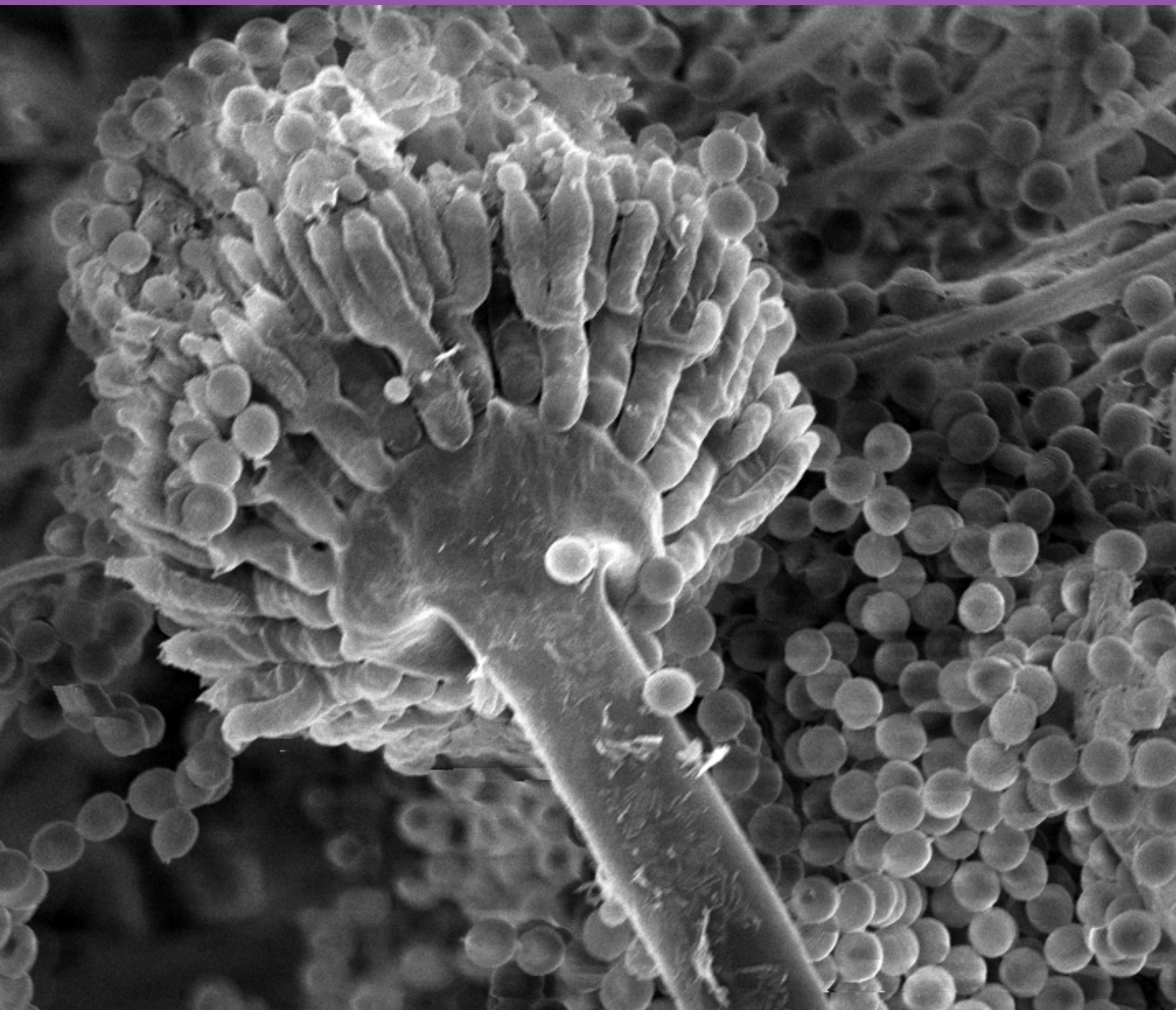


Mycology Proficiency Testing Program



Test Event Critique
September 2013

Wadsworth Center
NEW YORK STATE DEPARTMENT OF HEALTH
Mycology Laboratory

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Mycology Laboratory

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

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

Mycology Laboratory Staff and Contact Details

| Name | Responsibility | Phone | Email |
|-----------------------|---|--------------|--|
| Dr. Vishnu Chaturvedi | Director (on leave of absence) | 518-474-4177 | vishnu@wadsworth.org |
| Dr. Sudha Chaturvedi | Deputy Director | 518-474-4177 | schaturv@wadsworth.org |
| Dr. Ping Ren | PT Program Coordinator | 518-474-4177 | mycologypt@wadsworth.org or renp@wadsworth.org |
| Ms. Xiaojiang Li | Research Scientist (Diagnostic Section) | 518-486-3820 | mycologydiagnostics@wadsworth.org |
| Ms. Tanya Victor | Research Scientist (Molecular Section) | 518-474-4177 | mycologydiagnostics@wadsworth.org |

Mycology Proficiency Testing Program (PTP)

CATEGORY DESCRIPTION

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

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

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

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

Molecular methods: This category is for laboratories that use FDA-approved or lab-developed molecular methods for detection, identification, typing, characterization or determination of drug resistance against fungal pathogens. Laboratories using molecular methods under another Restricted permit category (e.g. Restricted: Antigen detection) or those holding a Comprehensive category permit are exempt from this-category.

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

PROFICIENCY TESTING ANALYTES OFFERED

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

Comprehensive

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

Restricted

Identification Yeast Only

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

Antigen Detection

- Antigen detection of *Cryptococcus neoformans**

Molecular Methods

- No proficiency testing is offered at this time.

TEST SPECIMENS & GRADING POLICY

Test Specimens

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

Grading Policy

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

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

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

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

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

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

TEST ANALYTE MASTER LISTS

Mold Master List

The mold master list is intended to provide guidance to the participating laboratories about the scope of the Mycology (Comprehensive) Proficiency Testing Program. The list includes most common pathogenic and non-pathogenic fungi likely to be encountered in the 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
Microsporium audouinii
Microsporium canis
Microsporium cookei
Microsporium gypseum species complex
Microsporium nanum
Microsporium persicolor
Microsporium species
Mucor circinelloides
Mucor plumbeus
Mucor racemosus
Mucor species
Nigrospora species
Paecilomyces lilacinus
Paecilomyces species
Paecilomyces variotii
Penicillium marneffei
Penicillium species
Phaeoannellomyces werneckii (Hortaea werneckii)
Phialophora richardsiae
Phialophora species
Phialophora verrucosa species complex
Phoma species
Pithomyces species
Pseudallescheria boydii species complex
Pseudallescheria species
Rhizomucor pusillus
Rhizomucor species
Rhizopus oryzae
Rhizopus species
Scedosporium apiospermum (Pseudallescheria apiospermum)
Scedosporium prolificans (inflatum)
Scedosporium species
Scopulariopsis brevicaulis
Scopulariopsis brumptii
Scopulariopsis species
Scytalidium hyalinum
Scytalidium species
Sepedonium species
Sporothrix schenckii species complex
Stachybotrys atra (chartarum / alternans)
Stachybotrys species
Syncephalastrum racemosum
Syncephalastrum species
Trichoderma species
Trichophyton ajelloi
Trichophyton interdigitale
Trichophyton mentagrophytes species complex
Trichophyton rubrum
Trichophyton schoenleinii
Trichophyton species
Trichophyton terrestre
Trichophyton tonsurans
Trichophyton verrucosum
Trichophyton violaceum
Trichothecium species
Ulocladium species
Ustilago species
Verticillium species

Yeast Master List

The yeast master list is intended to provide guidance to the participating laboratories about the scope of the Mycology - Restricted to Yeasts Only Proficiency Testing Program. This list includes most common pathogenic and non-pathogenic yeasts likely to be encountered in the clinical laboratory. The list is compiled from published peer-reviewed reports as well as current practices in other proficiency testing programs. The list is meant to illustrate acceptable identifications used in grading of responses received after each test event. 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 | <i>Aspergillus terreus</i> | <i>Aspergillus terreus</i> | <i>Aspergillus terreus</i> group | 60/60 (100%) |
| M-2 | <i>Beauveria</i> species | <i>Beauveria</i> species | | 59/61 (97%) |
| M-3 | <i>Aureobasidium pullulans</i> | (Not validated) | <i>Aureobasidium</i> species | 16/61 (26%) |
| M-4 | <i>Cunninghamella</i> species | <i>Cunninghamella</i> species | <i>Cunninghamella bertholletiae</i> <i>Cunninghamella elegans</i> | 60/61 (98%) |
| M-5 | <i>Cladosporium</i> species | <i>Cladosporium</i> species | | 59/61 (97%) |

Mycology – Yeast Only

| | Specimen key | Validated specimen | Other acceptable answers | Laboratories with correct responses / Total laboratories (% correct responses) |
|-----|-----------------------------|-----------------------------|---|--|
| Y-1 | <i>Rhodotorula minuta</i> | <i>Rhodotorula minuta</i> | | 46/49 (94%) |
| Y-2 | <i>Candida parapsilosis</i> | <i>Candida parapsilosis</i> | <i>Candida parapsilosis</i> species complex | 55/55 (100%) |
| Y-3 | <i>Candida glabrata</i> | <i>Candida glabrata</i> | | 55/55 (100%) |
| Y-4 | <i>Candida albicans</i> | <i>Candida albicans</i> | | 55/55 (100%) |
| Y-5 | <i>Candida tropicalis</i> | <i>Candida tropicalis</i> | | 54/55 (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 (1:8) | (Not validated) | 1:2 – 1:32 | 50/67 (75%) | 48/48 (100%) |
| Cn-Ag-2 | Positive (1:512) | Positive (1:512) | 1:64 – 1:2048 | 67/67 (100%) | 60/62 (97%) |
| Cn-Ag-3 | Negative | Negative | | 67/67 (100%) | NA |
| Cn-Ag-4 | Positive (1:256) | Positive (1:256) | 1:32 – 1:1024 | 67/68 (99%) | 60/62 (97%) |
| Cn-Ag-5 | Negative | Negative | | 66/67 (99%) | NA |

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

| Drugs | Acceptable MIC (µg/ml) Range | Acceptable interpretation | Laboratories with acceptable responses/ Total laboratories (% correct responses) |
|--------------------|-------------------------------------|------------------------------------|---|
| Amphotericin B | 0.25 – 1 | Susceptible / No interpretation | 21/21 (100%) |
| Anidulafungin | 0.25 – 1 | Susceptible | 16/16 (100%) |
| Caspofungin | 0.125 – 1 | Susceptible | 22/22 (100%) |
| Flucytosine (5-FC) | 0.03 – 0.125 | Susceptible / No interpretation | 25/25 (100%) |
| Fluconazole | 0.5 – 4 | Susceptible | 32/32 (100%) |
| Itraconazole | 0.03 – 0.125 | Susceptible / No interpretation | 29/29 (100%) |
| Ketoconazole | 0.03 – 0.125 | Susceptible / No interpretation | 5/5 (100%) |
| Micafungin | 0.5 – 1 | Susceptible | 17/17 (100%) |
| Posaconazole | 0.03 – 0.125 | Susceptible / No interpretation | 15/15 (100%) |
| Voriconazole | 0.008 – 0.125 | Susceptible | 27/27 (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 | 28 |
| | |
| Antifungal Susceptibility* | |
| Disk diffusion | 1 |
| Etest | 1 |
| Vitek II | 1 |
| YeastOne – Mold | 2 |
| YeastOne – Yeast | 26 |
| 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 | 7 |
| Meridien BioScience Cryptococcal Antigen Latex Agglutination System (CALAS) | 44 |
| Meridien BioScience Premier Cryptococcal Antigen Detection (EIA) | 3 |
| Remel Cryptococcal Antigen Latex Test | 9 |

*Include multiple systems used by some laboratories

MOLD DESCRIPTIONS

M-1 *Aspergillus terreus*

Source: Sputum / Abscess / Nail

Clinical Significance: *Aspergillus terreus* is an uncommon pathogen that causes allergic broncho-pulmonary aspergillosis or invasive aspergillosis. It is also reported from cutaneous, ophthalmic, pulmonary, and disseminated infections. Keratitis, arthritis, spondylodiscitis, and suppurative otitis are also reported to be caused by *A. terreus*. *Aspergillus terreus*, an emerging opportunistic fungal pathogen, is among very few fungi that are reported to exhibit intrinsic resistance to amphotericin B.

Colony: *A. terreus* colonies grow rapidly, cinnamon to brown, powdery on Sabouraud's dextrose agar at 25°C (Figure 1).

Microscopy: Lactophenol – Lactophenol cotton blue mount shows hyaline hyphae with solitary aleurioconidia attached and phialides biserial and conidial head in the form of compact column. Conidia are in chains, round, and smooth walled (Figure 1).

Differentiation: *A. terreus* forms cinnamon to brown colony, which differentiates it from *A. fumigatus* (dark greenish to gray colony), *A. niger* (black colony), *A. flavus* (yellow to green colony), *A. versicolor* (yellow, tan, to green colored colony) *A. nidulans* (usually green colony), *A. glaucus* (green colony), and *A. clavatus* (blue-green colony). *A. terreus* phialides are biserial, but *A. fumigatus*, *A. glaucus*, and *A. clavatus* are uniserial. *A. flavus* phialides are both uniserial and biserial. Cleistothecia are usually present in *A. nidulans* and *A. glaucus*, but not in *A. terreus*. Conidial head is loosely radiate and covers most of vesicle for *A. versicolor*, but it is more compact and phialides limited mainly to the upper part of the vesicle in *A. terreus*. Please refer to Table 1 for details.

Molecular test: Molecular typing with the random amplified polymorphic DNA (RAPD) technique can be used to differentiate various *A. terreus* genotypes. Nested PCR with a mixture of specific primers to DNA topoisomerase II gene was also reported to identify pathogenic *Aspergillus* species including *A. terreus*.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *A. terreus* ATCC 20542 isolate (GenBank accession no. AJ001338).

Antifungal susceptibility: *A. terreus* is resistant to fluconazole and 5 fluorocytosine (5 FC) but susceptible to itraconazole, ketoconazole, and caspofungin.

Participant performance:

| | |
|---------------------------------------|----|
| Referee Laboratories with correct ID: | 10 |
| Laboratories with correct ID: | 59 |
| Laboratories with incorrect ID: | 0 |

Illustrations:

Figure 1. Seven-day-old, pale yellow colony of *Aspergillus terreus* on Sabouraud's dextrose agar, the reverse of colony appears yellow to brown with age (upper panel). Microscopic morphology of *Aspergillus terreus* showing hyaline hyphae with solitary aleurioconidia attached (Lower left panel, bar = 25 nm) and typical compact column formed conidial head with biseriate phialides and round, smooth conidia (lower right panel, bar = 50 nm).

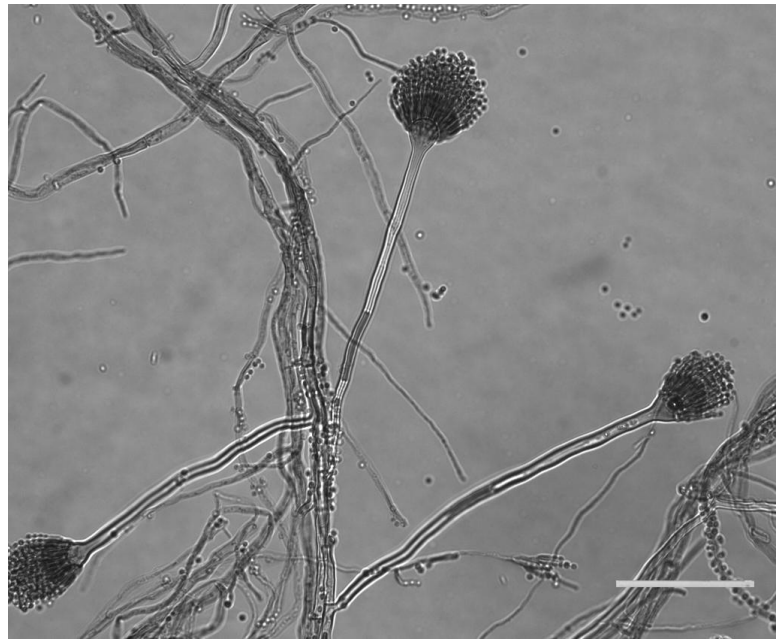
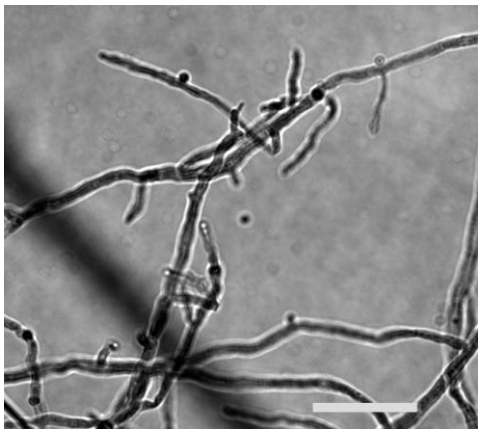
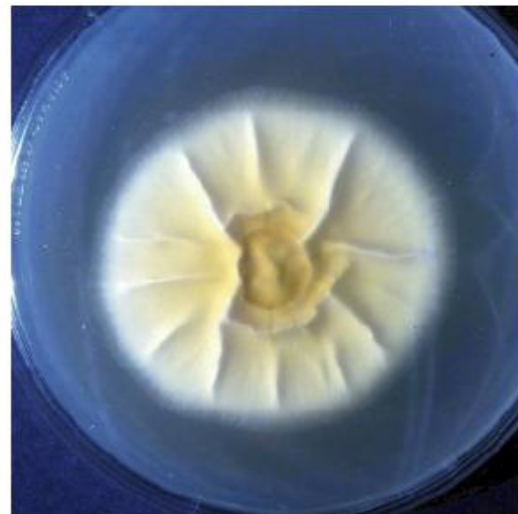
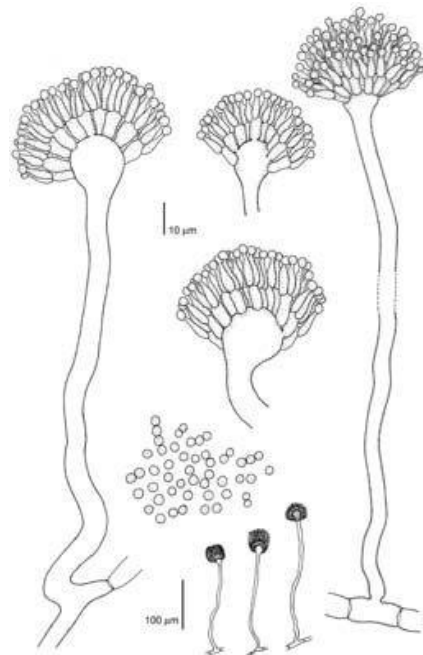
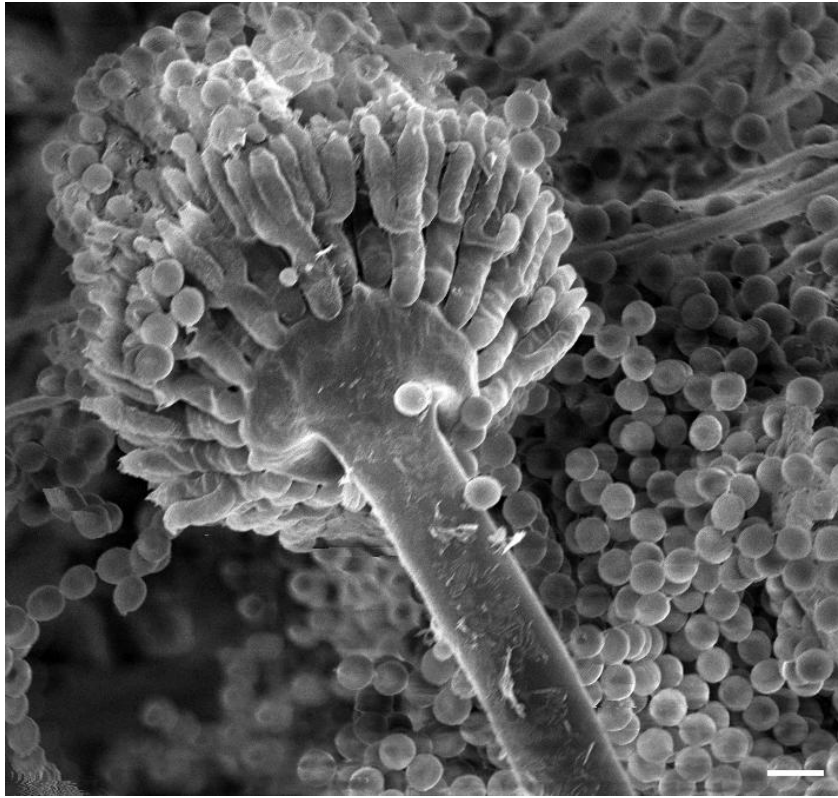


Figure 1A. Scanning electron micrograph of *Aspergillus terreus* (bar = 3 μ m, upper panel). Line drawings of *A. terreus* (lower panel).






























<http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=3690>

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

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

M-2 *Beauveria* species

Source: Sputum / Bronchial Wash / Eye

Clinical significance: *Beauveria* sp. is a very rare human pathogen, which may be associated with keratitis. Pneumonia in an immunocompromised patient due to *Beauveria* has also been reported.

Colony: *Beauveria* sp. grows moderately fast. Colonies are cottony, white to yellowish white with white to pale reverse on Sabouraud's dextrose agar (Figure 2).

Microscopy: Lactophenol cotton blue mount shows hyaline and septate hyphae. Conidiophore is flask-shaped with inflation at the base and narrow zigzagging filaments at the apex. The conidiogenous cells tend to form dense clusters. The unicellular, round to oval conidia are produced from each bending point called sympodial geniculate growth (Figure 2).

Differentiation: *Beauveria* species can be differentiated from other fungal species by its zigzagging conidiogenous formation.

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

The ribosomal ITS2 region of the test isolate showed 100% nucleotide identity with *Beauveria bassiana* isolate IMI 382723 (GenBank accession no. AJ560692.1).

Antifungal susceptibility: *Beauveria* species are generally susceptible to amphotericin B and itraconazole.

Participant performance:

| | |
|---------------------------------------|-----|
| Referee Laboratories with correct ID: | 10 |
| Laboratories with correct ID: | 59 |
| Laboratories with incorrect ID: | 02 |
| (<i>Gliocladium</i> species) | (1) |
| (<i>Malbranchea</i> species) | (1) |

Illustrations:

Figure 2. Seven-day-old, white, cotton textured colony of *Beauveria* sp. on Sabouraud's dextrose agar (upper panels). Microscopic morphology of *Beauveria* species showing the zigzag conidiogenous cell (lower panel).

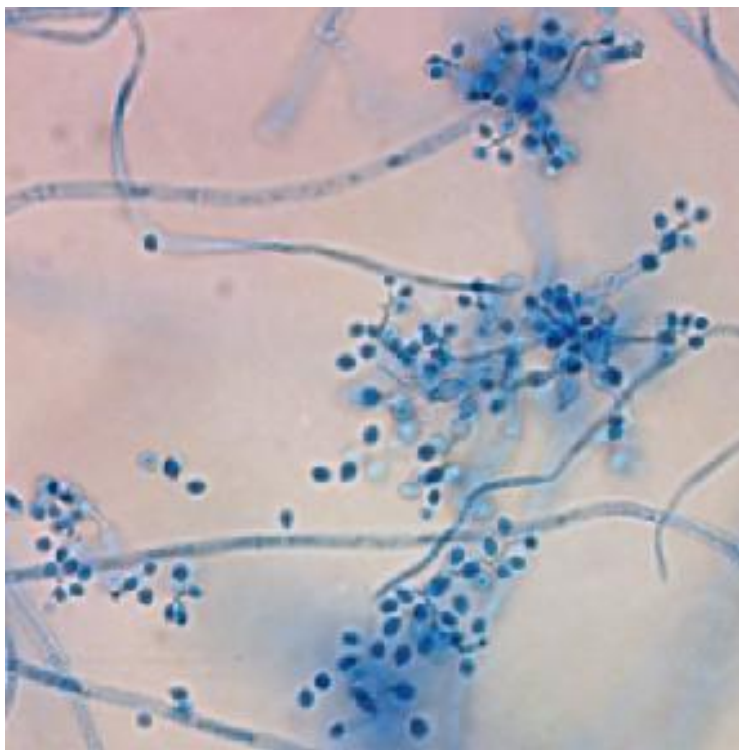
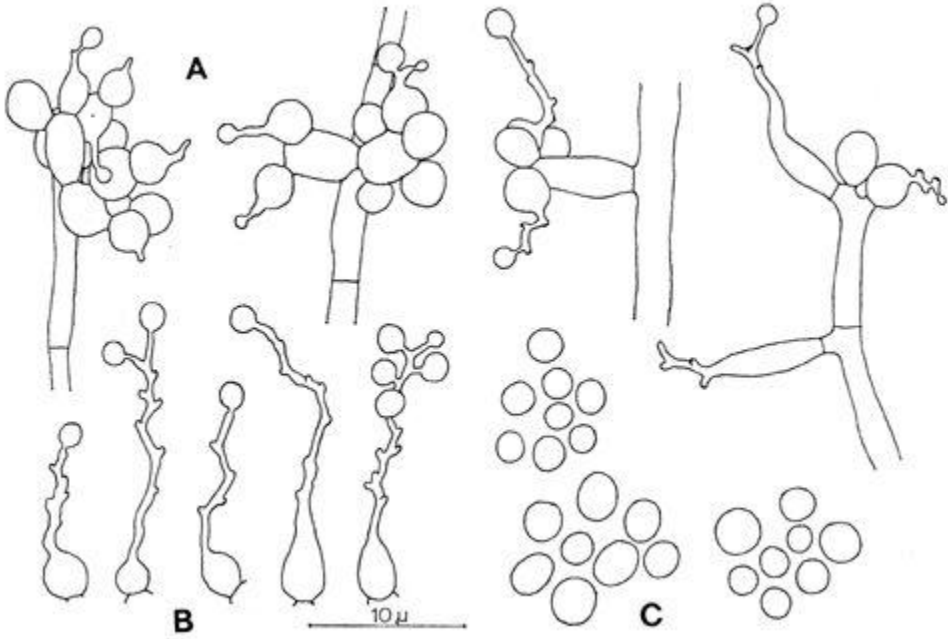
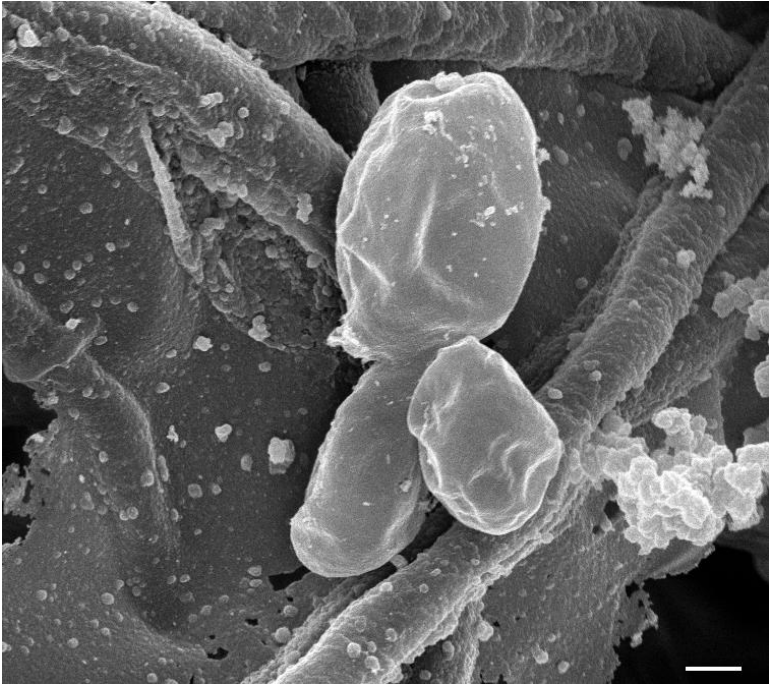


Figure 2A. Scanning electron micrograph of conidia of *Beauveria* species on Sabouraud's dextrose agar (bar = 1 μ m, upper panel). Line drawings of *Beauveria* species (lower panel).



<http://www.mycobank.org/BioMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=1805>

Further reading:

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Tu EY, Park AJ. 2007. Recalcitrant *Beauveria bassiana* keratitis: confocal microscopy findings and treatment with posaconazole (Noxafil). *Cornea.* 26: 1008-1010.

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M-3 *Aureobasidium pullulans*

Source: Blood / Skin / Bronchial Wash

Clinical significance: *Aureobasidium pullulans* is a black-yeast like fungus, which occasionally causes phaeohyphomycosis, keratomycosis, pulmonary mycosis, and cutaneous mycoses.

Colony: Generally *A. pullulans* grows fast on Sabouraud's dextrose agar, white colony turning black with age, mucoid or slimy texture (Figure 3).

Microscopy: Lactophenol cotton blue mount shows mostly hyaline septate hyphae. Blastoconidia develops synchronously (i.e. conidia formed simultaneously from separate fertile points on hyphal tips), producing clusters of conidia (Figure 3). Dark arthroconidia and chlamydoconidia also appears with age.

Differentiation: *A. pullulans* is differentiated from other black-yeast like fungi by its rapid growth, white or pink colonies, turning black with age, blastoconidia produced synchronously in tufts and formation of dark chlamydospores and arthroconidia. *Hormonema dematioides* is also a black yeast-like fungus that produces blastoconidia successively from a single opening, unlike synchronous blastoconidia formation by *A. pullulans*. *Aureobasidium pullulans* is methyl- α -D-glucoside assimilation positive, which can be tested with the API 20C AUX panel, and used for differentiation between *A. pullulans* and *H. dematioides*. *Phaeoannellomyces werneckii*, another black yeast, is currently called *Hortaea werneckii* that forms dark colonies, annelloconidia sympodially or percurrently from undifferentiated conidiogenous cells, and it does not grow on methyl- α -D-glucoside.

Molecular test: Analysis of genes coding for small subunit rRNA sequences of dematiaceous fungal pathogens provided means of accurate identification. An oligonucleotide probe for *A. pullulans* was developed based on the small subunit rRNA gene for identification from leaf surfaces and other microbial communities. The nuclear subunit rRNA genes of various black molds were amplified by PCR and directly sequenced. RAPD technique was used to discriminate among the strains of *A. pullulans* isolated from rocks and other habitats.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Aureobasidium pullulans* Y126 (Genebank accession number: KC161971.1).

Antifungal susceptibility: Susceptibility testing studies suggested that *A. pullulans* is susceptible to amphotericin B, flucytosine, itraconazole, and ketoconazole, but less susceptible to fluconazole.

Participant performance:

| | |
|---------------------------------------|------|
| Referee Laboratories with correct ID: | 02 |
| Laboratories with correct ID: | 16 |
| Laboratories with incorrect ID: | 44 |
| (<i>Hormonema dematioides</i>) | (36) |
| (<i>Arthrographis</i> species) | (1) |

| | |
|---|-----|
| (<i>Cunninghamella</i> species) | (1) |
| (<i>Emmonsia parva</i>) | (1) |
| (<i>Phoma</i> species) | (1) |
| (<i>Pseudallescheria boydii</i> species complex) | (1) |
| (<i>Scytalidium hyalinum</i>) | (1) |
| (<i>Sporothrix schenckii</i> species complex) | (1) |
| (<i>Trichophyton</i> species) | (1) |

Illustrations:

Figure 3. *Aureobasidium pullulans* colony is white to black, texture mucoid on Sabouraud's dextrose agar (upper panel). Microscopic morphology of *Aureobasidium pullulans* showing hyphae and blastoconidia, produced synchronously, dark arthroconidia and chlamydoconidia appearing with age (lower panel).

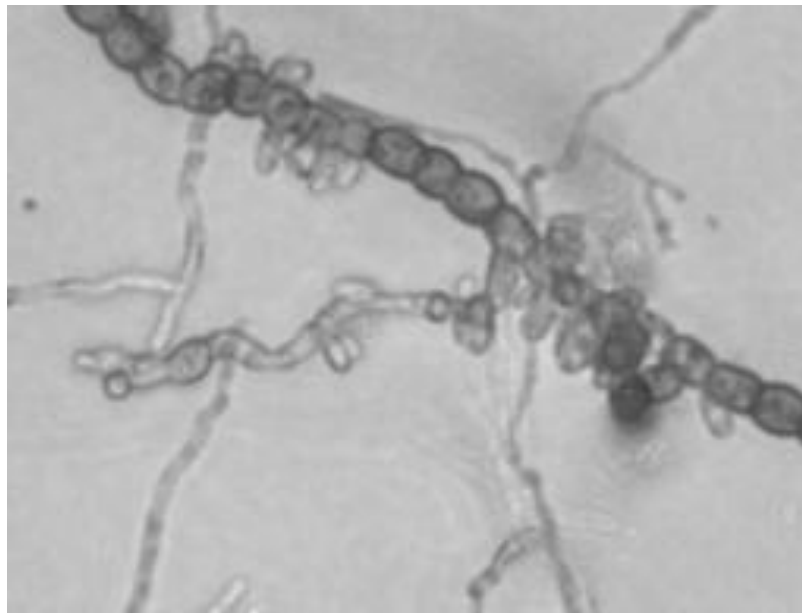
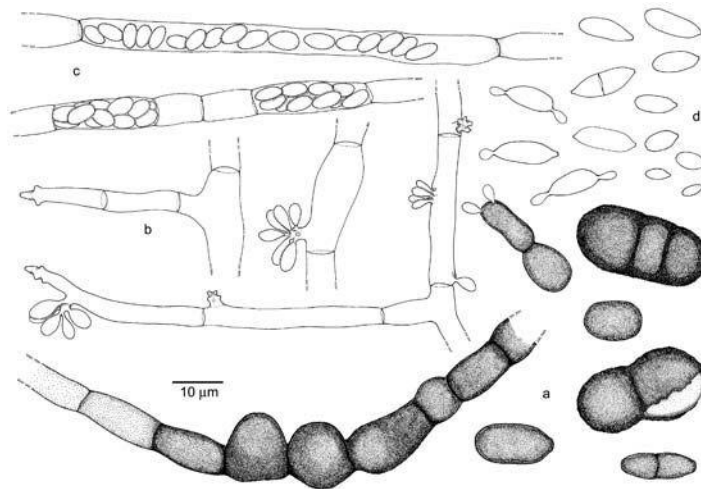
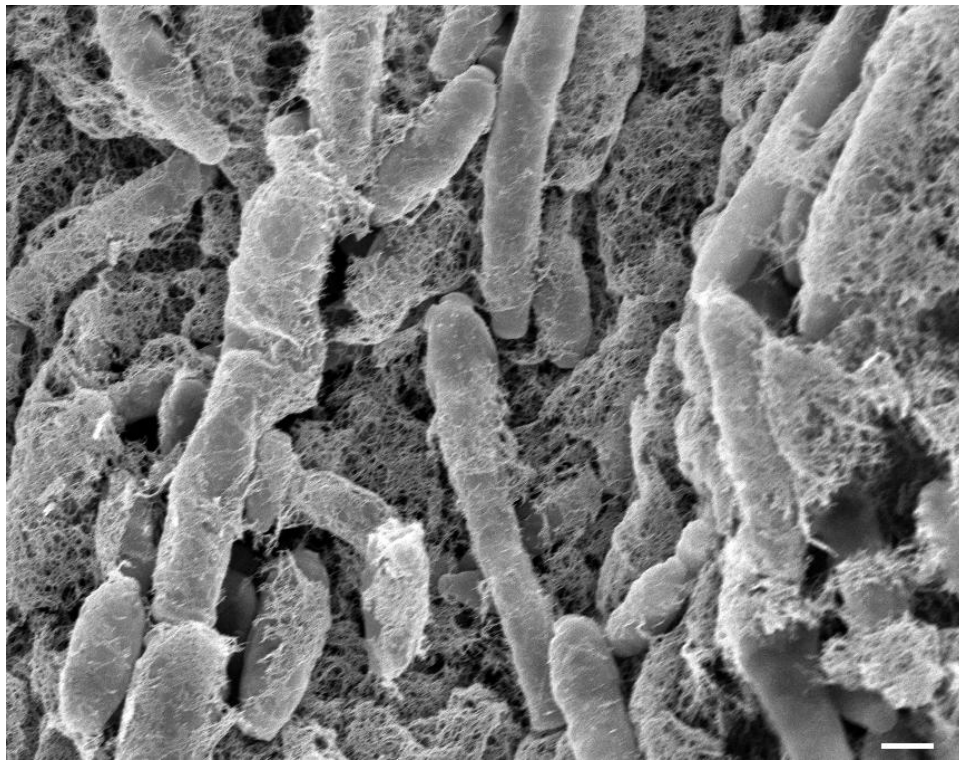


Figure 3A. Scanning electron micrograph of *Aureobasidium pullulans* (bar = 1 μm ; upper panel). Line drawing depicting details of *Aureobasidium pullulans* (lower panel).



<http://www.mycobank.org/BioLomics.aspx?Link=T&TargetKey=14682616000002126&Rec=3701>

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M-4 *Cunninghamella* species

Source: Sputum / Toenail / Blood

Clinical significance: *Cunninghamella* sp. is occasionally reported as a causal agent of pulmonary zygomycosis or as an agent of nosocomial mycosis.

Colony: *Cunninghamella* sp. grows relatively rapidly. Colony is white to gray with cottony texture, pale or buff on reverse on Sabouraud's dextrose agar (Figure 4).

Microscopy: Lactophenol cotton blue mount shows broad, hyaline, and aseptate hyphae. Sporangiohores are branched with swollen vesicles at the end. Vesicles are covered with single-spored sporangioles supported by short denticles projecting from the vesicle (Figure 4).

Differentiation: *Cunninghamella* sp. is distinct from other common Zygomycetes or mucorales fungi by their single-spored sporangia formed on denticles on the vesicle surface.

Molecular test: *Cunninghamella* spp. can be identified by means of panfungal polymerase chain reaction (PCR), direct DNA sequencing of the PCR products, and homology search with nucleotide basic local alignment search tool.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Cunninghamella elegans* isolate 19-M-1 (Genbank accession number: EU076936).

Antifungal susceptibility: *Cunninghamella* sp. is susceptible to amphotericin B, posaconazole, and itraconazole, but resistant to ketoconazole and miconazole.

Participant performance:

| | |
|---------------------------------------|-----|
| Referee Laboratories with correct ID: | 10 |
| Laboratories with correct ID: | 60 |
| Laboratories with incorrect ID: | 01 |
| (<i>Aureobasidium pullulan</i>) | (1) |

Illustrations:

Figure 4. White to gray cottony texture colony of *Cunninghamella* sp. on Sabouraud's dextrose agar (upper panel). Microscopic morphology of *Cunninghamella* sp. showing broad, hyaline, and aseptate hyphae. Sporangiohores branched and end with a swollen vesicle covered with single-spored sporangioles (lower panel)

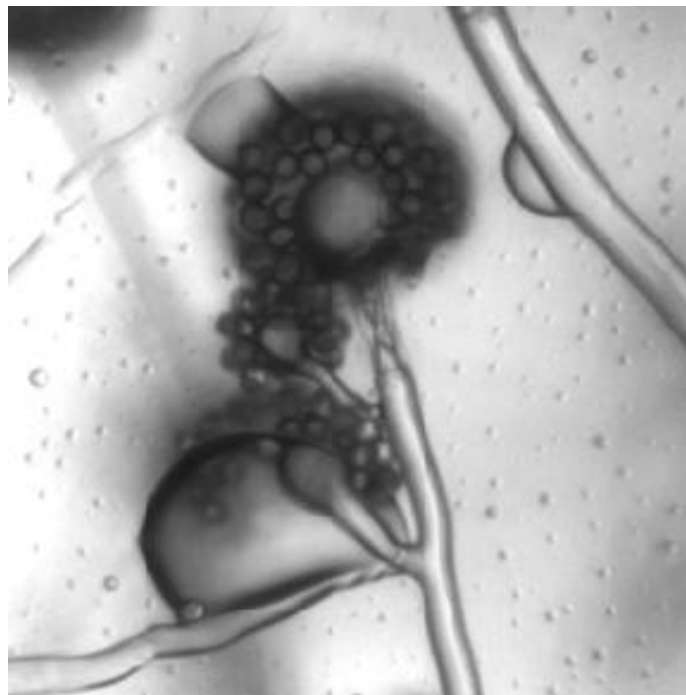
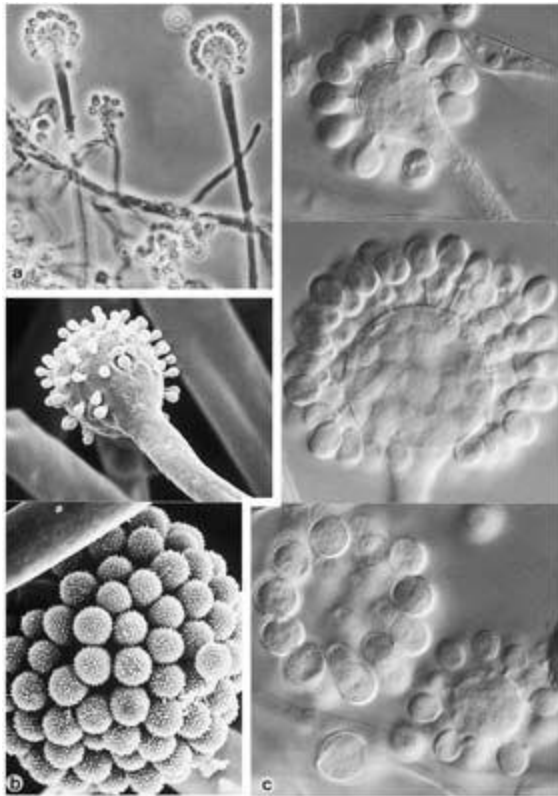
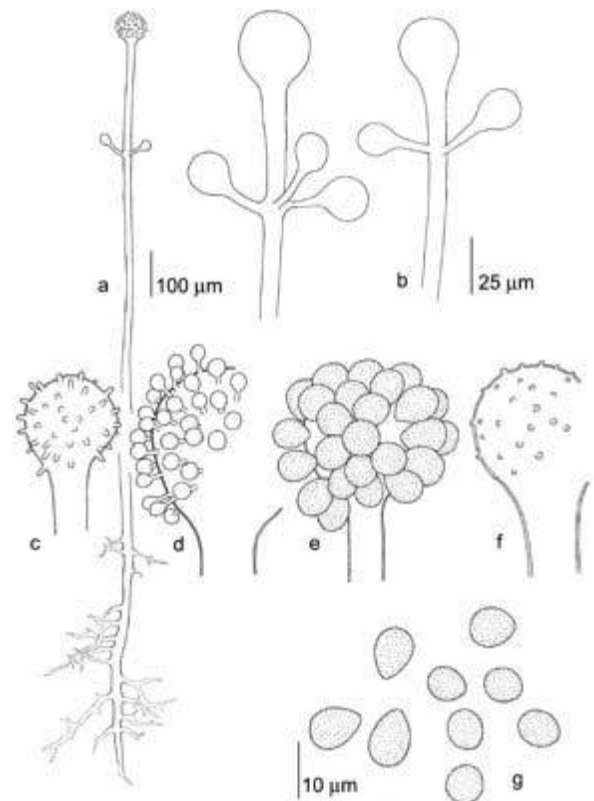


Figure 4A. Light microscopic and scanning electron micrograph of *Cunninghamella* species (upper panel). Line drawing depicting details of *Cunninghamella* species (lower panel).



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<http://www.mycobank.org/BioMICS.aspx?Link=T&TargetKey=1468261600002126&Rec=3556>

Further reading:

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M-5 *Cladosporium* species

Source: Tissue / Scalp / Nail

Clinical significance: *Cladosporium* sp. is a common airborne mold, but rarely causes human disease such as allergic fungal sinusitis. This fungus has also been implicated in cutaneous infection.

Colony: *Cladosporium* sp. grows rapidly with grayish green, powdery or velvety surface and greenish-black to brownish-black reverse on Sabouraud's dextrose agar (Figure 5).

Microscopy: Lactophenol cotton blue mount shows conidia often in long, branched chains with variable size. Conidia are unicellular and ellipsoidal to round at the tip. Prominent scars are visible at the points of attachment (Figure 5).

Differentiation: Generally, *Cladosporium* species have longer chains of conidia than *Fonsecaea* spp. and have small dark scars of attachment. On the contrary, the distal end of the conidiaophore of *Fonsecaea* develops swollen denticles that bore primary single-celled ovoid conidia. *Xylohypha bantiana* is differentiated from *Cladosporium* by its lack of disjuncture scars on conidia.

Molecular test: Restriction fragment length polymorphisms (RFLP) of the ribosomal small subunit gene and internal transcribed spacer (ITS) regions were used to distinguish *Cladosporium* species from other closely related molds such as *Fonsecaea*, *Phialophora*, and *Rhinochadiella* spp.

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with *Cladosporium cladosporioides* strain CBS 169.54 (Genebank accession number: AJ300335.1).

Antifungal susceptibility: *Cladosporium* species are generally susceptible to fluconazole.

Participant performance:

| | |
|---------------------------------------|-----|
| Referee Laboratories with correct ID: | 10 |
| Laboratories with correct ID: | 59 |
| Laboratories with incorrect ID: | 02 |
| (<i>Cladophialophora</i> species) | (2) |

Illustrations:

Figure 5. Grayish green colony of *Cladosporium* species on Sabouraud's dextrose agar (upper panel). Microscopic morphology of *Cladosporium* species showing conidia in long, branched chains with variable size with the scars at the points of attachment of conidia (lower panel).

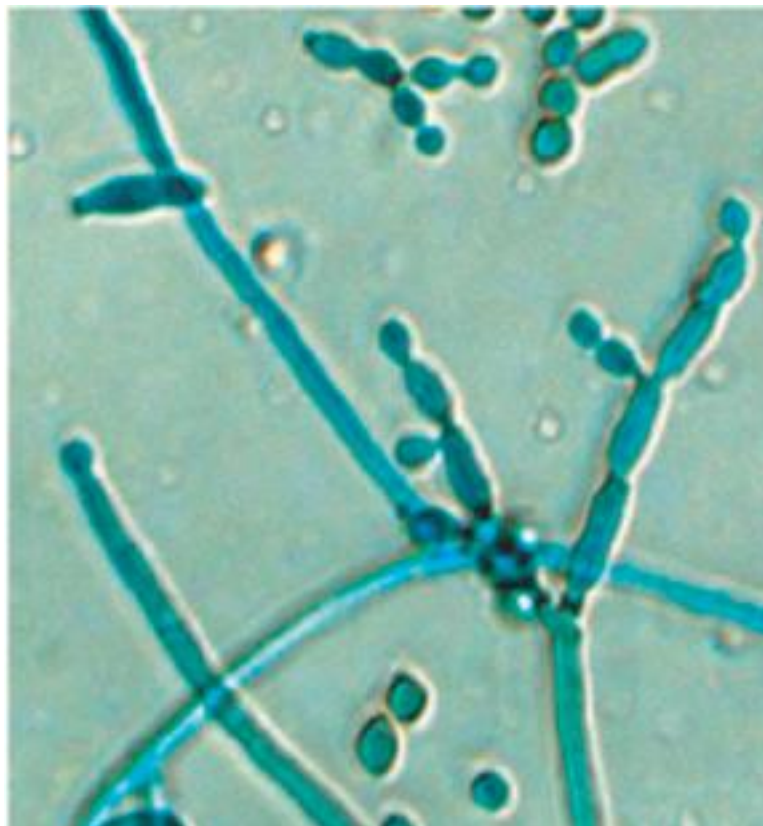
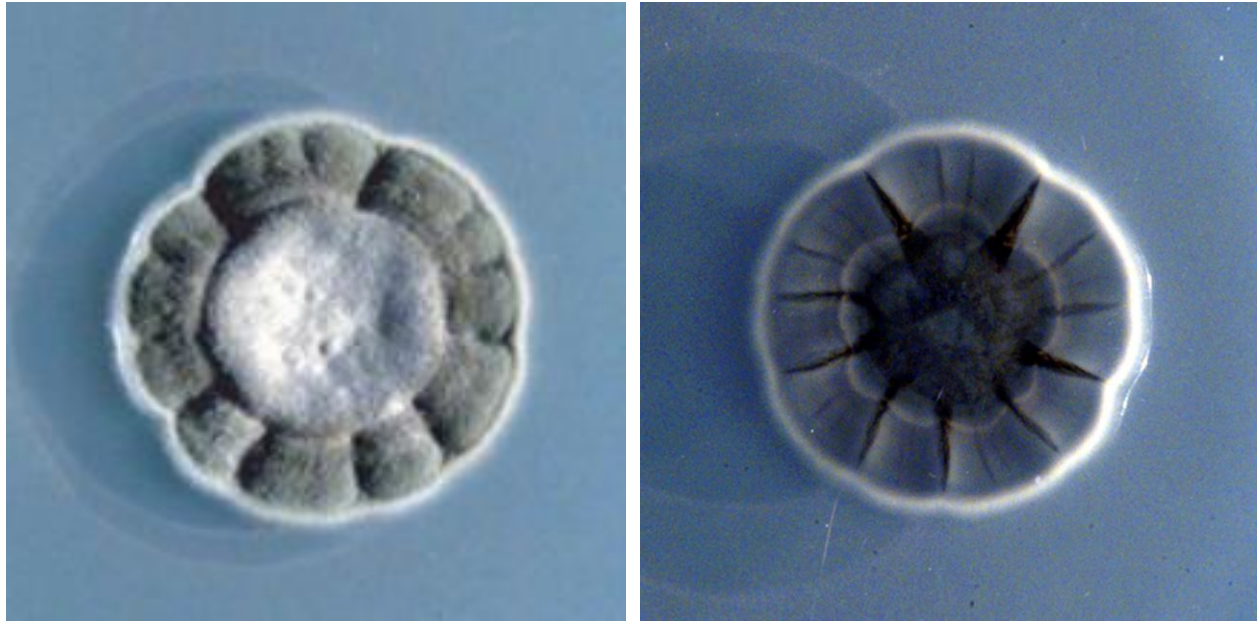
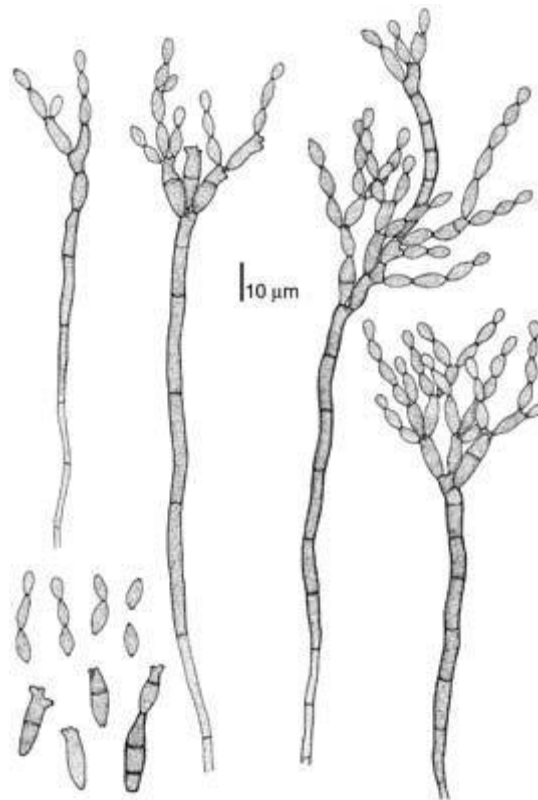
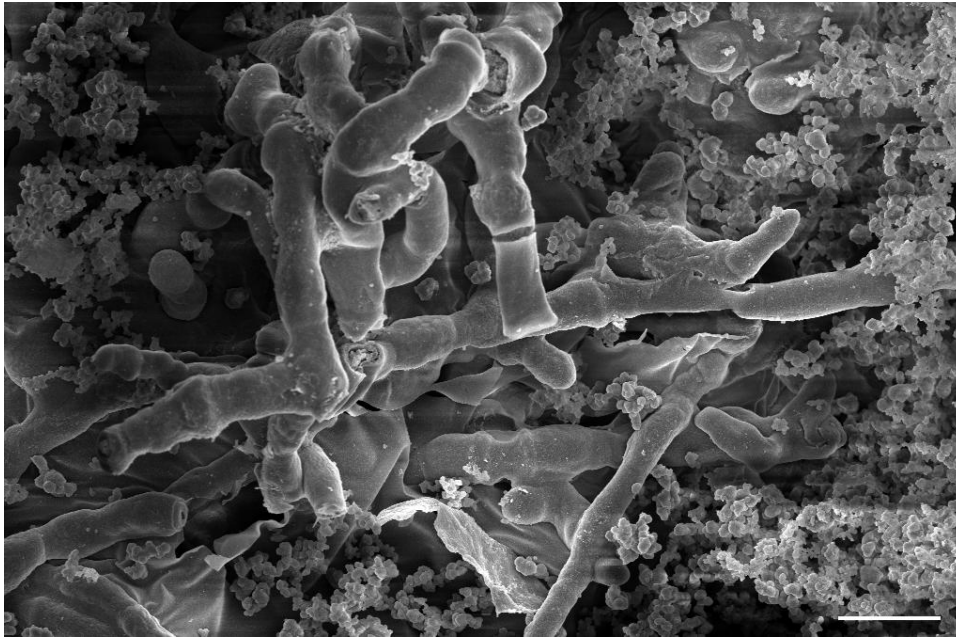


Figure 5A. Scanning electron micrograph of *Cladosporium* species (bar = 10 μm ; upper panel). Line drawing depicting details of *Cladosporium* species (lower panel).



<http://www.mycobank.org/BioloMICS.aspx?Link=T&TargetKey=14682616000002126&Rec=3744>

Further reading:

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- Kantarcioglu AS, Yucel A, De Hoog GS. 2002. Case report. Isolation of *Cladosporium cladosporioides* from cerebrospinal fluid. *Mycoses* 45: 500-503.
- Matsuwaki Y, Nakajima T, Iida M, Nohara O, Haruna S, Moriyama H. 2001. A case report of allergic fungal sinusitis caused by *Penicillium* sp. and *Cladosporium* sp. *Nippon Jibiinkoka Gakkai Kaiho* 104: 1147-1150.
- Sang H, Zheng XE, Zhou WQ, He W, Lv GX, Shen YN, Kong QT, Liu WD. 2012. A case of subcutaneous phaeohyphomycosis caused by *Cladosporium cladosporioides* and its treatment. *Mycoses.* 55: 195-197.
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YEAST DESCRIPTIONS

Y-1 *Rhodotorula minuta*

Source: Urine / Catheter / Sputum

Clinical significance: *Rhodotorula minuta* is reported as a rare/unusual causative agent of systemic infections in patients with AIDS and leukemia. It is isolated from blood, sputum, throat swabs, and feces.

Colony: *R. minuta* colony is pink, smooth, and soft on Sabouraud's dextrose agar at 25°C (Figure 6).

Microscopy: *R. minuta* does not produce pseudohyphae, round blastoconidia are seen on corn meal agar with Tween 80 (Figure 6).

Differentiation: *R. minuta* does not assimilate maltose, which differentiates it from *R. glutinis* and *R. mucilaginosa*.

Molecular test: ITS sequences information is available to be used for molecular identification.

The ribosomal ITS1 region of the test isolate showed 100% nucleotide identity with *Rhodotorula minuta* (synonyms: *Rhodotorula slooffii*) CBS 5706 (Genbank accession number: AF444627).

Antifungal susceptibility: *R. minuta* was susceptible to amphotericin B, 5-flucytosine, and itraconazole, but resistant to fluconazole.

Participant performance:

| | |
|---------------------------------------|-----|
| Referee Laboratories with correct ID: | 10 |
| Laboratories with correct ID: | 46 |
| Laboratories with incorrect ID: | 03 |
| (<i>Rhodotorula mucilaginosa</i>) | (3) |

Illustrations:

Figure 6. *Rhodotorula minuta*, colony smooth, soft, pink on Sabouraud's dextrose agar, 25°C (left panel). Microscopic morphology on corn meal agar with Tween 80, showing oval to round blastoconidia (right panel).

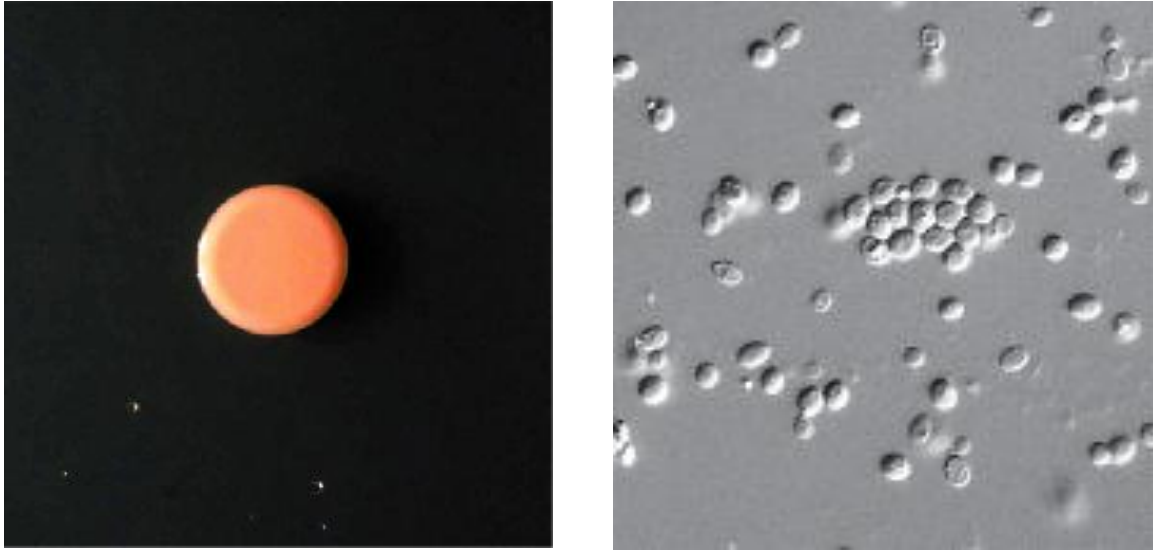
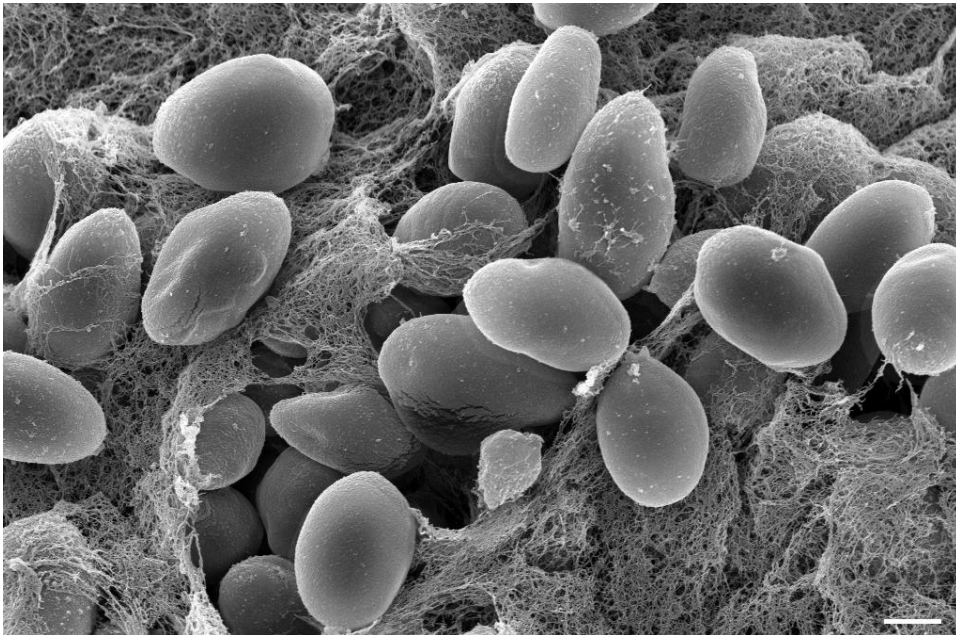


Figure 6A. Scanning electron micrograph of *Rhodotorula minuta* illustrates blastoconidia (bar = 1 μm).



Further reading:

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Y-2 *Candida parapsilosis*

Source: Body fluid / Urine / Stool

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

Colony: *Candida parapsilosis* colony is white to cream, dull with smooth surface on Sabouraud's dextrose agar after 5 days at 25°C (Figure 7).

Microscopy: *Candida parapsilosis* shows long, multibranched pseudohyphae, together with small elongated blastoconidia on corn meal agar with Tween 80 (Figure 7).

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

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

The ribosomal ITS1 and ITS2 regions of the test isolate showed 100% nucleotide identity with a reference strain of *Candida parapsilosis* CBS 604 (Genebank accession no: AY391843).

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

Participant performance:

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

Illustrations:

Figure 7. *Candida parapsilosis* white to cream, smooth colony on Sabouraud's dextrose agar, 25°C. Microscopic morphology of *Candida parapsilosis* with long, multibranched pseudohyphae together with small cluster of elongated blastoconidia on Corn meal agar with Tween 80 (bar = 5 µm).

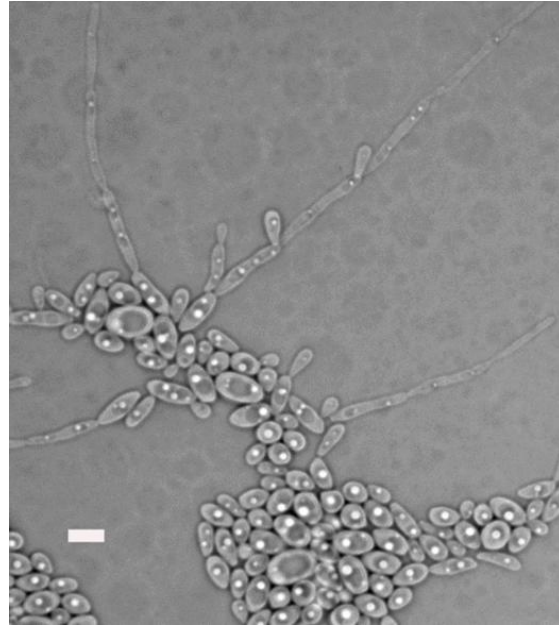
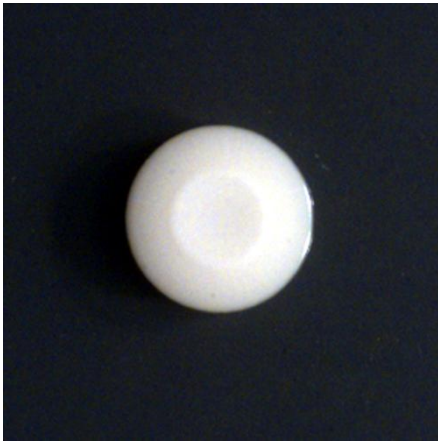
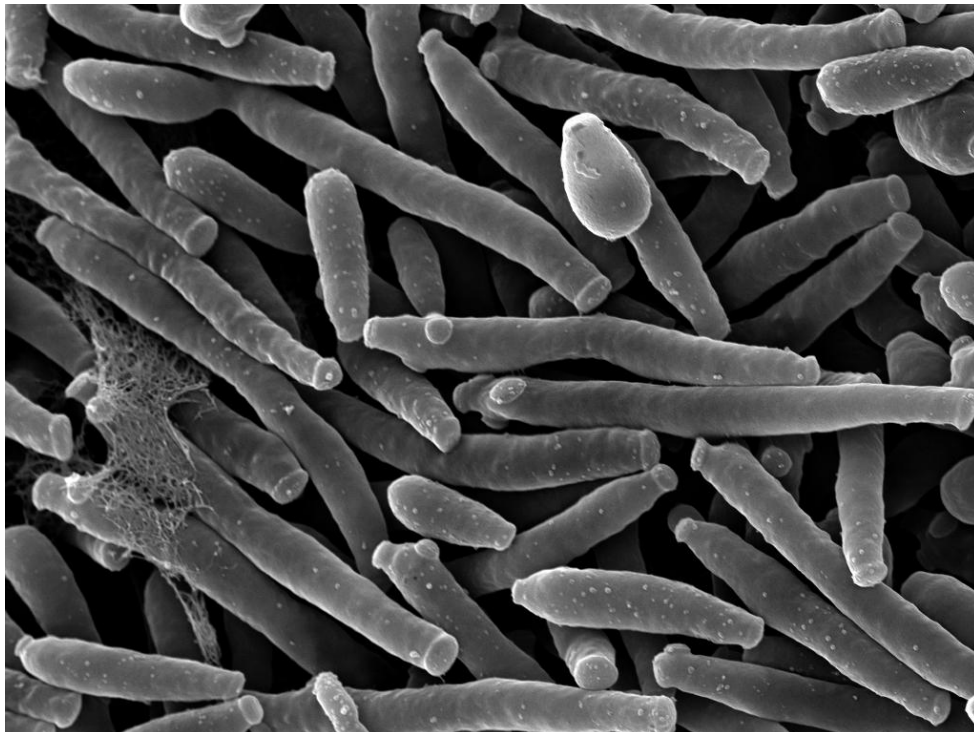


Figure 7A. Scanning electron micrograph with pseudohyphae and blastoconidia.



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Y-3 *Candida glabrata*

Source: Urine / Blood / Nail

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

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

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

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

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

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

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

Participant performance:

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

Illustrations:

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

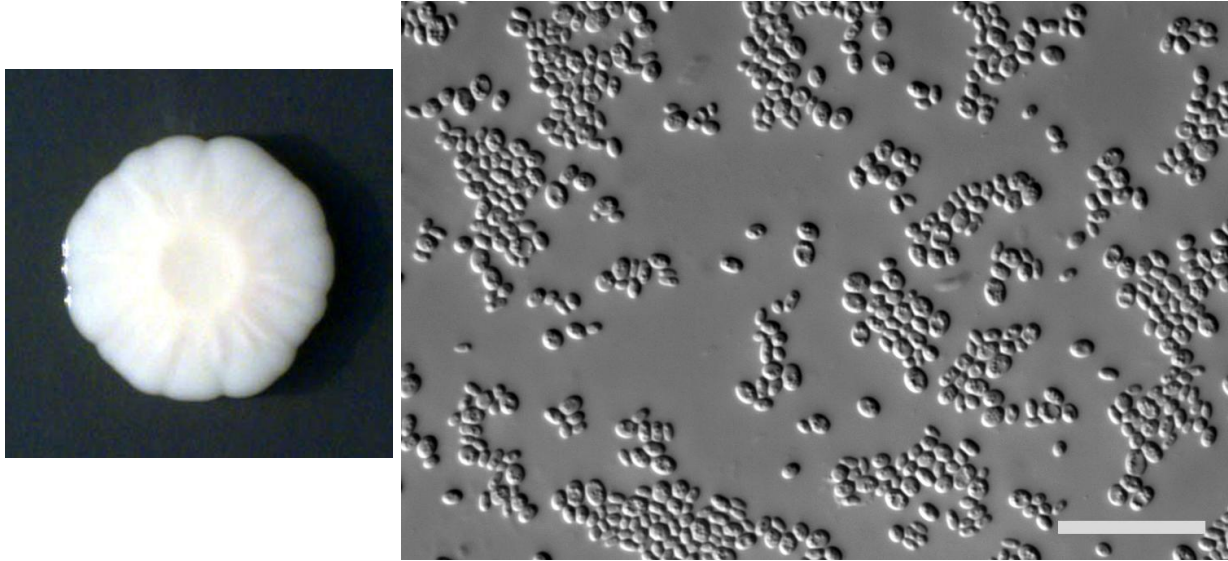
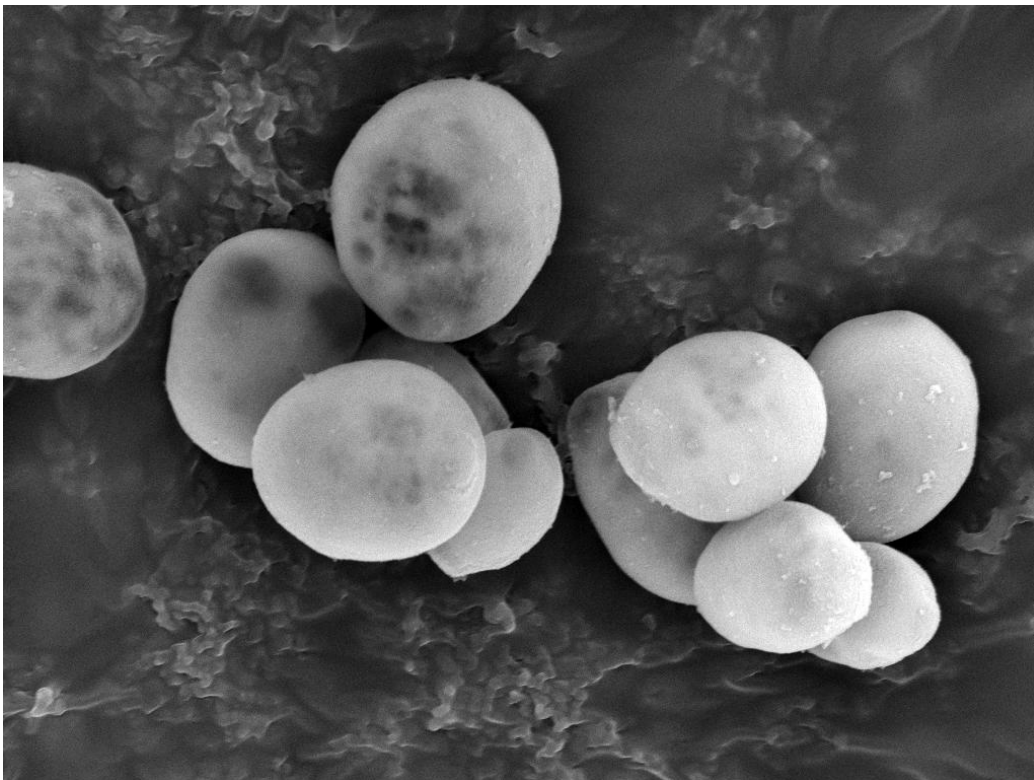


Figure 8A. Scanning electron micrograph with blastoconidia of *Candida glabrata*.



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Y-4 *Candida albicans*

Source: Sputum / Urine / Stool / Tissue

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

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

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

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

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

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

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

Participant performance:

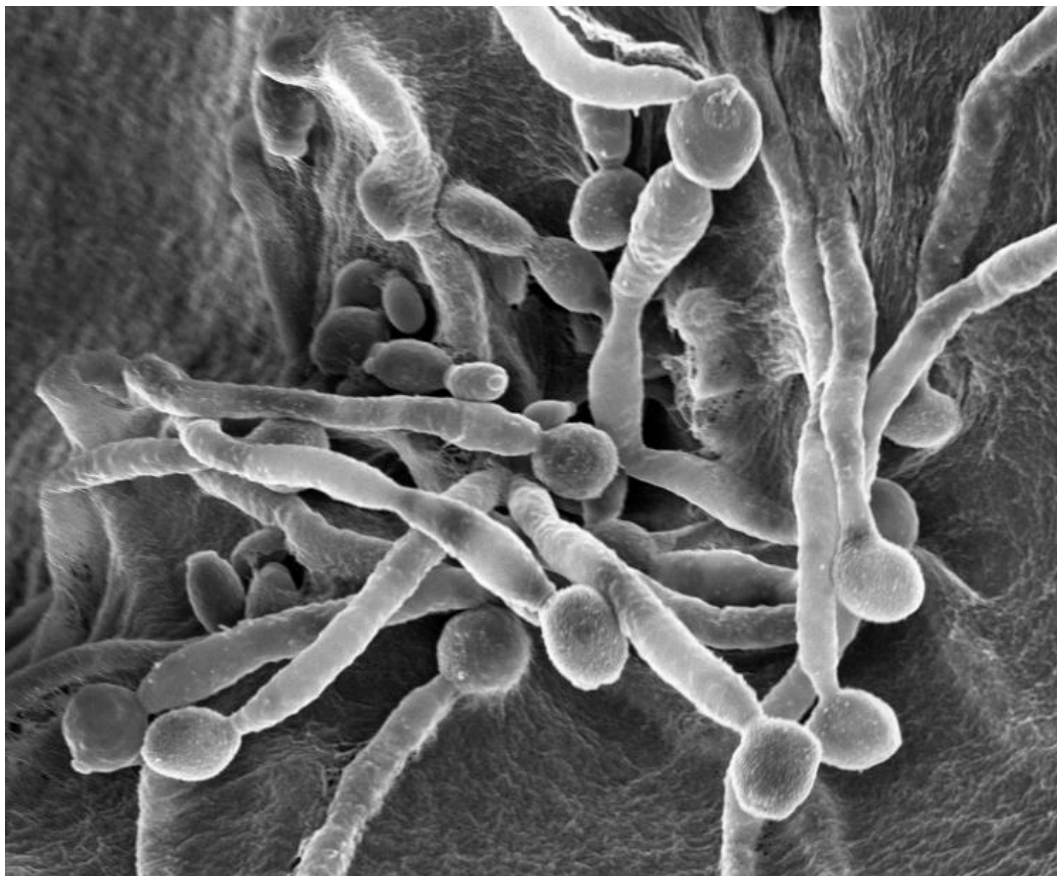
| | |
|---------------------------------------|----|
| Referee Laboratories with correct ID: | 10 |
| Laboratories with correct ID: | 55 |
| Laboratories with incorrect ID: | 0 |

Illustrations:

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



Figure 9A. Scanning electron micrograph illustrating pseudohyphae with blastoconidia of *Candida albicans*.



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Y-5 *Candida tropicalis*

Source: Stool / Blood / Urine

Clinical significance: *Candida tropicalis* causes sepsis, wound infections, and disseminated infections in immunocompromised patients.

Colony: *C. tropicalis* colony is smooth to wrinkled, cream-colored and rapid-growing on Sabouraud's dextrose agar after 7 days of incubation at 25°C, (Figure 10).

Microscopy: *C. tropicalis* shows long true hyphae and pseudohyphae, with either single or small clusters of blastoconidia on Corn meal agar with Tween 80 (Figure 10).

Differentiation: *C. tropicalis* is differentiated from *C. albicans* and *C. dubliniensis* by variable growth on media containing cycloheximide, and by its fermentation of glucose, maltose, sucrose, and trehalose. Occasionally, *C. tropicalis* produces chlamydospores on corn meal agar.

Molecular test: Reverse-hybridization line probe assay combined with PCR amplification of internal transcribed-spacer (ITS) regions are used for rapid identification of clinically significant fungal pathogens including *C. tropicalis*. The combination of pan-fungal PCR and multiplex liquid hybridization of ITS regions are developed for detection and identification of fungi in tissue specimens.

The ribosomal *ITS1* and *ITS2* regions of the test isolate showed 100 % nucleotide identity with *C. tropicalis* CBL Cd-3 (Genebank accession no. EU924133)

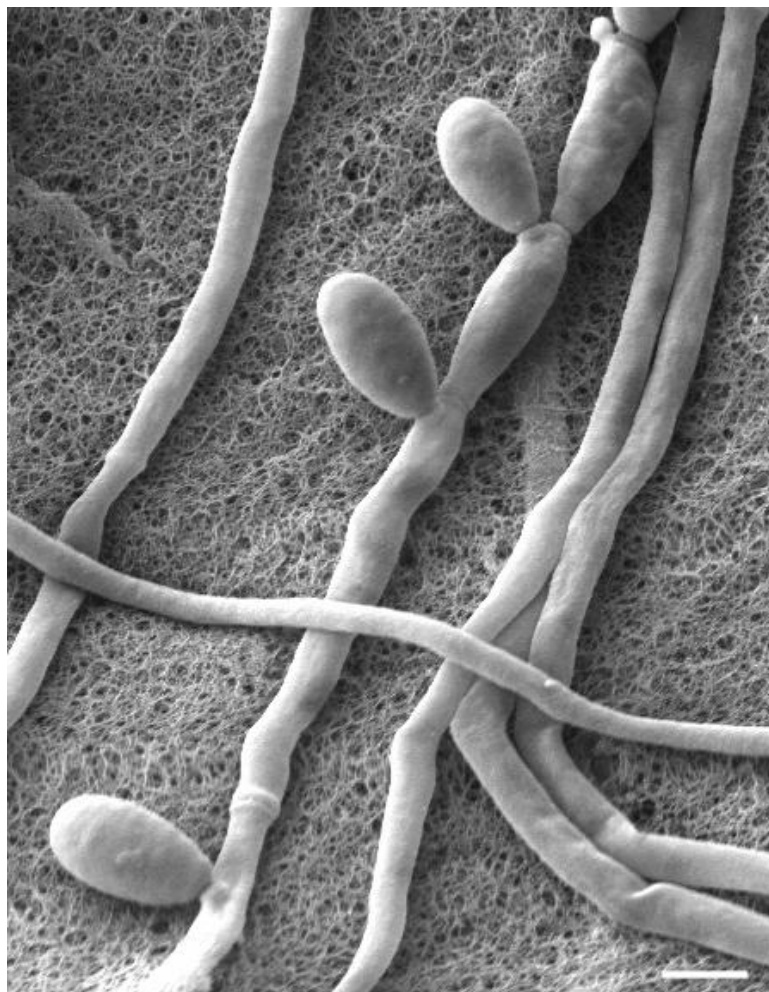
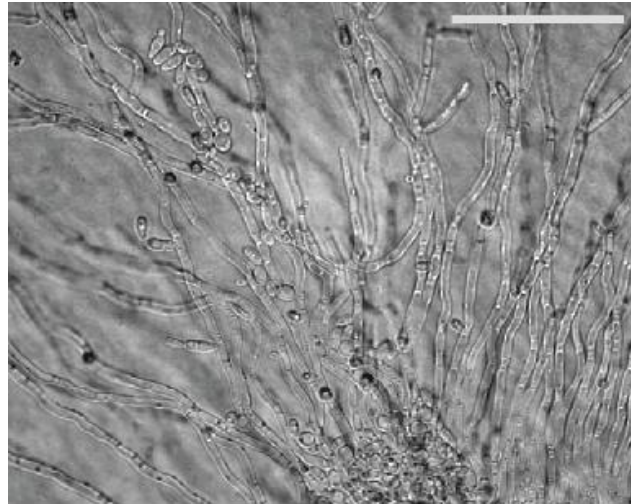
Antifungal susceptibility: *C. tropicalis* is generally susceptible to azoles and echinocandins, but variably susceptible to flucytosine. Few strains of *C. tropicalis* have been reported with high amphotericin B MIC.

Participant performance:

| | |
|---------------------------------------|-----|
| Referee Laboratories with correct ID: | 10 |
| Laboratories with correct ID: | 54 |
| Laboratories with incorrect ID: | 1 |
| (<i>Candida famata</i>) | (1) |

Illustrations:

Figure 10. *Candida tropicalis*, smooth-to-wrinkled, creamish colony, Sabouraud's dextrose agar 7-days, 25°C. Microscopic morphology on corn meal agar with Tween 80, showing long true hyphae and pseudohyphae with clusters of blastoconidia (bar = 50 μm). Scanning electron micrograph illustrates true and pseudohyphae (with constrictions) and blastoconidia (bar = 2 μm).



Further reading:

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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 September 25, 2013 direct antigen detection test event. Two negative (Cn-Ag-3 and Cn-Ag-5) and three positive serum samples (Cn-Ag-1, Cn-Ag-2, and Cn-Ag-4) with the titer of 1:8, 1:512, and 1:256, respectively for cryptococcal antigen were included.

Results: The consensus results for specimens Cn-Ag-3 and Cn-Ag-5 were negative as expected. There was only one laboratory reported Cn-Ag-5 positive, which was not acceptable. Cn-Ag-2 and Cn-Ag-4 were reported positive by all the participating laboratories except only one laboratory reported Cn-Ag-4 negative. Specimen Cn-Ag-1 was not validated since there was no consensus on qualitative results. Of 67 laboratories, 50 reported Cn-Ag-1 specimen to be positive with the titer ranged from 1:2 to 1:32, which were all in the acceptable range. Seventeen (25%) laboratories reported this specimen to be ~~it~~negative, which makes the sample invalidated. The summary of laboratory performance for semi-quantitative detection of cryptococcal antigen is shown in Table 2. It has been reported that cryptococcal antigens are unstable at elevated temperatures. Therefore, samples should be processed as quickly as possible or should be stored at 4°C prior to processing.

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

| Method | | Cn-Ag-1 Titers | | | | | | | |
|---------------------|----------------------------|-----------------------|-----------|-----------|----------|-----------|----------|----------|----------|
| No. laboratories | | Negative | 2 | 4 | 5 | 8 | 10 | 16 | 32 |
| EIA | 3 | 3 | | | | | | | |
| Latex Agglutination | 57 | 13 | 9 | 16 | | 17 | | 1 | 1 |
| | <i>Immuno-Mycologics</i> | 7 | 2 | 1 | 1 | 3 | | | |
| | <i>Meridien Diagnostic</i> | 42 | 10 | 6 | 14 | 12 | | | |
| | <i>Remel</i> | 8 | 1 | 2 | 1 | 2 | | 1 | 1 |
| Lateral Flow Assay | 5 | 1 | 1 | 1 | 1 | | 1 | | |
| Total | 65 | 17 | 10 | 17 | 1 | 17 | 1 | 1 | 1 |

| Method | | Cn-Ag-2 Titers | | | | | | | | | | |
|---------------------|----------------------------|-----------------------|----------|-----------|----------|----------|-----------|----------|-----------|----------|----------|----------|
| No. laboratories | | 32 | 64 | 128 | 160 | 200 | 256 | 320 | 512 | 1024 | 1280 | 2048 |
| EIA | 1 | 1 | | | | | | | | | | |
| Latex Agglutination | 56 | 1 | 4 | 12 | | 1 | 14 | | 19 | 4 | | 1 |
| | <i>Immuno-Mycologics</i> | 7 | 1 | | | | 3 | | 3 | | | |
| | <i>Meridien Diagnostic</i> | 41 | 4 | 10 | | 1 | 9 | | 13 | 3 | | 1 |
| | <i>Remel</i> | 8 | | 2 | | | 2 | | 3 | 1 | | |
| Lateral Flow Assay | 5 | | | | 2 | | 1 | 1 | | | 1 | |
| Total | 62 | 2 | 4 | 12 | 2 | 1 | 15 | 1 | 19 | 4 | 1 | 1 |

| Method | | Cn-Ag-4 Titers | | | | | | | | | | | |
|---------------------|----------------------------|-----------------------|----------|----------|----------|-----------|----------|-----------|----------|-----------|----------|----------|----------|
| No. laboratories | | 2 | 16 | 32 | 40 | 64 | 80 | 128 | 160 | 256 | 320 | 512 | 1024 |
| EIA | 1 | | 1 | | | | | | | | | | |
| Latex Agglutination | 55 | 1 | | 4 | | 18 | 1 | 13 | | 13 | | 3 | 2 |
| | <i>Immuno-Mycologics</i> | 6 | | | | 1 | | 2 | | 2 | | 1 | |
| | <i>Meridien Diagnostic</i> | 41 | | 4 | | 14 | 1 | 10 | | 9 | | 2 | 1 |
| | <i>Remel</i> | 8 | 1 | | | 3 | | 1 | | 2 | | | 1 |
| Lateral Flow Assay | 5 | | | | 1 | 1 | 1 | | 1 | | 1 | | |
| Total | 61 | 1 | 1 | 4 | 1 | 19 | 2 | 13 | 1 | 13 | 1 | 3 | 2 |

Further Reading:

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ANTIFUNGAL SUSCEPTIBILITY TESTING FOR YEASTS

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

Materials: *Candida parapsilosis* (S-1) was the analyte in the September 25, 2013 antifungal proficiency testing event. The interpretation of MIC values for antifungal susceptibility testing of yeasts and molds is in a state of constant change. These changes are necessitated by new information emerging from clinical trials and laboratory susceptibility testing. NYSDOH Mycology Laboratory uses latest CLSI and EUCAST documents to score proficiency testing results. However, the participating laboratories are advised to regularly consult these organizations for the latest version of their standard documents.

Comments: Acceptable results were MICs +/-2 dilutions of the reference laboratory results for any single drug. Only 2 of the 32 laboratories participating in this test event tested all 10 antifungal drugs. The reported results were as follows: itraconazole (29 laboratories), voriconazole (27 laboratories), flucytosine (25 laboratories), caspofungin (22 laboratories), amphotericin B (21 laboratories), micafungin (17 laboratories), anidulafungin (16 laboratories), posaconazole (15 laboratories), and ketoconazole (5 laboratories). Fluconazole was the only drug tested by all 32 laboratories.

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

S-1: *Candida parapsilosis* (M2554)

| Drug | No. labs | MIC (µg/ml) | | | | | | | | | |
|--------------------|----------|-------------|-------|------|------|-------|------|-----|----|---|---|
| | | 0.008 | 0.016 | 0.03 | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 |
| Amphotericin B | 22 | | | | | | 1 | 19 | 2 | | |
| Anidulafungin | 16 | | | | | | 1 | 8 | 7 | | |
| Caspofungin | 22 | | | | | 2 | 14 | 4 | 2 | | |
| Flucytosine (5-FC) | 25 | | | 5 | 17 | 3 | | | | | |
| Fluconazole | 31* | | | | | | | 3 | 20 | 7 | 1 |
| Itraconazole | 28* | | | 2 | 8 | 18 | | | | | |
| Ketoconazole | 4* | | | 2 | 1 | 1 | | | | | |
| Micafungin | 17 | | | | | | | 7 | 10 | | |
| Posaconazole | 15 | | | 6 | 8 | 1 | | | | | |
| Voriconazole | 27 | 11 | 11 | 4 | | 1 | | | | | |

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

Colors represent the testing method used:

- CLSI microdilution method
- Vitek II method
- YeastOne Colorimetric method
- Both CLSI microdilution and Vitek II methods
- Both CLSI microdilution and YeastOne Colorimetric methods
- Both Etest and YeastOne Colorimetric methods
- 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 parapsilosis* (M2554)

| Drug | No. laboratories | Susceptible | Susceptible-dose dependent | Intermediate | Resistant | Non-susceptible | No interpretation |
|----------------|------------------|-------------|----------------------------|--------------|-----------|-----------------|-------------------|
| Amphotericin B | 21 | 8 | | | | | 13 |
| Anidulafungin | 16 | 16 | | | | | |
| Caspofungin | 22 | 22 | | | | | |
| Flucytosine | 25 | 21 | | | | | 4 |
| Fluconazole | 32 | 32 | | | | | |
| Itraconazole | 29 | 25 | | | | | 4 |
| Ketoconazole | 5 | 2 | | | | | 3 |
| Micafungin | 17 | 17 | | | | | |
| Posaconazole | 15 | 7 | | | | | 8 |
| Voriconazole | 27 | 27 | | | | | |

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR MOLDS (EDUCATIONAL)

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

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

Comments: Five out of thirty-two 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 ($\mu\text{g/ml}$) Values of Mold Antifungal Susceptibility: *Aspergillus fumigatus* M2040

| Drugs ($\mu\text{g/ml}$) | Total # of labs | 0.008 | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1.0 | 2.0 | 4.0 | 8.0 | 16 | 32 | 64 | 256 |
|----------------------------|-----------------|-------|-------|------|------|------|------|-----|-----|-----|-----|-----|----|----|----|-----|
| Amphotericin B | 5 | | | | | | 1 | 2 | 1 | 1 | | | | | | |
| Anidulafungin | 4 | | 3 | | 1 | | | | | | | | | | | |
| Caspofungin | 4 | 1 | 1 | | 2 | | | | | | | | | | | |
| Fluconazole | 4 | | | | | | | | | | | | | | 2 | 2 |
| Itraconazole | 4 | | | | | | | | 1 | | | 1 | 2 | | | |
| Ketoconazole | 2 | | | | | | | | | | | | 1 | 1 | | |
| Micafungin | 4 | 2 | 1 | | 1 | | | | | | | | | | | |
| Posaconazole | 4 | | | | | | 1 | 1 | 1 | 1 | | | | | | |
| Voriconazole | 4 | | | | | | 2 | | | 1 | | | | | | |

Colors represent the testing method used:



CLSI microdilution method

YeastOne Colorimetric method

Both CLSI microdilution and YeastOne Colorimetric methods

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