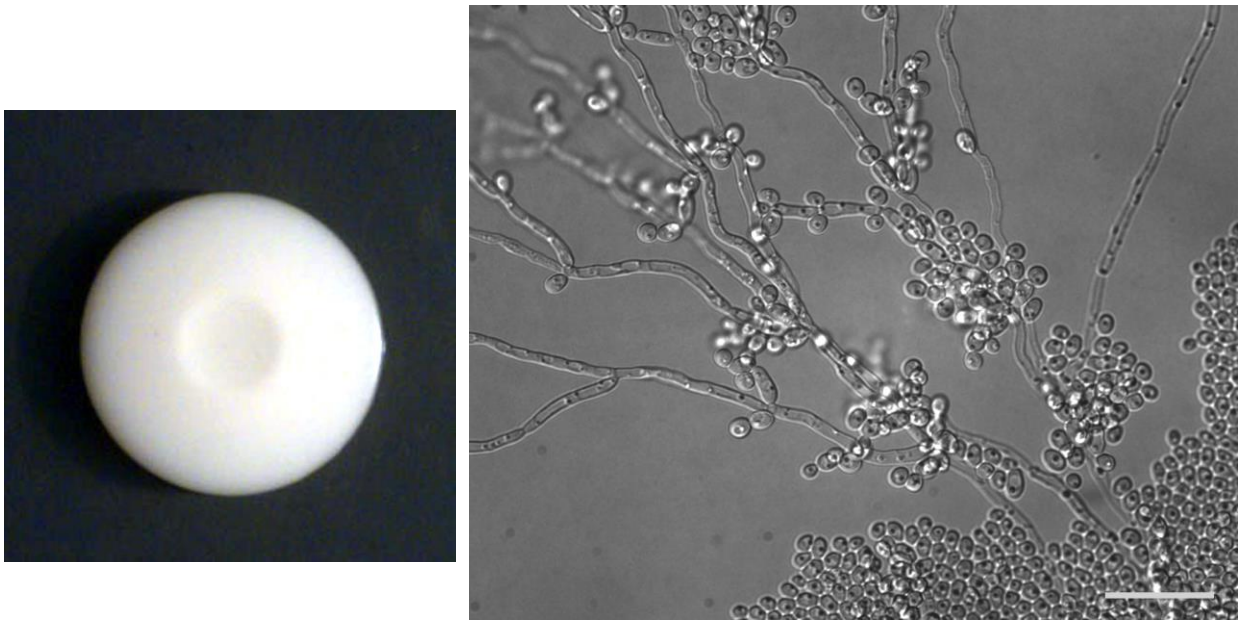
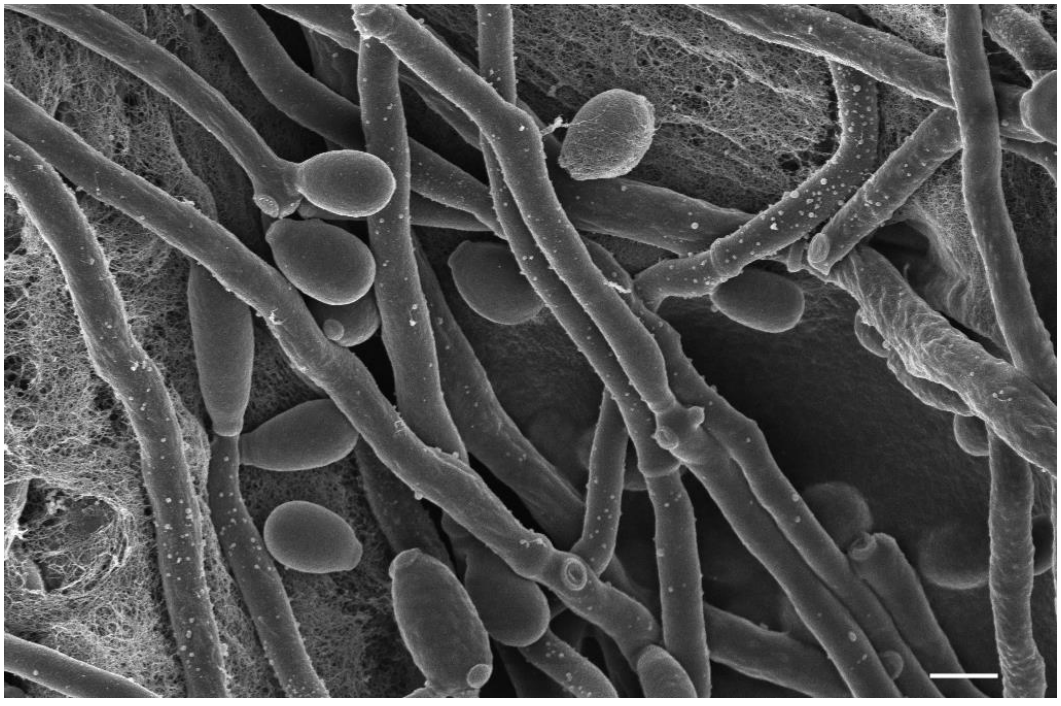


Figure 8A. Scanning electron micrograph of *Candida dubliniensis* illustrates pseudohyphae and blastoconidia (bar = 2 μm)



Further reading:

Bosco-Borgeat ME, Taverna CG, Cordoba S, Isla MG, Murisengo OA, Szusz W, Vivot W, Davel G. 2011. Prevalence of *Candida dubliniensis* fungemia in Argentina: identification by a novel multiplex PCR and comparison of different phenotypic methods. Mycopathologia. 172(5):407-414.

Cardenes-Perera CD, Torres- Lana A, Alonso-Vargas R, Moragues-Tosantas MD, Emeterio JP, Quindos-Andres G, Arevalo-Morales MP. 2004. Evaluation of API ID 32C® and Vitek-2® to identify *Candida dubliniensis*. *Diagn Microbiol & Infect Dis*. 50: 219 – 221.

Ellepola AN, Khan ZU. 2012. Rapid Differentiation of *Candida dubliniensis* from *Candida albicans* by Early D-Xylose Assimilation. *Med Princ Pract.* 21: 375-378.

Espinosa-Heidmann DG, McMillan BD, Lasala PR, Stanley J, Larzo CR. 2012. *Candida dubliniensis* endophthalmitis: first case in North America. *Int Ophthalmol*. 32: 41-45.

Khan Z, Ahmad S, Chandy R, Joseph L. 2012. A simple xylose-based agar medium for the differentiation of *Candida dubliniensis* and *Candida albicans*. *Diagn Microbiol Infect Dis*. 72: 285-287.

Khan Z, Ahmad S, Joseph L, Chandy R. 2012. *Candida dubliniensis*: an appraisal of its clinical significance as a bloodstream pathogen. *PLoS One.* 7:e32952.

Mirhendi H, makimura K, Zomorodian K, Maeda N, Ohshima T, Yamaguchi H. 2005. Differentiation of *Candida albicans* and *Candida dubliniensis* using a single enzyme PCR-RFLP method. *Jpn J Infect Dis.* 58: 235 – 237.

Romeo O, Criseo G. 2009. Molecular epidemiology of *Candida albicans* and its closely related yeasts *Candida dubliniensis* and *Candida africana*. *J Clin Microbiol*. 47: 212-214.

Salgado-Parreno FJ, Alcoba-Florez J, Arias A, Moragues MD, Quindos G, Ponton J, Arevalo MP. 2006. *In vitro* activities of voriconazole and five licensed antifungal agents against *Candida dubliniensis*: comparison of CLSI M27-A2, Sensititre YeastOne, disk diffusion, and Etest methods. *Microb Drug Resist*. 12: 246-51.

Scheid LA, Mario DA, Kubiça TF, Santurio JM, Alves SH. 2012. *In vitro* activities of antifungal agents alone and in combination against fluconazole-susceptible and -resistant strains of *Candida dubliniensis*. *Braz J Infect Dis*. 16: 78-81.

Sullivan DJ, Moran GP, Pinjon E, Al-Mosaid A, Stokes C, Vaughan C, Coleman DC. 2004. Comparison of the epidemiology, drug resistance mechanisms and virulence of *Candida dubliniensis* and *Candida albicans*. *FEMS Yeast Research*. 4: 369 – 376.

Tsuruta R, Oda Y, Mizuno H, Hamada H, Nakahara T, Kasaoka S, Maekawa T. 2007. *Candida dubliniensis* isolated from the sputum of a patient with end-stage liver cirrhosis. *Intern Med*. 46: 597-600.

Us E, Cengiz SA. 2007. Prevalence and phenotypic evaluation of *Candida dubliniensis* in pregnant women with vulvovaginal candidosis in a university hospital in Ankara. *Mycoses.* 50: 13-20.

Yu N, Kim HR, Lee MK. 2012. The First Korean Case of Candidemia due to *Candida dubliniensis*. *Ann Lab Med*. 32: 225-228.

Y-4 *Candida lipolytica*

Source: Nail / Urine / Catheter

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 9).

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

Differentiation: *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 the genus *Candida*. Randomly amplified polymorphic DNA (RAPD) PCR has been used for the identification of *C. lypolytica* isolates from dairy products.

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

Antifungal susceptibility: *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:	50
Laboratories with incorrect ID:	3
(*Candida krusei*)	(2)
(*Candida* species)	(1)

Illustrations:

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

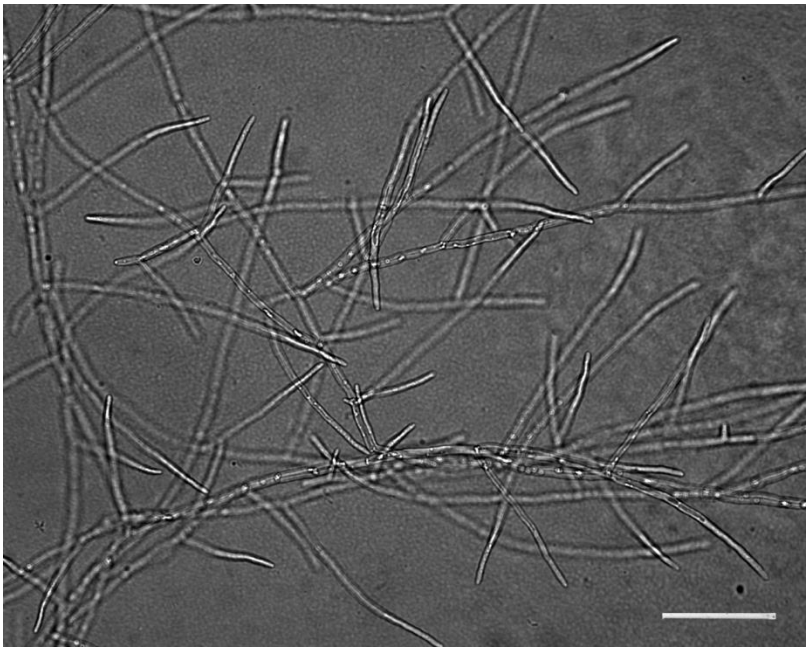
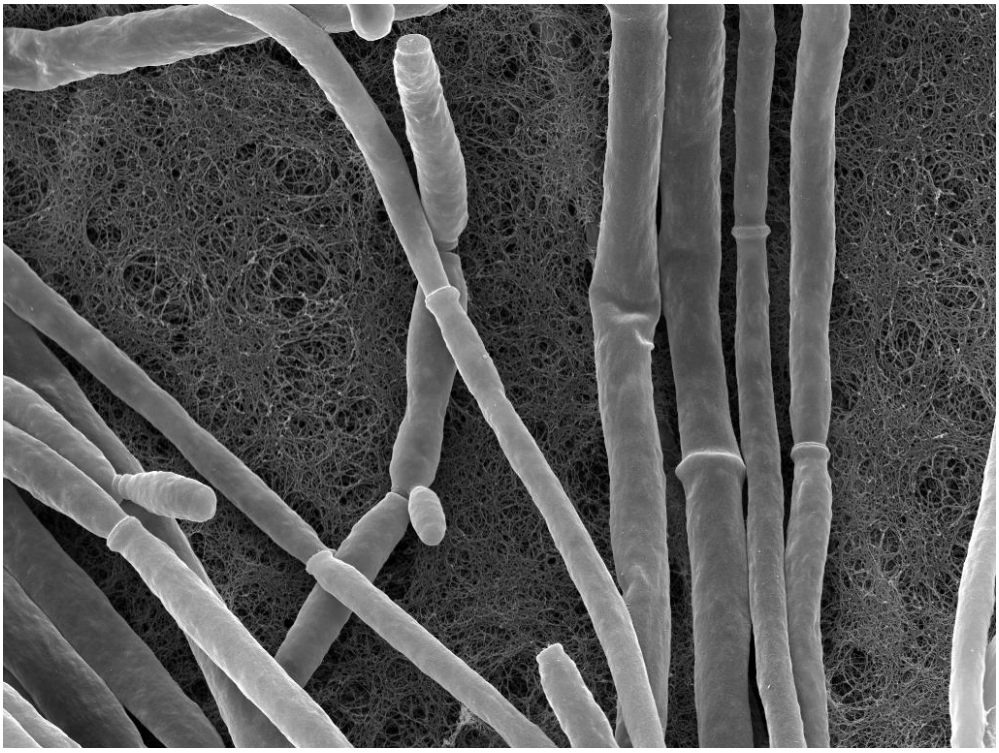


Figure 9A. Scanning electron micrograph illustrates pseudohyphae and blastoconidia.



Further reading:

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

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

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

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

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

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

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

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

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

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

Y-5 *Cryptococcus laurentii*

Source: Stool / CSF / Urine

Clinical significance: *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.

Colony: *C. laurentii* colony ranged from cream, yellow, tan, or pink, and the color intensified as the culture aged (Figure 10).

Microscopy: *C. laurentii* yeasts are round to oval on Corn meal agar with Tween 80. There is no discernible capsule (Figure 10).

Differentiation: *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.

Molecular test: *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).

Antifungal susceptibility: 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.

Participant performance:

Referee Laboratories with correct ID:	10
Laboratories with correct ID:	52
Laboratories with incorrect ID:	1
(*Cryptococcus albidus*)	(1)

Illustrations:

Figure 10. *Cryptococcus laurentii*, white creamy colony on Sabouraud's dextrose agar, 25°C. *Cryptcoccus laurentii* on corn meal agar with Tween 80 showing blastoconidia (BAR = 20 μm).

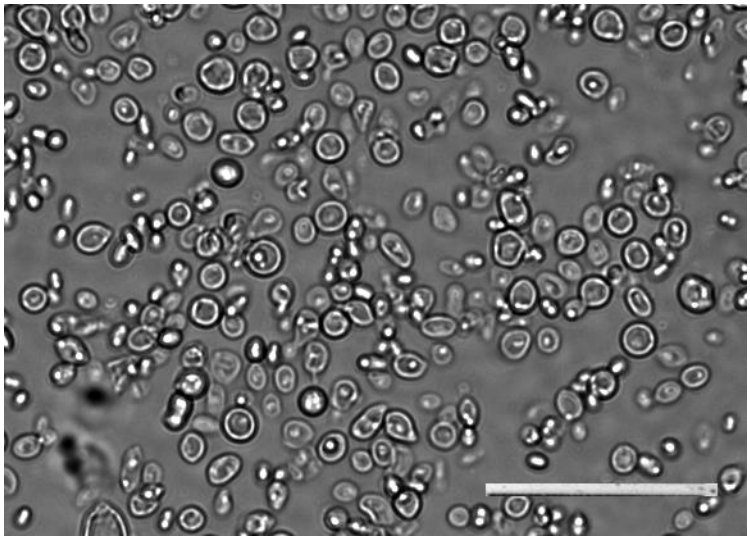
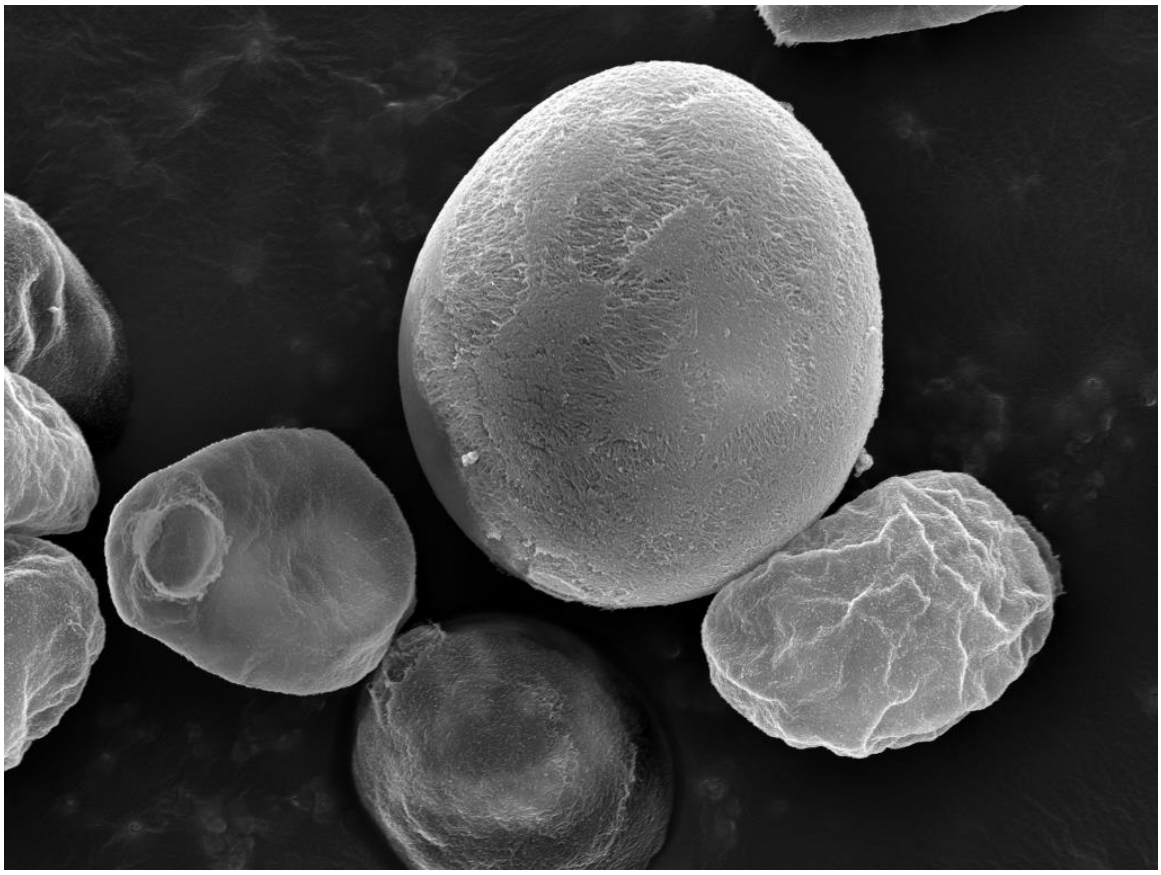


Figure 10. Scanning electron micrograph illustrating blastoconidia.



Further reading:

Andrade-Silva L, Ferreira-Paim K, Silva-Vergara ML, Pedrosa AL. 2010. Molecular characterization and evaluation of virulence factors of *Cryptococcus laurentii* and *Cryptococcus neoformans* strains isolated from external hospital areas. *Fungal Biol.* 114: 438-445.

Averbuch D, Boekhoutt T, Falk R, Engelhard D, Shapiro M, Block C, Polacheck I. 2002. Fungemia in a cancer patient caused by fluconazole-resistant *Cryptococcus laurentii. Med Mycol.* 40: 479-484.

Banerjee P, Haider M, Trehan V, Mishra B, Thakur A, Dogra V, Loomba P. 2013. *Cryptococcus laurentii* fungemia. *Indian J Med Microbiol.* 31:75-77.

Bauters TG, Swinne D, Boekhout T, Noens L, Nelis HJ. 2002. Repeated isolation of C*ryptococcus laurentii* from the oropharynx of an immunocompromized patient. *Mycopathologia.* 153: 133-135.

Cheng MF, Chiou CC, Liu YC, Wang HZ, Hsieh KS. 2001. *Cryptococcus laurentii* fungemia in a premature neonate. *J Clin Microbiol*. 39: 1608-1611.

Furman-Kuklińska K, Naumnik B, Myśliwiec M. 2009. Fungaemia due to *Cryptococcus laurentii* as a complication of immunosuppressive therapy--a case report. *Adv Med Sci.* 54: 116-119.

Khawcharoenporn T, Apisarnthanarak A, Mundy LM. 2007. Non-*neoformans* cryptococcal infections: a systematic review. *Infection.* 35: 51-58.

Khawcharoenporn T, Apisarnthanarak A, Kiratisin P, Mundy LM, Bailey TC. 2006. Evaluation of *Cryptococcus laurentii* meningitis in a patient with HIV infection: a case report and review of the literature. *Hawaii Med J.* 65: 260-263.

Kulkarni A, Sinha M, Anandh U. 2012. Primary cutaneous cryptococcosis due to *Cryptococcous laurentii* in a renal transplant recipient. *Saudi J Kidney Dis Transpl*. 23: 102-105.

Manfredi R, Fulgaro C, Sabbatani S, Legnani G, Fasulo G. 2006. Emergence of amphotericin B-resistant *Cryptococcus laurentii* meningoencephalitis shortly after treatment for *Cryptococcus neoformans* meningitis in a patient with AIDS. *AIDS Patient Care STDS*. 20: 227-232.

Molina-Leyva A, Ruiz-Carrascosa JC, Leyva-Garcia A, Husein-Elahmed H. 2013. Cutaneous *Cryptococcus laurentii* infection in an immunocompetent child. *Int J Infect Dis.* 17:e1232-3.

Shankar EM, Kumarasamy N, Bella D, Renuka S, Kownhar H, Suniti S, Rajan R, Rao UA. 2006. Pneumonia and pleural effusion due to *Cryptococcus laurentii* in a clinically proven case of AIDS. *Can Respir J.* 13: 275-278.

Sugita T, Takashima M, Ikeda R, Nakase T, Shinoda T. 2000. Intraspecies diversity of *Cryptococcus laurentii* as revealed by sequences of internal transcribed spacer regions and 28S rRNA gene and taxonomic position of *C. laurentii* clinical isolates. *J Clin Microbiol*. 38: 1468-1471.

Vlchkova-Lashkoska M, Kamberova S, Starova A, Goleva-Mishevska L, Tsatsa-Biljanovska N, Janevska V, Petrovska M. 2004. Cutaneous *Cryptococcus laurentii* infection in a human immunodefi ciency virus-negative subject*. J Eur Acad Dermatol Venereol.* 18: 99-100.

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 January29, 2014 direct antigen detection test event. Three negative (Cn-Ag-2,Cn-Ag-3, and Cn-Ag-4) and two positive serum samples (Cn-Ag-1and Cn-Ag-5) with the titer of 1:32 and 1:8, respectively for cryptococcal antigen were included.

Results: The consensus results for specimensCn-Ag-2,Cn-Ag-3, and Cn-Ag-4were negative, Cn-Ag-1and Cn-Ag-5 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 ~ 1:128 and 1:2 ~ 1:64 for Cn-Ag-1 and Cn-Ag-5 respectively. One laboratory each reported the titer higher or lower than the acceptable range for Cn-Ag-1. All the laboratories reported the titers within the range for Cn-Ag-2.

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

Method		Cn-Ag-1 Titers								
No. laboratories		4	16	20	32	40	64	80	128	256
EIA	2					1	1			
Latex Agglutination	55	1	12		23		17		1	1
Immuno-Mycologics	6		1		1		4			
Meridien Diagnostic	40	1	11		16		10		1	1
Remel	9				6		3			
Lateral Flow Assay	5			1		2		2		
Total	62	1	12	1	23	3	18	2	1	1

Method		Cn-Ag-5 Titers								
No. laboratories		2	4	5	8	10	16	20	32	40
EIA	2									2
Latex Agglutination	55	2	5		21		21		6	
Immuno-Mycologics	6		1		2		3			
Meridien Diagnostic	40	2	3		16		15		4	
Remel	9		1		3		3		2	
Lateral Flow Assay	5			3		1		1		
Total	62	2	5	3	21	1	21	1	6	2

Further Reading:

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

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

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

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

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

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

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

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

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

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

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

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

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

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR YEASTS

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

Materials: *Candida krusei* (S-1) was the analyte in the January 29, 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 latest CLSI and EUCAST documents to score proficiency testing results. However, the participating laboratories are advised to regularly consult these organizations for the latest version of their standard documents.

Comments: Acceptable results were MICs +/-2 dilutions of the reference laboratory results for any single drug. Only 2 of the 32 laboratories participating in this test event tested all 10 antifungal drugs. The reported results were as follows: fluconazole (27 laboratories), itraconazole (27 laboratories), voriconasole (26 laboratories), flucytosine (23 laboratories), caspofungin (20 laboratories), amphotericin B (21 laboratories), micafungin (16 laboratories), anidulafungin (16 laboratories), posacoanazole (15 laboratories), and ketocoanzole (5 laboratories).

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

S-1: *Candida krusei* (M2559)

Drug	No. labs	MIC (µg/ml)													
		0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	256
Amphotericin B	21						4	16	1						
Anidulafungin	16	2	8	4	1	1									
Caspofungin	20				4	16									
Flucytosine (5-FC)	23									11	12				
Fluconazole	24*									1		2	15	4	1
Itraconazole	26*				5	18	2								
Ketoconazole	5*					2	2								
Micafungin	16			7	8	1									
Posaconazole	15				6	9									
Voriconazole	26			1	7	18									

* 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 Vitek II 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 krusei* (M2559)

Drug	No. laboratories	Susceptible	Susceptible-dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	21	6					15
Anidulafungin	16	16					
Caspofungin	20	20					
Flucytosine	23	7		10			6
Fluconazole	27	1	1		19		6
Itraconazole	29	2	17				7
Ketoconazole	5	1	1				3
Micafungin	16	16					
Posaconazole	15	6					9
Voriconazole	26	26					

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 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. Three laboratories used CLSI broth microdilution method while the remaining two used TREK YeastOne Colorimetric method.

Comments: Five 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	5							3	1	1				
Anidulafungin	4		2	1	1									
Caspofungin	4	1		1	1	1								
Fluconazole	4											2	1	1
Itraconazole	5		1			1	1		2					
Ketoconazole	2									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:

Canton E, Peman J, Gobernado M, Alvarez E, Baquero F, Cisterna R, Gil J, Martin-Mazuelos E, Rubio C, Sanchez-Sousa A, Settano C. 2005. Sensititre YeastOne caspofungin susceptibility testing of *Candida* clinical isolates: correlation with results of NCCLS M27-A2 multicenter study. *Antimicrobiol Agents Chemother*. 49: 1604-1607.

Clinical and Laboratory Standards Institute. 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard - Third Edition. CLSI document M27-A3 (ISBN 1-56238-666-2).

Clinical and Laboratory Standards Institute. 2008. Quality Control Minimal Inhibitory Concentration (MIC) Limits for Broth Microdilution and MIC Interpretive Breakpoints; Informational Supplement - Third Edition. CLSI document M27-S3 (ISBN 1-56238-667-0).

Clinical and Laboratory Standards Institute. 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard – Second Edition. CLSI document M38-A2 (1-56238-668-9).

Clinical and Laboratory Standards Institute. 2009. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline – Second Edition. CLSI document M44-A2 (ISBN 1-56238-703-0).

Clinical and Laboratory Standards Institute. 2009. Zone Diameter Interpretive Standards, Corresponding Minimal Inhibitory Concentration (MIC) Interpretive Breakpoints, and Quality Control Limits for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Informational Supplement. CLSI document M44-S3.

Clinical and Laboratory Standards Institute. 2010. Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Approved Guideline. CLSI document M51-A (ISBN 1-56238-725-1).

Clinical and Laboratory Standards Institute. 2010. Performance Standards for Antifungal Disk Diffusion Susceptibility Testing of Filamentous Fungi; Informational Supplement. CLSI document M51-S1 (ISBN 1-56238-725-1).

Clinical and Laboratory Standards Institute. 2012. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Fourth Informational Supplement. CLSI document M27-S4 (ISBN 1-56238-863-0).

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST technical note on fluconazole. *Clin Microbiol Infect.* 14: 193-195.

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST definitive document Edef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. *Clin Microbiol Infect.* 14: 398-405.

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST technical note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia–forming moulds. *Clin Microbiol Infect.* 14: 982-984.

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST technical note on voriconazole. *Clin Microbiol Infect.* 14: 985-987.