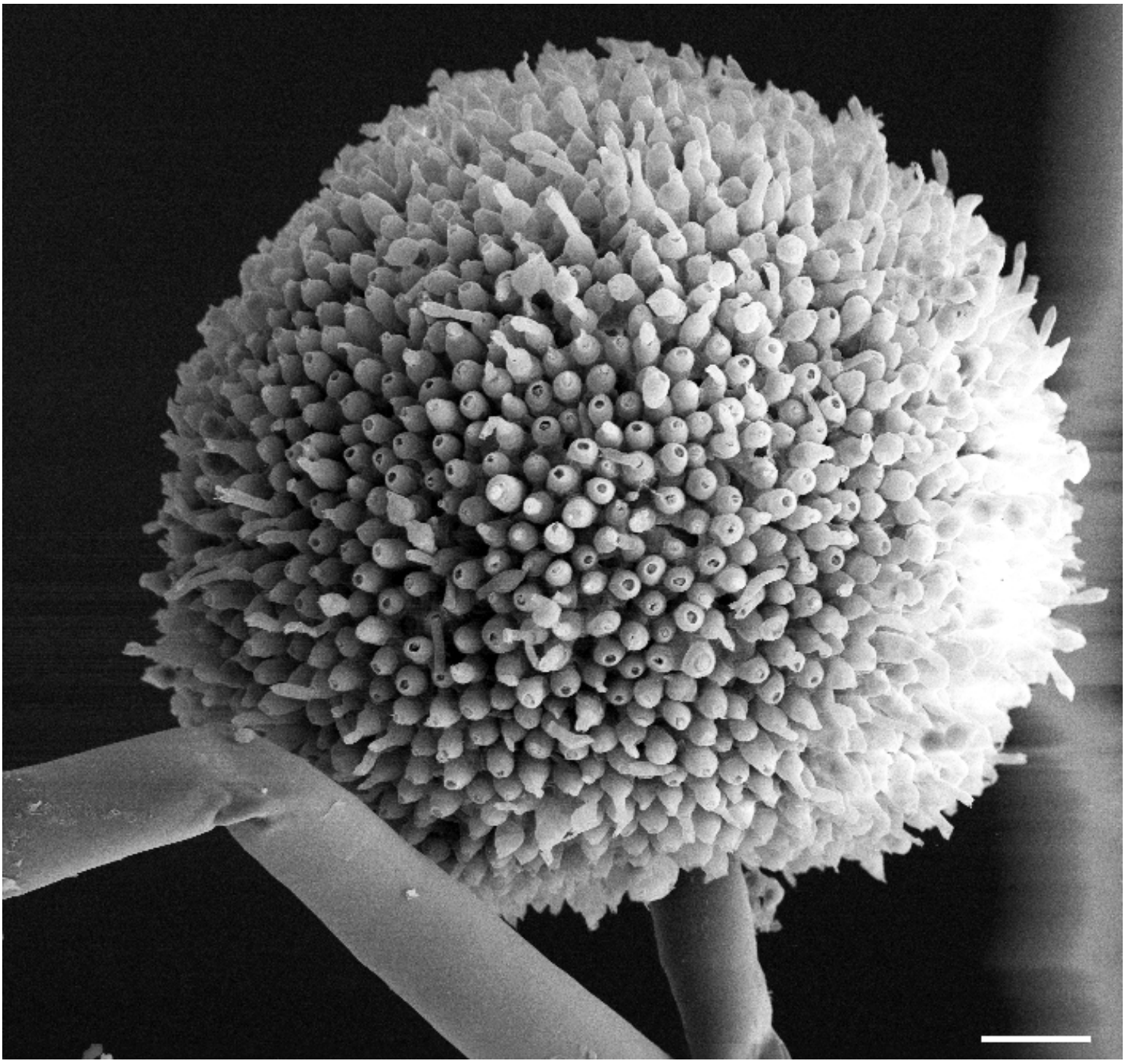
Mycology Proficiency Testing Program



Test Event Critique
September 2015

Wadsworth Center
New York State Department of Health

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Mycology Laboratory

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

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

Mycology Laboratory Contact Details

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Mycology Proficiency Testing Program (PTP)

CATEGORY DESCRIPTION

MYCOLOGY

This category is for laboratories that perform any technique for the detection and identification of molds and yeast to the extent of their abilities, including antigen detection assays, culture, molecular techniques, and drug susceptibility testing.

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

MYCOLOGY
- Culture and Identification of Molds*
- Culture and Identification of Yeasts*
- Susceptibility testing of Yeasts
- Susceptibility Testing of Molds
- *Cryptococcus neoformans* Antigen Detection

TEST SPECIMENS& GRADING POLICY

Test Specimens

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

Grading Policy

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

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

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

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

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

TEST ANALYTE MASTER LISTS

Mold Master List

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

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

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

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

Yeast Master List

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

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

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

Blastoschizomyces capitatus (*Geotrichum capitatum*)
Blastoschizomyces species
Candida albicans
Candida dubliniensis
Candida famata
Candida glabrata complex
Candida lipolytica (*Yarrowia lipolytica*)
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)
Kluyveromyces marxianus (formerly *Candida kefyr*)
Malassezia furfur
Malassezia pachydermatis
Malassezia species
Meyerozyma guilliermondii (formerly *Candida guilliermondi*
Pichia kudriazevii (formerly *Candida krusei*)
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
Wickerhamomyces anomalus (formerly *Candida pelliculosa*)

Summary of Laboratory Performance:

Mycology – Mold

	Specimen key	Validated specimen	Other acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
M-1	*Trichophyton rubrum*	*Trichophyton rubrum*	*Trichophyton* species	57/57 (100%)
M-2	*Microsporum canis*	*Microsporum canis*	*Microsporum species*	56/57 (98%)
M-3	*Aspergillus niger*	*Aspergillus niger*		56/57 (98%)
M-4	*Syncephalastrum* species	*Syncephalastrum* species		47/57 (82%)
M-5	*Biopolaris* species	*Biopolaris* species	*Bipolaris hawaiiensis*	54/57 (95%)

Mycology – Yeast Only

	Specimen key	Validated specimen	Other acceptable answers	Laboratories with correct responses / Total laboratories (% correct responses)
Y-1	*Candida parapsilosis species complex*	*Candida parapsilosis species complex*		50/52 (96%)
Y-2	*Candida zeylanoides*	*Candida zeylanoides*		47/50 (94%)
Y-3	*Rhodotorula mucilaginosa*	*Rhodotorula mucilaginosa*	*Rhodotorula species*	48/49 (98%)
Y-4	*Candida rugosa*	*Candida rugosa*	*Candida* species not *albicans*	49/51 (96%)
Y-5	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*	*Saccharomyces* species, Yeast not *Candida*	50/50 (100%)

Mycology – Direct detection (*Cryptococcus* Antigen Test)

	Specimen key (Titer)	Validated specimen	Correct responses / Total laboratories (% correct responses)	
			Qualitative	Quantitative
Cn-Ag-1	Positive	Positive	65/65 (98%)	NA
Cn-Ag-2	Negative	Negative	65/65 (100%)	NA
Cn-Ag-3	Negative	Negative	64/65 (98%)	NA
Cn-Ag-4	Negative	Negative	65/65 (100%)	NA
Cn-Ag-5	Positive	Positive	64/65 (98%)	NA

Antifungal Susceptibility Testing for Yeast (S-1: *Candida albicans* M954)

Drugs	Acceptable MIC (μg/ml) range	Interpretation	Laboratories with acceptable responses/ Total laboratories (% correct responses)
Amphotericin B	0. 25 – 1.0	Susceptible / No interpretation	21/21 (100%)
Anidulafungin	0.015 – 0.03	Susceptible	19/19 (100%)
Caspofungin	0.03 – 0.25	Susceptible	24/25 (96%)
Flucytosine (5-FC)	0.03 – 0.25	Susceptible / No interpretation	22/22 (100%)
Fluconazole	32 - 256	Resistant	30/32 (94%)
Itraconazole	0.5 – 1.0	Susceptible dose-dependent/Resistant/ No interpretation	23/24 (96%)
Ketoconazole	0.25-0.5	No interpretation	2/3 (67%)
Micafungin	0.03 – 0.06	Susceptible	18/18 (100%)
Posaconazole	0.5 – 2.0	Susceptible/No interpretation	17/18 (94%)
Voriconazole	0.25 – 4.0	Susceptible-Dose Dependent/Resistant	27/28 (96%)

Commercial Device Usage Statistics:

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

Device	**No. laboratories**
Yeast Identification*	
Vitek 2	23
API 20C AUX	18
Dade Behring MicroScan Rapid Yeast Identification Panel	3
MALDI-TOF	2
Remel RapID Yeast Plus System	2
Chrome Agar	1
Other	3
Antifungal Susceptibility*	
Disk diffusion	1
Etest	1
Vitek II	2
YeastOne– Mold	2
YeastOne –Yeast	23
CLSI Microbroth dilution method – Yeast	5
CLSI Microbroth dilution method – Mold	2
Cryptococcal antigen*	
Immuno-Mycologics Latex Cryptococcus Antigen Detection System	6
Immuno-Mycologics CrAg Lateral Flow Assay	12
Meridien BioScience Cryptococcal Antigen Latex Agglutination System (CALAS)	37
Immuno-Mycologics ALPHA Cryptococcal Antigen enzyme immunoassay(CrAg EIA)	1
Remel Cryptococcal Antigen Latex Test	9

*Include multiple systems used by some laboratories

MOLD DESCRIPTIONS

M-1 *Trichophyton rubrum*

Source: Skin / Nail

Clinical significance: A frequent causal agent of infections of the feet, toes, groin, finger nails, and skin. It rarely causes infection of scalp or hair.

Colony: *Trichophyton rubrum* grow slowly on Sabouraud dextrose agar. At 30°C, the colony is fluffy to powdery, white to buff, with wine-red to brown in color on reverse (**Figure 1 A-B**).

Microscopy: Lactophenol cotton blue mount shows hyaline septate hyphae with clavate microconidia and cigar-shaped macroconidia (**Figures 2 & 3**). The macroconidia are rare.

Differentiation: *Trichophyton rubrum* can be differentiated from *T. interdigital / mentagrophytes* by solitary microconidia, no urease activity, no hair perforation, and no specific growth requirements. It can be differentiated from *T. terrestre* by good growth at 37°C, and production of red reverse pigment. Three new species, *T. fischeri, T. raubitschekii, and T. kanei* were described to be closely related to *T. rubrum*. Two of these species, *T. raubitschekii* and *T. kanei,* have been isolated from skin lesions. *T. raubitschekii* and *T. kanei* produce urease; *T. kanei* lacks microconidia, while *T. raubitschekii* produces variably shaped microconidia. *T. fischeri* resembles *T. rubrum* closely, but is non-pathogenic for humans.

Molecular test: A species–specific DNA probe using highly variable internal transcribed spacer 2 (ITS2) region of the ribosomal gene was developed to detect *T. rubrum* in culture and in clinical samples. The PCR-RFLP and RAPD are techniques used for the differentiation of various clinical isolates of *T. rubrum*.

Antifungal susceptibility: *Trichophyton rubrum* is highly susceptible to terbinafine and variably susceptible to azoles.

Participant performance:

Laboratories with correct ID:	57
Laboratories with incorrect ID:	0

Illustrations:

Figure 1. Ten-day-old culture of *T. rubrum* on Sabouraud dextrose agar with appearance of fluffy colony (A), and reverse showing wine red to brown color pigment (B).

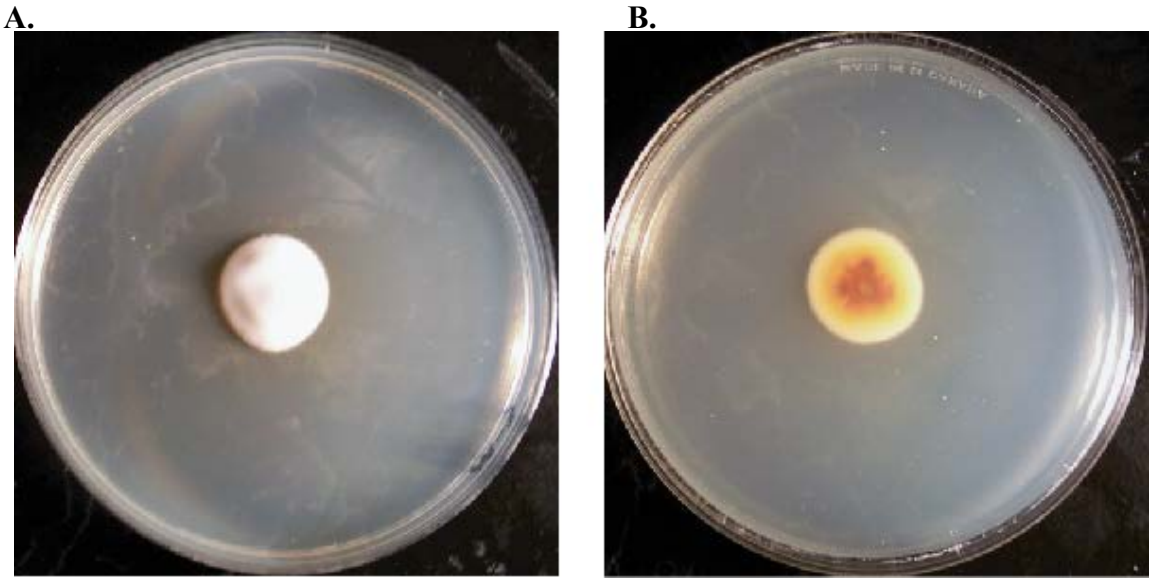


Figure 2. Microscopic morphology of *T. rubrum* showing moderate to abundant and clavate to pyriform shaped microconidia.

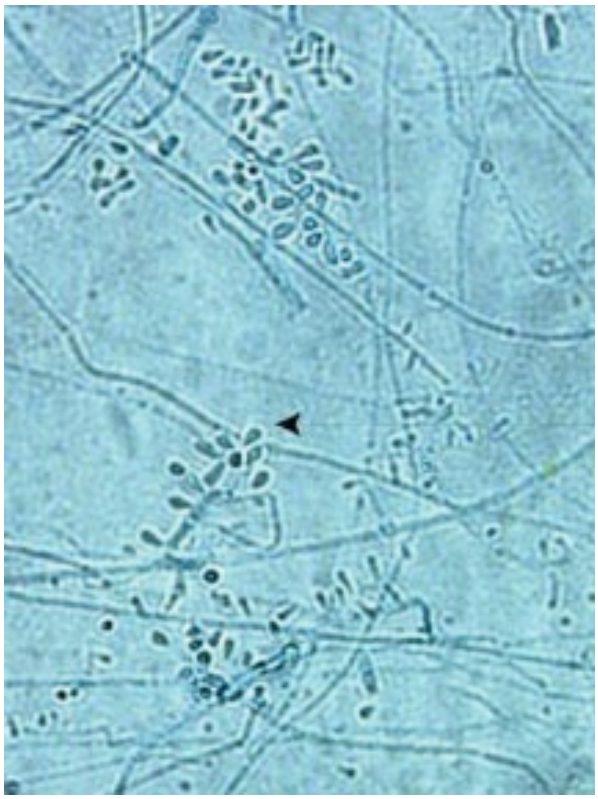
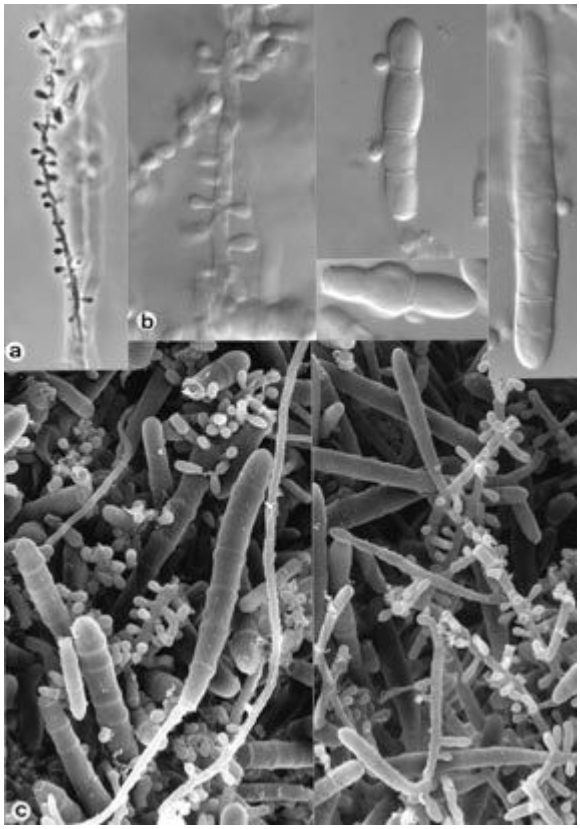


Figure 3. Scanning electron micrograph of *Trichophyton rubrum* showing clavate shaped microconidia and cigar shaped macroconidia



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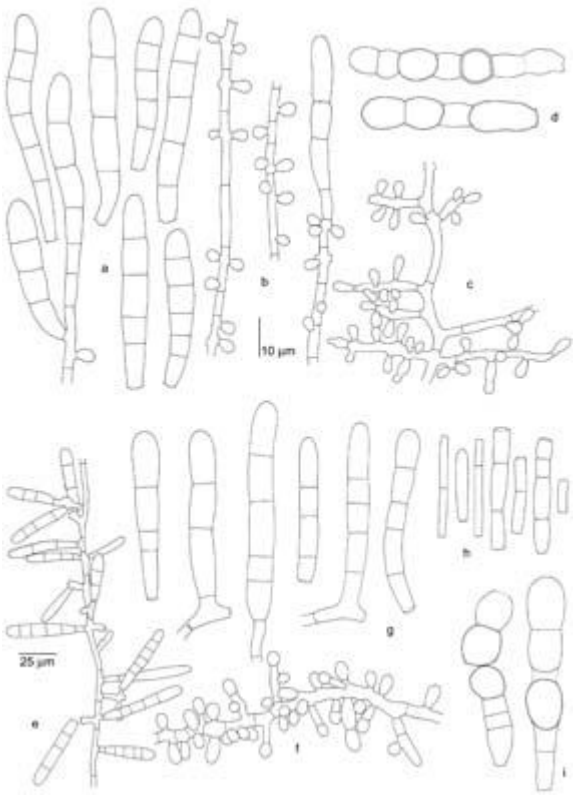

http://www.mycobank.org/TempFiles/20150904/gcaus5xoc25mi35o2iccrqat/TempF4023_atlas_p0974ab.jpg

Further reading:

Anju S, Kumar NS, Krishnakumar B, Kumar BS. 2015. Synergistic combination of violacein and azoles that leads to enhanced killing of major human pathogenic dermatophytic fungi *Trichophyton rubrum.* *Front Cell Infect Microbiol*. 5:57.

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Ran X, Zhuang K, Ran Y. 2015. Tinea corporis on the stump leg with *Trichophyton rubrum* infection. *Med Mycol Case Rep.* 9: 31-33.

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Smijs TG, Pavel S. 2011. The susceptibility of dermatophytes to photodynamic treatment with special focus on *Trichophyton rubrum*. *Photochem Photobiol*. 87: 2-13.

M-2 *Microsporum canis*

Source: Scalp / Skin

Clinical significance: *Microsporum canis* is a frequent causal agent of scalp and skin infections, most commonly in children. It rarely causes infection of the nail. Humans acquire infection through dogs or cats harboring this organism (zoophilic).

Colony: *M. canis* is a moderately fast-growing fungus. At 25°C, on Sabouraud dextrose agar, colonies are yellowish white, wooly, with yellow-orange reverse (**Figure 4 A-B**).

Microscopy: Lactophenol cotton blue mount shows hyaline septate hyphae with macroconidia and microconidia. The macroconidia are fusoid, thick-walled, with curved apex and up to 15 septations. The microconidia are club–shaped (**Figure 5 A-B**).

Differentiation: *M. canis* can be differentiated from other dermatophytic fungi by its macroscopic and microscopic features, notably yellowish colonies with yellowish-orange reverse and thick-walled macroconidia containing up to 15 cells.

Molecular test: Identification of *M. canis* by specific PCR and by the random amplification of polymorphic DNA (RAPD) method and Southern hybridization was reported.

Antifungal susceptibility: Most *M. canis* isolates are susceptible to griseofulvine, terbinafine, ketoconazole, itraconazole, and fluconazole.

Participant performance:

Laboratories with correct ID:	56
Laboratories with incorrect ID:	01
(*Microsporum gypseum* species complex*)*	(1)

Illustrations:

Figure 4. Seven-day-old culture of *Microsporum canis* on Sabouraud dextrose agar with white and wooly colony (A) with yellowish orange reverse (B).

A. **B.**

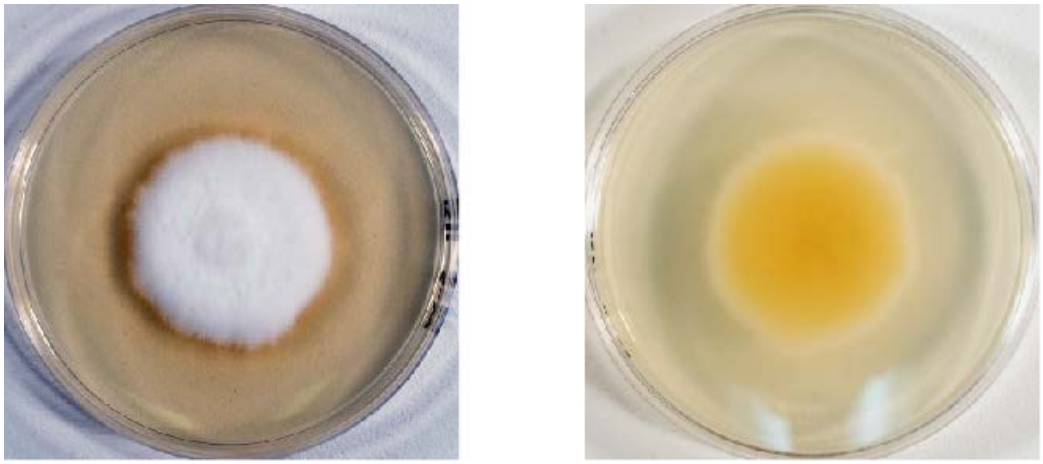
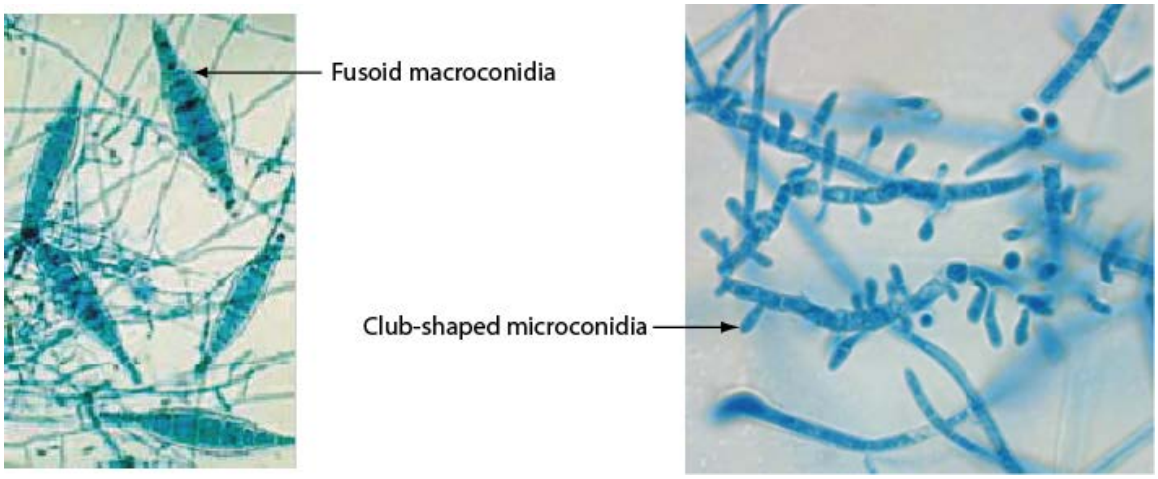


Figure 5. Microscopic morphology of *Microsporum canis* showing the fusoid, thick-walled macroconidia with curved apex (A, 200· magnification) and club-shaped microconidia (B, 1000· magnification).

A. **B.**



Further reading:

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M-3 *Aspergillus niger*

Source: Ear /Blood / Toe

Clinical significance: *Aspergillus niger* commonly causes ear infections. It is also implicated in allergic aspergillosis, pulmonary aspergilloma and rarely in primary cutaneous disease.

Colony: *Aspergillus niger* grows rapidly on Sabouraud dextrose agar at 30°C. The initial colony is white but with time, it becomes black giving salt and pepper appearance (**Figure 6 A**). It also grows well at 37^{o}C. The reverse of the colony is pale yellow (**Figure 6 B**).

Microscopy: Lactophenol cotton blue mount showed septate hyphae with smooth-walled, simple conidiophores measuring up to 1 mm in length. Conidiophores end in vesicle, which is globose and entirely covered (radiating) with two series (biseriate) sterigmata (**Figure 7 A-B & Table 1**).

Differentiation: *A. niger* can be easily differentiated from other *Aspergillus* species by its rapid growth, black colonies, biseriate sterigmata, radiating heads with black, round, and rough conidia.

Molecular test: ITS regions of the ribosomal gene can be used for the molecular identification of *A. niger*.

Antifungal susceptibility: Most clinical isolates are susceptible to amphotericin B, variably susceptible to itraconazole, and resistant to fluconazole. Posaconazole, ravuconazole, and voriconazole exhibit promising activity against *A. niger*. *A. niger* is also susceptible to caspofungin.

Participant performance:

Laboratories with correct ID:	56
Laboratories with incorrect ID:	01
(*Trichophyton* species)	(1)

Illustrations:

Figure 6. Five-day-old culture of *Aspergillus niger* on Sabouraud dextrose agar. The colony shows typical "salt and pepper appearance" due to darkly pigmented conidia (A). The reverse of the colony is pale yellow (B)

A. **B.**

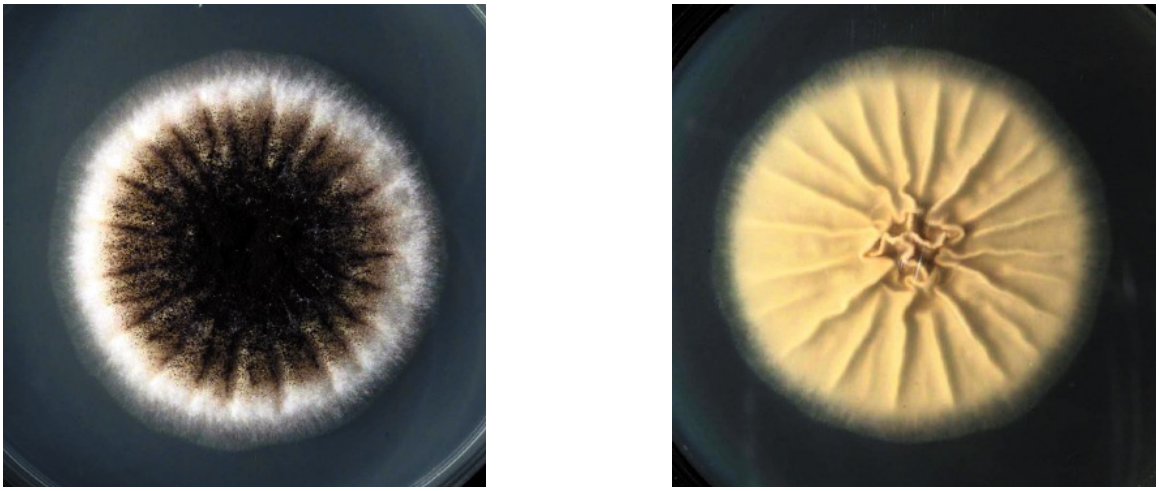


Figure 7. Microscopic morphology of *Aspergillus niger* showing globose vesicle with biseriate, radiating head ; conidia are dark, round, and rough (A, 400· magnification; B, line drawing not to scale).

A. **B.**

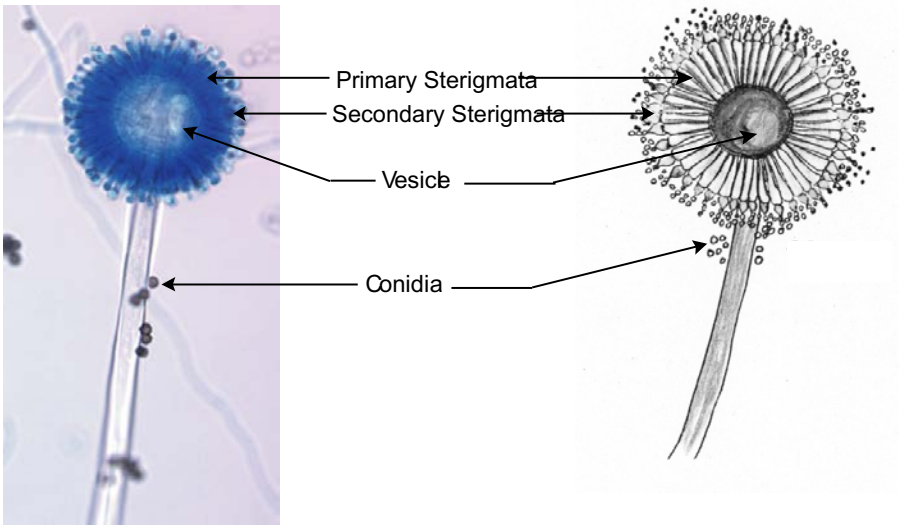
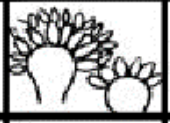
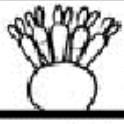
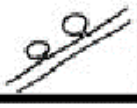


Table 1. Scheme for differentiation of *Aspergilli* most commonly involved in human diseases.

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

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M-4. *Syncephalestrum* species

Source: Wound / Skin

Clinical significance: *Syncephalastrum* species is a rare pathogen in humans. It is a saprobic fungus and has been isolated from environmental sources worldwide.

Colony: *Syncephelastrum* grows rapidly. On Sabouraud dextrose agar, the *Syncephalastrum* species grows very cottony to fluffy, white to light grey, becoming dark grey with the development of sporangia in 5-7 days (**Figure 8A**). The reverse of the colony is pale to light brown in color (**Figure 8B**).

Microscopy: Lactophenol cotton blue mount shows aseptate hyphae. Sporangiophores are branched and ends in round or oval terminal vesicle. The vesicles are surrounded by rod- or club-shaped structures called merosporangia in which multiple round to oval sporangiospores are developed in linear fashion (**Figure 9**).

Differentiation: The sporangiophore and merosporangia of *Syncephalastrum* species may also be mistaken for *Aspergillus* species, if the isolate is not examined carefully. *Syncephalastrum* differs from *Aspergillus* by the presence of merosporangia and absence of phialides. In contrast to *Aspergillus*, the hyphae of *Syncephalastrum* are aseptate. *Syncephalastrum* also differed from *Cunninghamella* by the terminal vesicle on the sporangiophore, which bears finger-shaped sporangia called merosporangium. Each merosporangium carries linearly arranged sporangiospores.

Molecular tests – The ITS and D1/D2 regions of the ribosomal gene can be used for the identification of this fungus to genus and species levels.

In vitro susceptibility testing – *S. racemosum* is susceptible to amphotericin B, but resistant to 5-FC, fluconazole, itraconazole and ketocoanzole.

Participant performance:

Laboratories with correct ID:	47
Laboratories with incorrect ID	10
(*Cunninghamella* species)	(9)
(*Cunnighamella bertholletiae*)	(1)

Illustrations:

Figure 8. White to gray and wooly colony of *Syncephalastrum* species on Sabouraud's dextrose agar (A) with reverse side of the colony pale brown (B)

A. **B.**

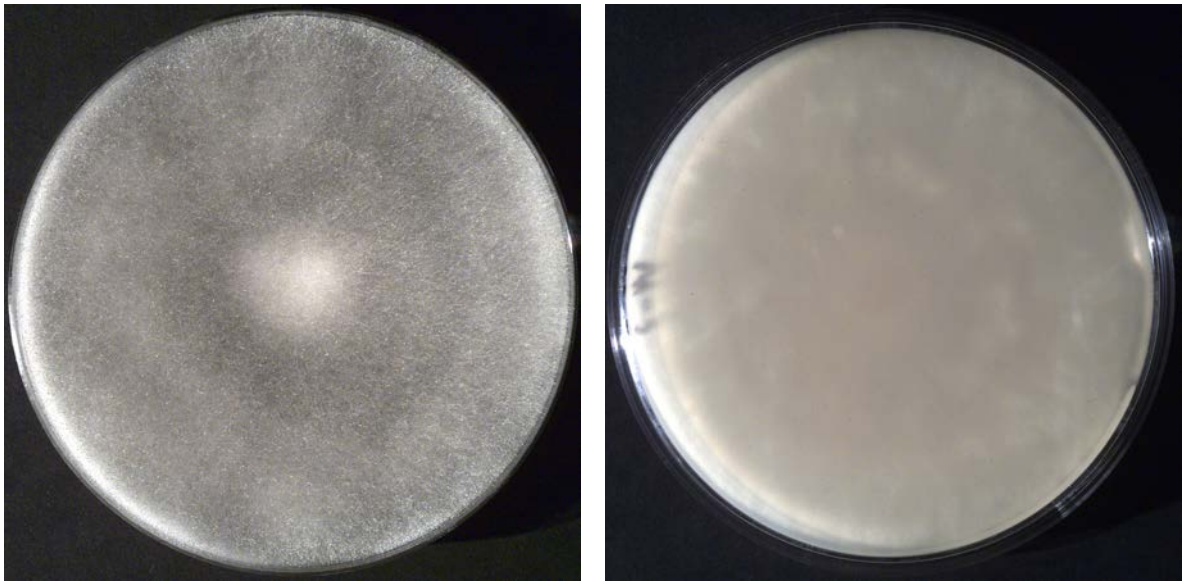
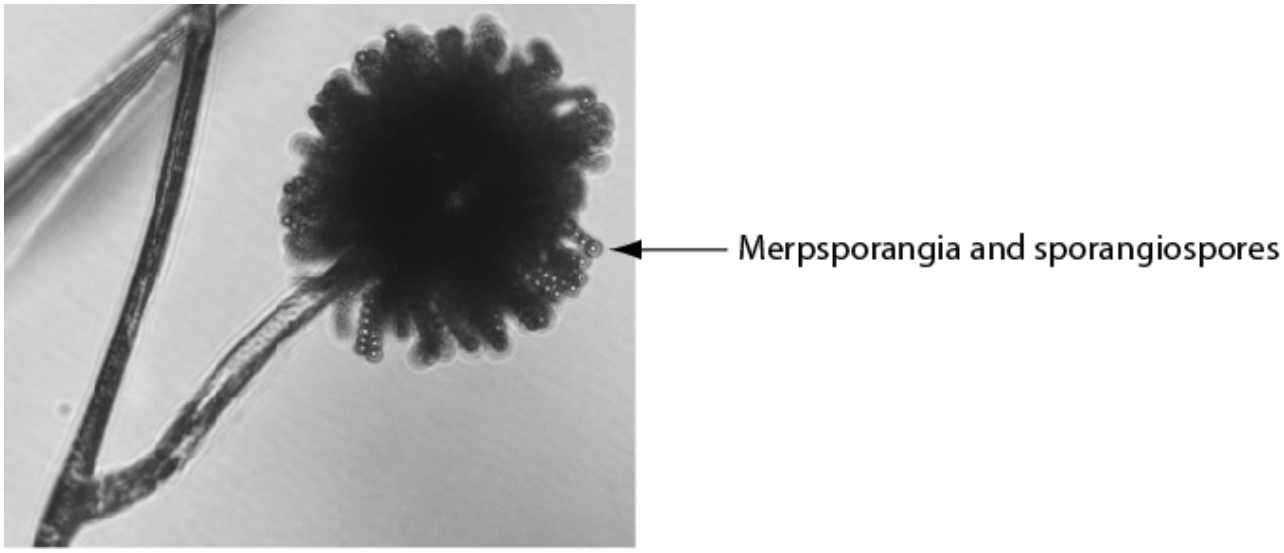


Figure 9. Microscopic morphology of *Syncephalastrum* showing merosporangia arranged around the vesicle at the apex of sporangiophore and round sporangiospores formed in a linear series in the interior of the merosporangia



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M-5 *Bipolaris* species

Source: Tissue / Nail

Clinical significance: *Bipolaris* sp. is reported as a causal agent for peritonitis, rhinosinusitis, keratitis, endocarditis, osteomyelitis, meningo-encephalitis, and cutaneous infection in the immunocompromised and healthy humans.

Colony: *Bipolaris* sp. was a fast grower. On Sabouraud's dextrose agar, after 7 days at 25°C, the colony was whitish becoming grayish black on the surface and downy texture (**Figure 10A**). Reverse was black with brownish edges (**Figure 10B**).

Microscopy: Lactophenol cotton blue mount showed dark septate hyphae. Elongated conidiophores bent at the point where each conidium was formed, which produced a zigzag appearance. This is called sympodial geniculate growth – conidiogenous structure that continues to increase in length by forming a new growing point just below each new terminal conidium, often resulting in a bent appearance. The conidia were brown, thick walled, with 3-5 septations (**Figure 11 A-B**). There was slightly protruding hilum at one end of the conidia attached to the conidiophore.

Differentiation: *Bipolaris* sp. can be distinguished from *Exserohilum* and *Drechslera* by the shape and size of conidia. *Bipolaris* conidia with hilum (a scar at the base of the conidium where it was attached to the conidiogenous cell) slightly protrudes, average size 8·26 μm, 3-5 septa, but conidia of *Exserohilum* with hilum strongly protrudes, average size 14·90 μm, 5-12 septa, and conidia of *Drechslera* lack protruded hilum, average in size 16·65 μm with 3-5 septa.

Molecular test: *Bipolaris* sp. can be identified to genus level by sequencing of the ITS region of the ribosomal gene.

Antifungal susceptibility: *Bipolaris* sp. is susceptible to amphotericin B, itraconazole, ketoconazole, and miconazole but resistant to 5 fluorocytosine.

Participant performance:

Laboratories with correct ID:	54
Laboratories with incorrect ID:	03
(*Curvularia* species)	(2)
(Specimen negative for fungi)	(1)

Illustrations:

Figure 10. Seven-day-old colony of *Bipolaris* species grown on Sabouraud dextrose agar showing white, gray to black downy texture (A). The reverse of colony with black pigment (B)

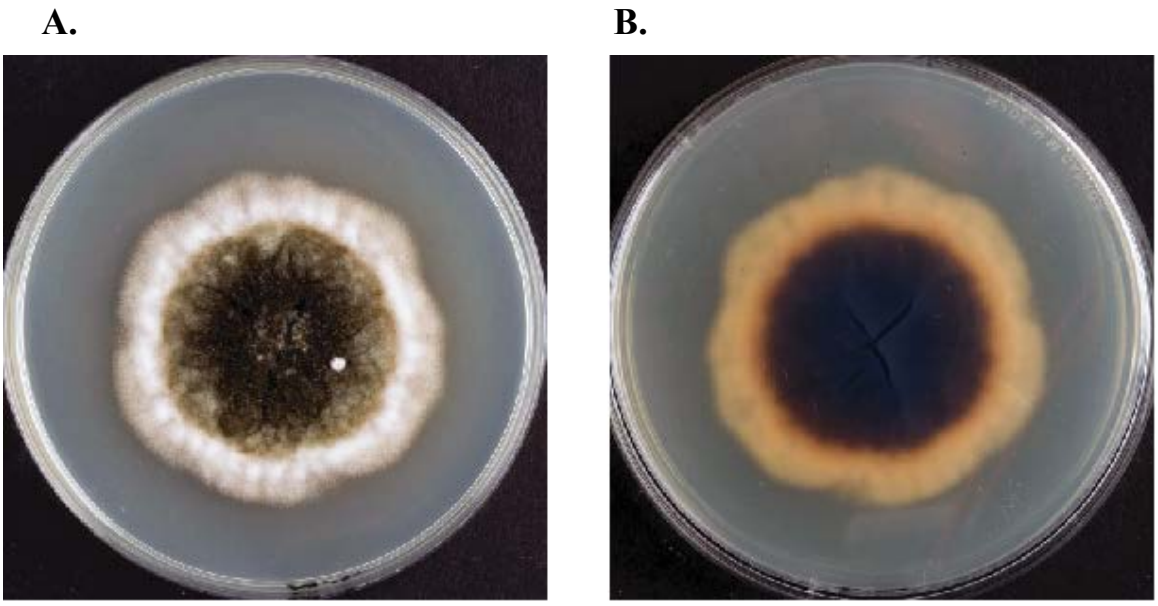
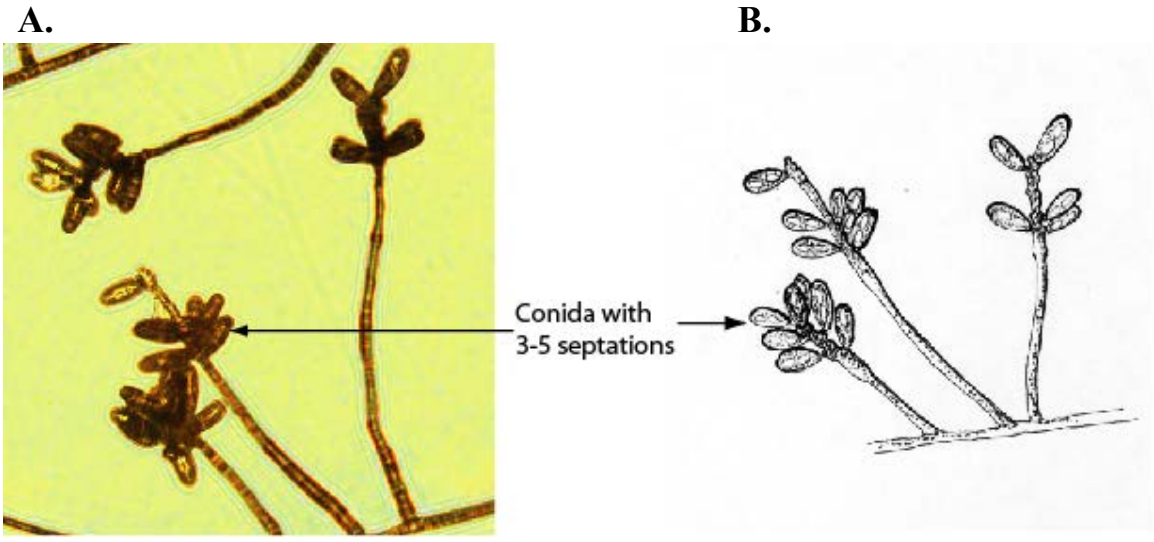


Figure 11 Microscopic morphology of *Bipolaris* sp. showing brown, thick walled conidia with 3-5 septations (A: 400· magnification; B: line drawing not to scale).



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YEAST DESCRIPTIONS

Y-1 *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 dextrose agar after 5 days of growth at 30°C (**Figure 12 A**).

Microscopy: *Candida parapsilosis* shows long, multibranched pseudohyphae, together with small elongated blastoconidia on cornmeal agar with Tween 80 (**Figure 12 B-C**).

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.

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

Participant performance:

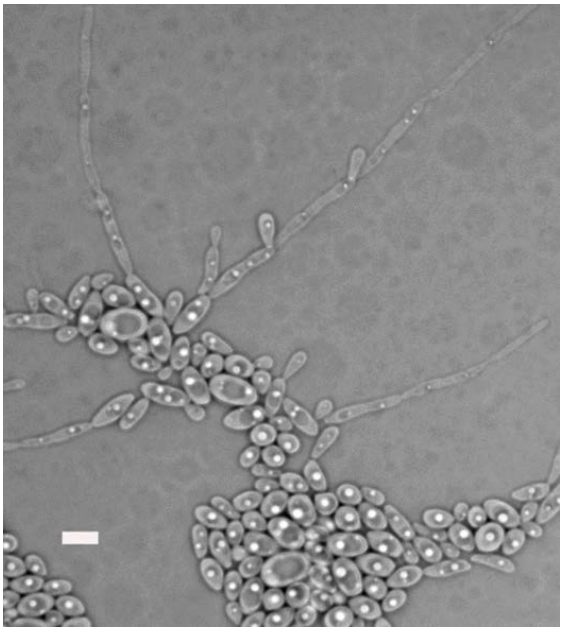
Laboratories with correct ID:	50
Laboratories with incorrect ID:	02
(*Candida tropicalis*)	(01)
(*Rhodotorula mucilaginosa*)	(01)

Illustrations:

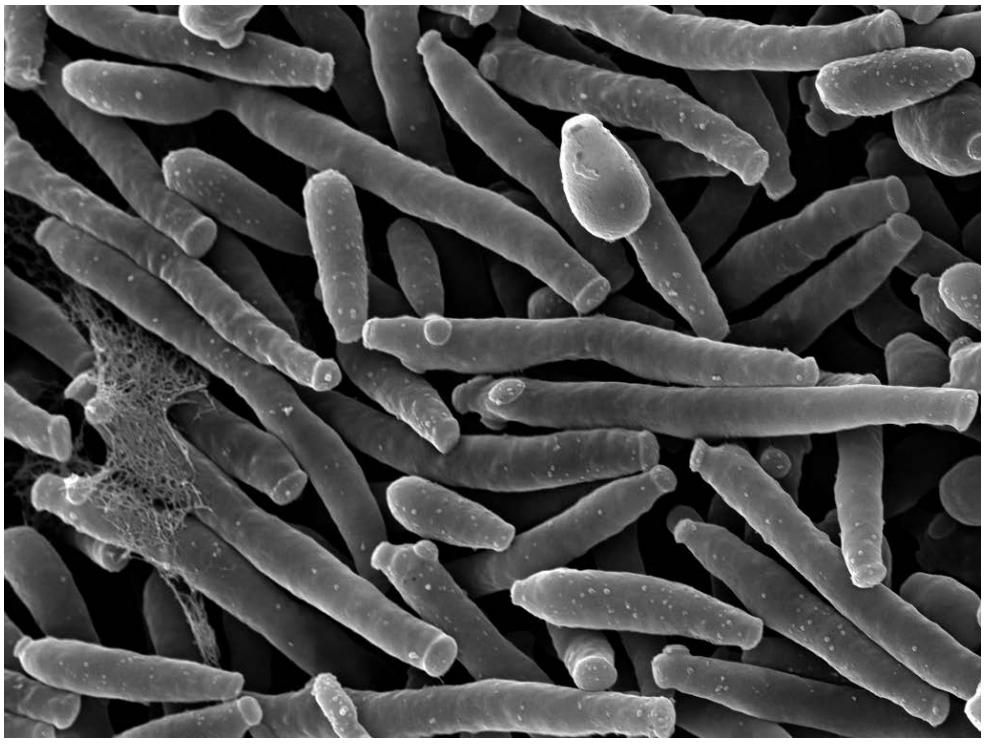
Figure 12. *Candida parapsilosis* white to cream, smooth colony on Sabouraud's dextrose agar, 25°C. Microscopic morphology of *Candida parapsilosis* with long, multibranched pseudohyphae together with small cluster of elongated blastoconidia on cornmeal agar with Tween 80 (bar = 5 μm**).**

A.

B.



C. Scanning electron micrograph of *Candida parapsilosis* with pseudohyphae and blastoconidia



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Y-2 *Candida zeylanoides*

Source: Nail / Urine

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

Colony: On Sabouraud dextrose agar after 7 days at 30^{o}C, colony was smooth, cream colored, butyrous raised (**Figure 13 A**)

Microscopy: On corn meal agar with Tween 80, *C. zeylanoides* forms long pseudohyphae, with verticillate, ovoid blastoconidia (**Figure 14 A-B**). Blastoconidia are produced in whorls around the pseudohyphae.

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

Molecular tests: ITS1 and ITS2 region of the ribosomal genes are used for the identification of this yeast species.

Antifungal susceptibility: *C. zeylanoides* is susceptible to amphotericin B and to the commonly used azoles.

Participant performance:

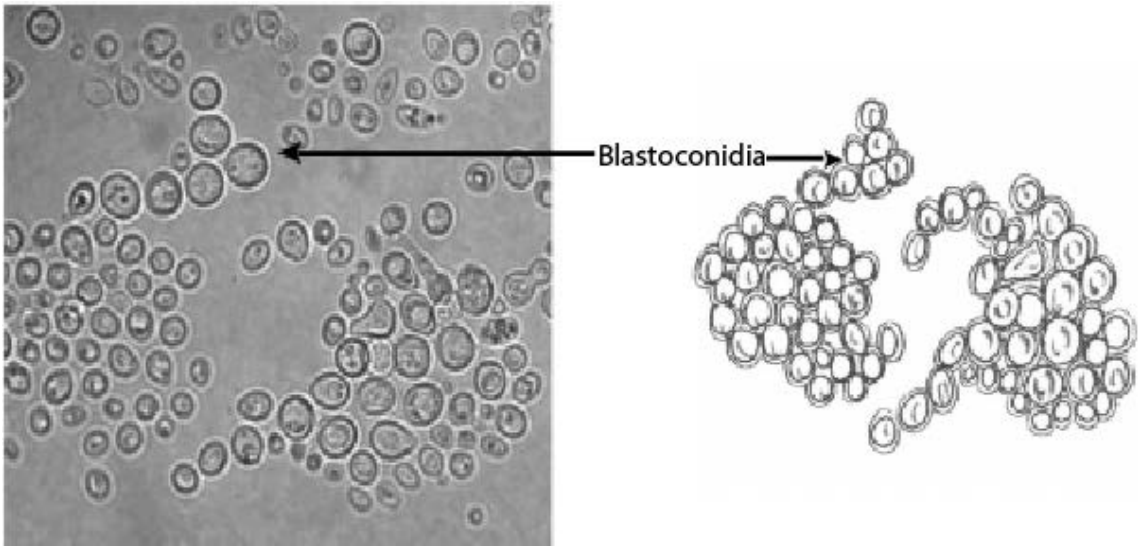
Laboratories with correct ID:	47
Laboratories with incorrect ID:	03
(Geotrichum capitatum)	(01)
(*Candida* species)	(01)
(Negative for fungi)	(01)

Figure 13. Seven-day-old culture of *Cryptococcus zeylanoides* on Sabouraud dextrose agar depicting mucoid to soft colony (A).

A.

Figure 14. Microscopic morphology of *Cryptococcus zeylanoides.* On cornmeal agar, large and round blastoconidia are seen (A, 400· magnification; B, line drawing not to scale).

A. **B.**



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Y-3 *Rhodotorula mucilaginosa*

Source: Blood / Nail / Stool

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

Colony: *R. mucilaginosa* colony is smooth, moist, soft, pink to coral red on Sabouraud dextrose agar at 30°C (**Figure 15 A**).

Microscopy: *Rhodotorula mucilaginosa* forms oval to round yeast cells, sometimes in short chains on cornmeal agar with Tween 80 (**Figure 15 B**). Rarely, a faint capsule and rudimentary pseudohyphae are also observed (**Figure 16**).

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

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

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

Participant performance:

Laboratories with correct ID:	48
Laboratories with incorrect ID:	01
(Cryptococcus albidus)	(01)

Illustrations:

Figure 15. *Rhodotorula mucilaginosa,* colony smooth, moist, soft, pink to coral red on Sabouraud dextrose agar at 30°C (A). Microscopic morphology on cornmeal agar with Tween 80, showing oval to round blastoconidia (B) (bar = 25 μm).

A.

B.

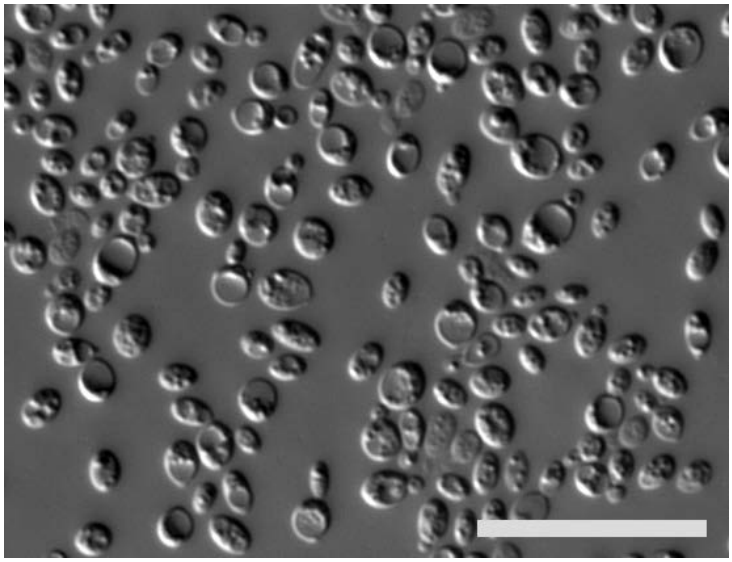
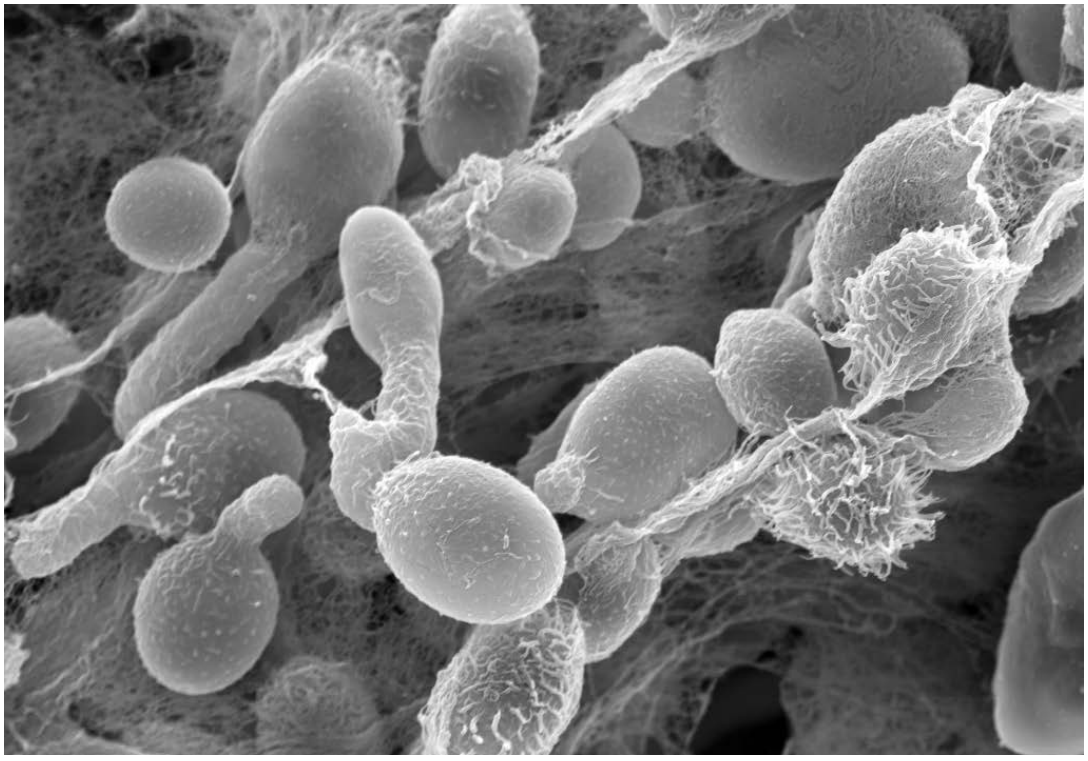


Figure 16. Scanning electron micrograph of *Rhodotorula mucilaginosa* illustrates blastoconidia.



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Y-4 *Candida rugosa*

Source: Blood / Catheter / Urine

Clinical significance: *Candida rugosa* is an infrequent causal agent of fungemia in patients with indwelling catheters. Also, it is reported to cause infection in burn patients.

Colony: *C. rugosa* colony is white to cream and wrinkled on Sabouraud dextrose agar after 7 days at 30°C (**Figure 17 A**).

Microscopy: *C. rugosa* showed branched pseudohyphae with chains of elongated blastoconidia (Figure 17 B). Pseudohyphae are seen in large numbers (**Figure 18**)

Differentiation: *C. rugosa* ferments only glucose, does not grow on media containing cycloheximide, shows variable growth at 42°C, and is urea and nitrate negative. Microscopically, it forms branched pseudohyphae that differentiates it from *C. lusitaniae* and *C. parapsilosis*. It does not form true hyphae, differentiating it from *Trichosporon beigelii*.

Molecular test: PCR assay of the ITS1 and ITS2 regions of the ribosomal gene was developed to identify *C. rugosa* in clinical specimens. A repetitive sequence-based PCR technique was developed to characterize the genotypic relatedness among *C. rugosa* isolates. Karyotyping by PFGE was developed as a typing tool to discriminate *C. rugosa* strains.

Antifungal susceptibility: Clinical isolates are susceptible to caspofungin, 5-flucytosine, and various azoles such as fluconazole, ketocoanzole, and itraconazole. It is less susceptible to polyene antifungals like amphotericin B and nystatin.

Participant performance:

Laboratories with correct ID:	49
Laboratories with incorrect ID:	02
(*Candida tropicalisi*)	(01)
(*Trichosporon* sp.)	(01)

Illustrations:

Figure 17. Cream colored and wrinkled colony of *Candida rugosa* on Sabouraud dextrose agar (A). Microscopic morphology depicting branched pseudohyphae with elongated blastoconidia on cornmeal agar with Tween 80 (B) (bar = 10 μm).

A.

B.

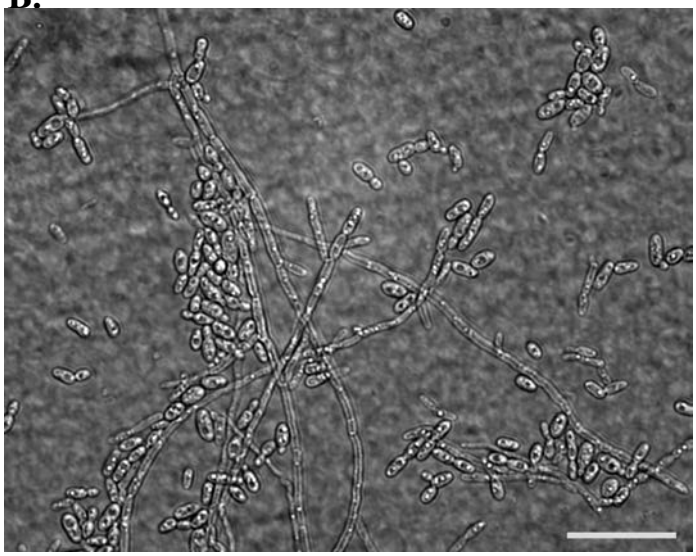
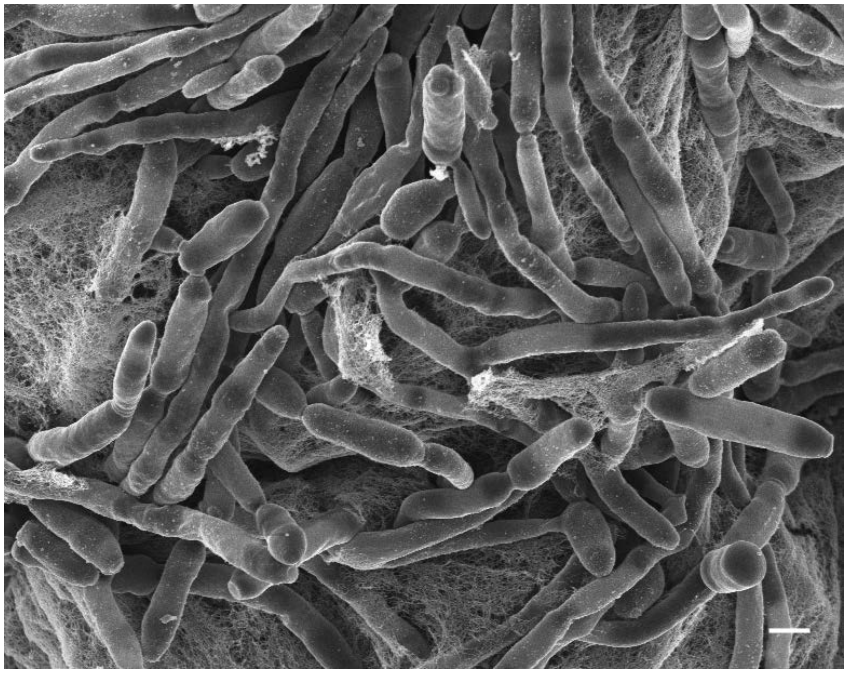


Figure 18. Scanning electron micrograph of *Candida rugosa* with extensive pseudohyphae (bar = 2 μm).



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Y-5 *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 dextrose agar at 25°C (**Figure 19 A**).

Microscopy: *Sacharomyces cerevisiae* are round to oval yeast cells with no pseudohyphae or rudimentary pseudohyphae on cornmeal agar with Tween 80 (**Figure 19 B**), and characteristic blastoconidia are seen (**Figure 20**).

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 and also being the first eukaryote to have its entire genome sequenced and mapped.

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

Participant performance:

Laboratories with correct ID:	50
Laboratories with incorrect ID:	0

Illustrations:

Figure 19. *Sacchromycetes cerevisiae*, creamy, smooth, dull butyrous colony on Sabouraud dextrose agar (A). Microscopic morphology showing round to oval blastoconidia on cornmeal agar with Tween 80 (B) (bar = 10 μm).

A.

B.

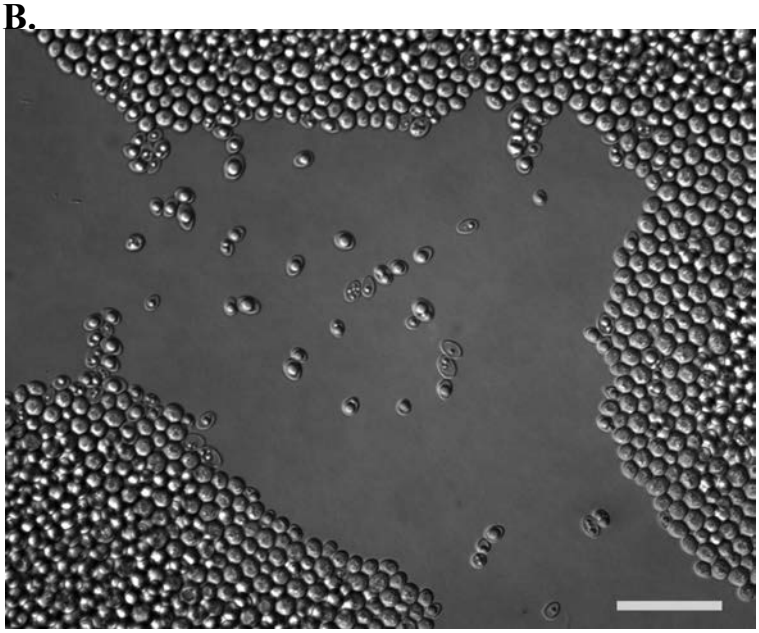
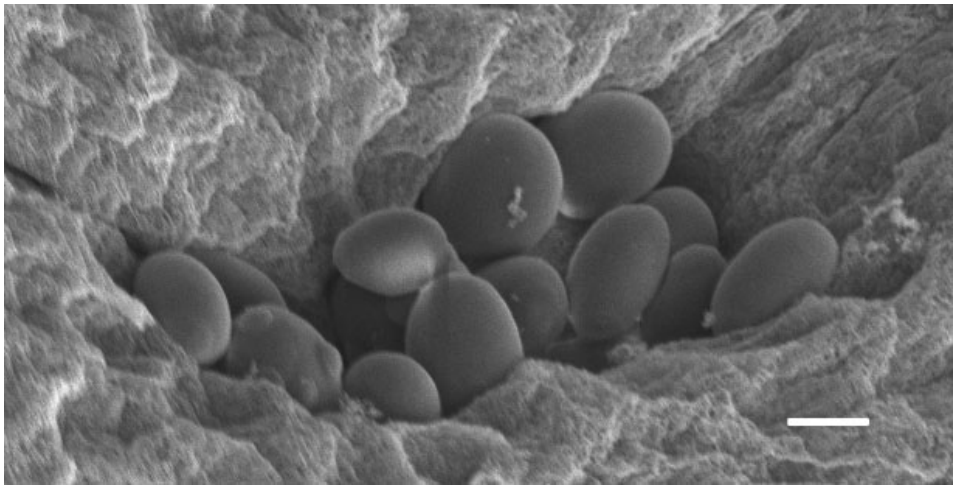


Figure 20. Scanning electron micrograph illustrating oval blastoconidia (bar = 2 μm).



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DIRECT DETECTION (*Cryptococcus neoformans* ANTIGEN TEST)

Introduction: In early 1960s, a simple, sensitive latex test, capable of detecting the capsular polysaccharide of *C. neoformans* in serum, was described. The test proved superior in sensitivity to the India ink mount of CSF from suspected patients. Further clinical studies established the prognostic value of the test and showed it to be a valuable aid in establishing a diagnosis when culture was negative. Paired serum and CSF specimens allowed detection of antigen in confirmed cases. In early 1990s, an enzyme immunoassay based upon monoclonal antibody against capsular polysaccharide, was described. More recently, a lateral flow immunoassay was described as an immunochromatographic test system for the qualitative or semi-quantitative detection of the capsular polysaccharide antigens of *C. neoformans* and *C. gattii* complex in serum and CSF.

Materials: Sixty-five laboratories participated in the September 30, 2015 direct antigen detection test event. Three negative (Cn-Ag-2, Cn-Ag-3 and Cn-Ag-4), and two positive (Cn-Ag-1 and Cn-Ag-5) serum samples cor cryptococcus antigen were included.

Results: There was consensus for specimen Cn-Ag-2, Cn-Ag-3 and Cn-Ag-4 as negative except that one laboratory reported Cn-Ag-3 as positive. The summary of laboratory performance for Cn-Ag-1 and Cn-Ag-5 is shown in Table 2. The acceptable titer ranges for Cn-Ag-2 were 1:8 to 1:128 or 1:10 to 1:160, and for Cn-Ag-5 were 1:32 ~ 1:1024 or 1:40 ~ 1:1280 based on kits used for antigen detection. Three laboratories reported the titer lower and two laboratories reported titer higher than the acceptable range for Cn-Ag-1. One laboratory reported lower and higher than the expected range for Cn-Ag-5. One laboratory reported negative titer for Cn-Ag-5.

Table 2. Summary of laboratories performance for cryptococcal antigen test

	Cn-Ag-1 Titers													
	4	8	10	16	20	32	40	64	80	128	160	132		
No. Labs	3	4	1	6	3	17	6	10	2	2	4	2		
	Cn-Ag-5 Titers													
	16	32	40	64	80	128	160	256	320	512	640	1024	1280	2048
No. Labs	1	3	1	9	2	13	5	12	2	2	3	2	4	1

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ANTIFUNGAL SUSCEPTIBILITY TESTING FOR YEASTS

Introduction: Clinical laboratories perform susceptibility testing of pathogenic yeasts to determine their *in vitro* resistance to antifungal drugs. This test is also useful in conducting surveillance for evolving patterns of antifungal drug resistance in a healthcare facility. The results are likely to facilitate the selection of appropriate drugs for treatment. Clinical Laboratory Standards Institute (CLSI) documents of M27-A3, M27-S3, 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 albicans* (S-1) was the analyte in the September 30, 2015 antifungal proficiency testing event. The interpretation of MIC values for antifungal susceptibility testing of yeasts and molds is in a state of constant change. These changes are necessitated by new information emerging from clinical trials and laboratory susceptibility testing. NYSDOH Mycology Laboratory uses the consensus/all participating laboratories' MIC values within +/- 2 dilutions and then the interpretation per latest CLSI and EUCAST documents to score proficiency testing results. Especially, when there is no interpretation, MIC values are the key judge points. However, the participating laboratories are advised to regularly consult these organizations for the latest version of their standard documents.

Comments: Acceptable results were MICs +/-2 dilutions of the reference laboratory results for any single drug. The reported results were as follows: fluconazole (32 laboratories), itraconazole (24 laboratories), voriconazole (28 laboratories), caspofungin (24 laboratories), 5-flucytosine (22 laboratories), amphotericin B (21 laboratories), anidulafungin (19 laboratories), micafungin (18 laboratories), posaconazole (18 laboratories), and ketoconazole (3 laboratories). The MIC results and MIC interpretations by participating laboratories are summarized in Table 3 & 4. The MIC interpretations for several antifungal drugs against *C. albicans* are significantly different based on CLSIM27-S3 and CLSI M27-S4 documents. Therefore, we strongly suggest that laboratories should follow the latest CLSI guidelines for interpretation of MIC data.

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

S-1: *Candida albicans*

Drug	No. labs	MIC (µg/ml)															
		0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
Amphotericin B	21						2	18	1								
Anidulafungin	19		13	6													
Caspofungin	25			1	3	10	10		1								
Flucytosine (5-FC)	22			1	13	5	3										
Fluconazole	32*											1		2	6	18	4
Itraconazole	24*							18	5								
Ketoconazole	3*						1	1									
Micafungin	18			16	2												
Posaconazole	18			1				5	11	1							
Voriconazole	28					1	1	2	13	10	1						

* One laboratory used disk diffusion method with MIC of 6.0 for fluconazole & itraconazole & MIC of 19.0 for ketoconazole

Colors represent the testing method used:

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

Table 4. Antifungal Susceptibility Interpretations Reported by the Participating Laboratories

S-1: *Candida albicans*

Drug	No. laboratories	Susceptible	Susceptible-dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	21	4					17
Anidulafungin	19	19					
Caspofungin	25	24			1		
Flucytosine (5-FC)	22	15					7
Fluconazole	32				32		
Itraconazole	24		12		5		7
Ketoconazole	3				1		2
Micafungin	18	18					
Posaconazole	18	5	2		1		10
Voriconazole	28	4	6	1	17		

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR MOLDS (EDUCATIONAL)

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

Materials: *Aspergillus fumigatus* 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 and one laboratory used TREK YeastOne Colorimetric method.

Comments: Four out of thirty-two laboratories, which hold yeast antifungal susceptibility testing permit, voluntarily participated in this test event for molds. Since too few laboratories have participated, no consensus could be generated (Table 5).

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	8.0	16	64	128	256
Amphotericin B	4							2	1	1						
Anidulafungin	4			2	2											
Caspofungin	4			1	2		1									
Fluconazole	4													2	1	1
Itraconazole	4												16			
Ketoconazole	3										1		2			
Micafungin	4		1	2	1			1	1	2						
Posaconazole	4							1	1	2						
Voriconazole	4							1	1	1	1					

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

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