

*M*ycology *P*roficiency *T*esting *P*rogram



*C*ritique *J*anuary 2002

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New York State Department of Health

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*T*est Specimens and Grading Policy

Test Specimens

A minimum of two strains of each of the proposed mold specimens were examined for inclusion in the proficiency test event of January 2002. The colony morphology of these strains was studied on Sabouraud dextrose agar. The microscopic morphologic features were examined by potato dextrose agar slide cultures. The physiological characteristics, such as cycloheximide sensitivity, and growth at higher temperatures, were investigated with the appropriate test media. The single strain that best demonstrated the morphologic and physiologic characteristics of the proposed fungal pathogen was used in the test. Similarly, two or more strains of each of the proposed yeast pathogens were examined for inclusion in the proficiency test. The morphology of all yeast isolates was studied on Cornmeal - Tween 80 agar plates inoculated by the Dalmou or streak-cut method. Carbohydrate assimilation was studied with the API 20C AUX identification kit. The fermentations of carbohydrates, i.e., glucose, maltose, sucrose, lactose, trehalose, and cellobiose, were also investigated. Additionally, physiologic characteristics, such as nitrate assimilation, urease activity, and cycloheximide sensitivity, were investigated with the appropriate test media. The single strain that best demonstrated the morphologic and physiologic characteristics of each of the proposed yeast pathogens was used in the test.

Grading Policy

A laboratory's response for each sample is compared with the response that reflects 90 percent agreement of 10 referee laboratories or 90 percent 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 grading formula used for each specimen is:

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

Participating laboratories must achieve a score of 80% or better on two (2) of three (3) consecutive test events to maintain acceptable proficiency levels.

Acceptable results for antifungal susceptibility testing are MICs within +/-2 dilutions from the reference results for a particular organism against single drug. If these results were outside the range, the lab gets a score of zero for that particular test component or set. The current testing format is based on the following two drugs; amphotericin B and fluconazole. Five yeasts are to be tested against these two drugs. A test component/set involving one yeast against both drugs receives a maximum score of 20 (10 for first drug + 10 for second drug). The total score being $5 \times 20 = 100$. However, a lab that routinely does not perform test with either of the two drugs, is scored with the maximum score for single isolate against one drug. Again for five yeasts isolates, the total will be $20 \times 5 = 100$.

Answer Key

Mycology - General

	Specimen Key	Validated Specimen	Acceptable Answers
M-1	<i>Scedosporium prolificans</i>	<i>Scedosporium prolificans</i>	<i>Scedosporium inflatum</i>
M-2	<i>Epicoccum</i> species	<i>Epicoccum</i> species	
M-3	<i>Curvularia</i> species	<i>Curvularia</i> species	
M-4	<i>Aureobasidium pullulans</i>		
M-5	<i>Sporothrix schenckii</i>	<i>Sporothrix schenckii</i>	
Ed.sp.	<i>Stachybotrys</i> species		

Mycology - Yeast Only

	Specimen Key	Validated Specimen	Acceptable Answers
Y-1	<i>Candida famata</i>	<i>Candida famata</i>	<i>Torulopsis candida</i>
Y-2	<i>Candida rugosa</i>	<i>Candida rugosa</i>	
Y-3	<i>Candida krusei</i>	<i>Candida krusei</i>	<i>Candida krusei/inconspicua</i>
Y-4	<i>Cryptococcus uniguttulatus</i>	<i>Cryptococcus uniguttulatus</i>	
Y-5	<i>Candida lambica</i>		
Ed.sp.	<i>Prototheca zopfii</i>		

Mycology - Antifungal Susceptibility Testing for Yeast

	Specimen Key
S-1	<i>Candida krusei</i> ATCC 6258
S-2	<i>Candida albicans</i> ATCC 24433
S-3	<i>Candida albicans</i> ATCC 90028
S-4	<i>Candida parapsilosis</i> ATCC 22019
S-5	<i>Candida parapsilosis</i> ATCC 90018

Laboratory Results

Mycology - General

	Correct Responses / Total # Labs (%)	Referees (%)
M- 1 <i>Scedosporium prolificans</i>	71/79 (90)	10/10 (100)
M- 2 <i>Epicoccum</i> species	79/79 (100)	10/10 (100)
M- 3 <i>Curvularia</i> species	77/79 (97)	10/10 (100)
M- 4 <i>Aureobasidium pullulans</i>	67/79 (85)	9/10 (90)
M- 5 <i>Sporothrix schenckii</i>	77/79 (97)	10/10 (100)

Mycology - Yeast Only

	Correct Responses / Total # Labs (%)	Referees (%)
Y - 1 <i>Candida famata</i>	60/70 (86)	10/10 (100)
Y - 2 <i>Candida rugosa</i>	69/70 (99)	10/10 (100)
Y - 3 <i>Candida krusei</i>	67/70 (96)	9/10 (90)
Y - 4 <i>Cryptococcus uniguttulatus</i>	70/70 (100)	10/10 (100)
Y - 5 <i>Candida lambica</i>	16/70 (23)	0/10 (0)

Mycology - Antifungal Susceptibility Testing for Yeasts

	Correct Responses / Total # Labs (%) Amphotericin B	Correct Responses / Total # Labs (%) Fluconazole
S- 1 <i>Candida krusei</i> ATCC 6258	14/14 (100)	14/14 (100)
S- 2 <i>Candida albicans</i> ATCC 24433	14/14 (100)	13/14 (93)
S- 3 <i>Candida albicans</i> ATCC 90028	12/14 (86)	14/14 (100)
S- 4 <i>Candida parapsilosis</i> ATCC 22019	13/14 (93)	14/14 (100)
S- 5 <i>Candida parapsilosis</i> ATCC 90018	12/14 (86)	14/14 (100)

Mycology - General

Number of participating laboratories	79
Number of referee laboratories	10
Number of laboratories responding by deadline	79
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	78
Number of laboratories unsuccessfully completing this test	1

Mycology - Yeast Only

Number of participating laboratories	70
Number of referee laboratories	10
Number of laboratories responding by deadline	70
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	69
Number of laboratories unsuccessfully completing this test	1

Mycology - Antifungal Susceptibility Testing for Yeasts

Number of participating laboratories	14
Number of referee laboratories	14
Number of laboratories responding by deadline	14
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	13
Number of laboratories unsuccessfully completing this test	1

Commercial Identification Systems Used*

AMS Vitek system.....	27
API 20C AUX.....	37
Microscan.....	05
Remel Uni-Yeast-Tek.....	04
Other	01

(* Includes multiple systems used by some labs)

M-1 *Scedosporium prolificans*

Source: Bone

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	71
Labs with incorrect ID:	8
<i>(Scedosporium apiospermum)</i>	<i>(8)</i>

Clinical Significance: A frequent cause of osteomyelitis and arthritis following traumatic inoculation. Few cases of endocarditis have been reported (1). Disseminated diseases have been reported in immunocompromised and transplant patients. Colonization of respiratory tract has been reported (3).

Ecology: Cosmopolitan, found in soil.

Laboratory Diagnosis:

- Culture** – *Scedosporium prolificans* is a moderately fast growing fungus; colonies measuring up to 3 cm in a week. At 25° C, on Sabouraud's dextrose agar, initial growth is white, later becoming brownish-gray to black, wooly in texture, and reverse turning pale brown (Fig. 1).
- Microscopic morphology** – Lactophenol cotton blue or Calcofluor mounts show hyaline septate hyphae. Conidiophores: flask-shaped with swollen bases and annellations at the apex. Conidia: smooth, oval shaped with truncated bases, brown, formed in clusters (Fig. 2).
- Differentiation from other fungi** – *Scedosporium prolificans* is differentiated from *S. apiospermum* by its conidiophores with inflated base and annellations, positive growth at 45° C, and inhibition of growth by cycloheximide. *S. prolificans* does not convert to yeast phase on rich media at 37° C, unlike *Sporothrix schenckii* and *Blastomyces dermatitidis*.
- Molecular tests** – Ribosomal DNA internal transcribed spacers (ITS) regions were analyzed for *Scedosporium inflatum* and *Lomentospora prolificans*. It was found to be similar and hence the nomenclature *Scedosporium prolificans* (5). ITS restriction fragment length polymorphism differentiates *S. prolificans* from *S. apiospermum* (5). Based on large subunit ribosomal RNA sequences, *S. prolificans* and *Pseudallescheria boydii* were closely related (4). Randomly amplified polymorphic DNA (RAPD) and PCR-fingerprinting with phage M13 provided a means of typing *S. prolificans* isolates (6,7).
- In vitro susceptibility testing** – Almost all clinical isolates of *S. prolificans* has high MIC values against amphotericin B, flucytosine, fluconazole, itraconazole, ketoconazole, but they are variably susceptible to miconazole (2).

Comments: Eight labs reported this organism as *S. apiospermum* based on the microscopic features such as conidiophores with annellides and single conidia. Perhaps these labs did not perform additional tests to differentiate between *S. prolificans* and *S. apiospermum* i.e. growth on Mycosel agar and at 45° C.

Further reading:

- Carreter de Granda, M. E., C. Richard, E. Conde, A. Iriondo, F. Marco de Lucas, R. Salesa, and A. Zubizarreta. 2001. Endocarditis caused by *Scedosporium prolificans* after autologous peripheral blood stem cell transplantation. *Eur J Clin Microbiol Infect Dis.* **20**: 215-7.
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- Idigoras, P., E. Perez-Trallero, L. Pineiro, J. Larruskain, M. C. Lopez-Lopategui, N. Rodriguez, and J. M. Gonzalez. 2001. Disseminated infection and colonization by *Scedosporium prolificans* a review of 18 cases, 1990-1999. *Clin Infect Dis.* **32**: E158-65.
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- Lennon, P. A., C. R. Cooper, Jr., I. F. Salkin, and S. B. Lee. 1994. Ribosomal DNA internal transcribed spacer analysis supports synonymy of *Scedosporium inflatum* and *Lomentospora prolificans*. *J Clin Microbiol.* **32**: 2413-6.
- Ruiz-Diez, B., F. Martin-Diez, J. L. Rodriguez-Tudela, M. Alvarez, and J. V. Martinez-Suarez. 1997. Use of random amplification of polymorphic DNA (RAPD) and PCR-fingerprinting for genotyping a *Scedosporium prolificans* (inflatum) outbreak in four leukemic patients. *Curr Microbiol.* **35**: 186-90.
- San Millan, R., G. Quindos, J. Garaizar, R. Salesa, J. Guarro, and J. Ponton. 1997. Characterization of *Scedosporium prolificans* clinical isolates by randomly amplified polymorphic DNA analysis. *J Clin Microbiol.* **35**: 2270-4.

M - 2 *Epicoccum* species

Source: Nail

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	79

Clinical Significance: *Epicoccum* species has not been reported as a causative agent of infections in humans. It is implicated as an allergen (1,2).

Ecology: Cosmopolitan, found in soil.

Laboratory Diagnosis:

1. **Culture** – *Epicoccum* species is a fast growing fungus; colonies measure up to 3 cm in 3 days. At 25° C, on Sabouraud's dextrose agar, colonies are yellowish orange, later becoming brown, wooly in texture, with deep brown reverse, with diffuse orange pigment (Fig.3).
2. **Microscopic morphology** – Lactophenol cotton blue or Calcofluor mounts show hyaline septate hyphae, often brownish in color. Conidiophores are short, aggregate with vegetative hyphae to form sporodochia. Rough, black to brown muriform conidia, measuring up to 25 µm in diameter are formed along the sporodochium (Fig. 4).
3. **Differentiation from other fungi** – *Epicoccum* species is distinguished from other molds by its rapid growth, orange-brown colony; microscopically, clumps of dark brown muriform conidia are formed along the sporodochia.
4. **Molecular tests** – N/A
5. **In vitro susceptibility testing** – N/A

Comments: All the participating labs correctly identified this common contaminant.

Further reading:

1. Black, P. N., A. A. Udy, and S. M. Brodie. 2000. Sensitivity to fungal allergens is a risk factor for life-threatening asthma. *Allergy* 55: 501-4.

2. Bisht, V., B. P. Singh, N. Arora, S. Sridhara, and S. N. Gaur. 2000. Allergens of *Epicoccum nigrum* grown in different media for quality source material. *Allergy* 55: 274-80.

3. Noble, J. A., S. A. Crow, D. G. Ahearn, and F. A. Kuhn. 1997. Allergic fungal sinusitis in the south-eastern USA: involvement of a new agent *Epicoccum nigrum* Ehrenb. ex Schlecht. 1824. *J Med Vet Mycol* 35: 405-9.

4. Simmons, R. B., D. L. Price, J. A. Noble, S. A. Crow, and D. G. Ahearn. 1997. Fungal colonization of air filters from hospitals. *Am Ind Hyg Assoc J* 58: 900-4.

M-3 *Curvularia* species

Source: Eye

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	77
Labs with incorrect ID:	2
(<i>Bipolaris</i> species)	(2)

Clinical Significance: An infrequent cause of sinusitis, keratitis, endocarditis, mycetoma, and cerebral abscess. Few cases of disseminated infection have been reported in immunocompromised patients.

Ecology: Cosmopolitan, pathogen of tropical and subtropical plants.

Laboratory Diagnosis:

1. **Culture** – *Curvularia* is a fast growing fungus; colonies measure up to 5 cm in 3 days. At 25° C, on Sabouraud's dextrose agar, colonies are initially white, later becoming brownish black, woolly in texture, reverse turning black (Fig.5).
2. **Microscopic morphology** – Lactophenol cotton blue or Calcofluor mounts show brown septate hyphae: conidiophores brown, geniculate with poroconidia slightly curved, brown, 3-4 transverse septations, central cell larger and darker than the other cells (Fig. 6).
3. **Differentiation from other fungi** – *Curvularia* species is differentiated from other dark muriform fungi by its rapid growth. Microscopically, multicellular, subtly curved conidia with large and dark central cell. In *Bipolaris* species and *Drechslera* species, the conidia are distoseptate, while in *Curvularia* species, the conidia are transversely septate.
4. **Molecular tests** - Analysis of genes coding for small subunit rRNA sequences of dematiaceous fungal pathogens provided means of assessing relationships of pathogenic and non-pathogenic forms, and accurate identification (6). Electrophoretic karyotyping of *Curvularia lunata* demonstrated that there are 12 chromosomes, size ranging from 1.4 to 4.0 Mb (5).
5. **In vitro susceptibility testing** – Most clinical isolates are susceptible to amphotericin B, itraconazole, miconazole and ketoconazole, but resistant to flucytosine and fluconazole (2, 4).

Comments: Except two labs, all the labs reported correct identification. Microscopically, immature conidia of *Curvularia* species can be mistaken for those of *Bipolaris* species.

Further reading:

1. Canon, H. L., S. C. Buckingham, R. J. Wyatt, and D. P. Jones. 2001. Fungal peritonitis caused by *Curvularia* species in a child undergoing peritoneal dialysis. *Pediatr Nephrol.* 16: 35-7.
2. Guarro, J., T. Akiti, R. A. Horta, L. A. Morizot Leite-Filho, J. Gene, S. Ferreira-Gomes, C. Aguilar, and M. Ortoneda. 1999. Mycotic keratitis due to *Curvularia senegalensis* and in vitro antifungal susceptibilities of *Curvularia* spp. *J Clin Microbiol.* 37: 4170-3.
3. Kaushik, S., J. Ram, A. Chakrabarty, M. R. Dogra, G. S. Brar, and A. Gupta. 2001. *Curvularia lunata* endophthalmitis with secondary keratitis. *Am J Ophthalmol.* 131: 140-2.
4. Llop, C., J. Sala, M. D. Riba, and J. Guarro. 1999. Antimicrobial susceptibility testing of dematiaceous filamentous fungi: effect of medium composition at different temperatures and times of reading. *Mycopathologia.* 148: 25-31.
5. Osiewacz, H. D., and R. Ridder. 1991. Genome analysis of imperfect fungi: electrophoretic karyotyping and characterization of the nuclear gene coding for glyceraldehyde-3-phosphate dehydrogenase (gpd) of *Curvularia lunata*. *Curr Genet.* 20:151-5.
6. Spatafora, J. W. 1995. Analysis of genes coding for small-subunit rRNA sequences in studying phylogenetics of dematiaceous fungal pathogens. *J Clin Microbiol.* 33: 1322-26.

M - 4 *Aureobasidium pullulans*

Source: Sputum

Scoring:	No. Labs
Referee Labs with correct ID:	9
Labs with correct ID:	67
Labs with incorrect ID:	12
(<i>Exophiala</i> species)	(1)
(<i>Phaeoannellomyces</i> species)	(10)
(<i>Wangiella dermatitidis</i>)	(1)

Clinical Significance: An infrequent cause of keratitis, peritonitis, and pulmonary infection. Few cases of disseminated infection have been reported in immunocompromised patients.

Ecology: Cosmopolitan, commonly isolated from aerial parts of plants.

Laboratory Diagnosis:

1. **Culture** – *Aureobasidium pullulans* is a fast growing fungus; colonies measure up to 3 cm in 3 days. At 25° C, on Sabouraud's dextrose agar, colonies are creamy, moist, initially white, later becoming black (often in sectors) with pale reverse (Fig.7).
2. **Microscopic morphology** – Lactophenol cotton blue or Calcofluor mounts show hyaline septate hyphae turning dark with age. Pale blastoconidia are produced synchronously in clusters. Dark arthroconidia and chlamydoconidia are formed with age (Fig. 8).
3. **Differentiation from other fungi** – *A. pullulans* is differentiated from other dark fungi by its rapid growth, initially white-pink colonies, later turning black, blastoconidia produced synchronously in tufts and formation of dark chlamydoconidia and arthroconidia. *Hormonema dematioides* produces blastoconidia successively from a single opening, unlike synchronous blastoconidia formation by *A. pullulans* (8). *Phaeoannellomyces* species forms dark colonies, annelloconidia are formed sympodially or percurrently either from undifferentiated conidiogenous cells or from long well differentiated conidiophores. The annellations of *Phaeoannellomyces* species may be confused under the light microscope (1, 5).
4. **Molecular tests** – Analysis of genes coding for small subunit rRNA sequences of dematiaceous fungal pathogens provided means of accurate identification (7). Oligonucleotide probe for *Aureobasidium pullulans* was developed based on the small subunit rRNA gene for identification from leaf surfaces and other microbial communities (4). The nuclear subunit rRNA genes of various black molds were amplified by PCR and directly sequenced (2). Alignment with corresponding sequences was performed and a phylogenetic tree was constructed that demonstrated *Exophiala*, *Wangiella* are closely related (9). RAPD technique was sensitive to discriminate among the strains of *A. pullulans* isolated from rocks and other habitat (10).
5. **In vitro susceptibility testing** – Susceptibility testing results indicate that isolates are susceptible to amphotericin B, flucytosine, itraconazole, and ketoconazole, but less susceptible to fluconazole (6, 8).

Comments: This specimen was not validated as only 85% of the participating labs and 9/10 reference labs identified it correctly. Ten of the participating labs reported this organism as *Phaeoannellomyces* species based up on the annelloconidia formation and dark, thick walled chlamydoconidia. However, *A. pullulans* does not produce annelloconidia. It produces blastconidia that are formed synchronously in clusters. Also, one lab each reported this organism as *Exophiala* species or *Wangiella dermatitidis* based on the microscopic morphology.

Further reading:

1. Cole, C. T. 1976. Conidiogenesis in pathogenic hyphomycetes. I. *Sporothrix*, *Exophiala*, *Geotrichum* and *Microsporium*. *Sabouraudia*. **14**: 81-98.
2. Haase, G., L. Sonntag, Y. van de Peer, J. M. Uijthof, A. Podbielski, and B. Melzer-Krick. 1995. Phylogenetic analysis of ten black yeast species using nuclear small subunit rRNA gene sequences. *Antonie Van Leeuwenhoek*. **68**: 19-33.
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5. Nishimura, K., and M. Miyaji. 1985. Further studies on the phylogeny of the genus *Exophiala* and *Hortaea*. *Mycopathologia*. **92**: 101-9.
6. Shin, J. H., S. K. Lee, S. P. Suh, D. W. Ryang, N. H. Kim, M. G. Rinaldi, and D. A. Sutton. 1998. Fatal *Hormonema dematioides* peritonitis in a patient on continuous ambulatory peritoneal dialysis: criteria for organism identification and review of other known fungal etiologic agents. *J Clin Microbiol*. **36**: 2157-63.
7. Spatafora, J. W. 1995. Analysis of genes coding for small-subunit rRNA sequences in studying phylogenetics of dematiaceous fungal pathogens. *J Clin Microbiol*. **33**: 1322-26.
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9. Uijthof, J. M., and G. S. de Hoog. 1995. PCR-ribotyping of type isolates of currently accepted *Exophiala* and *Phaeococcomyces* species. *Antonie Van Leeuwenhoek*. **68**: 35-42.
10. Urzi, C., F. De Leo, C. Lo Passo, and G. Criseo. 1999. Intra-specific diversity of *Aureobasidium pullulans* strains isolated from rocks and other habitats assessed by physiological methods and by random amplified polymorphic DNA (RAPD). *J Microbiol Methods*. **36**: 95-105.

M - 5 *Sporothrix schenckii*

Source: Skin lesions

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	77
Labs with incorrect ID:	2
(<i>Acremonium</i> species)	(1)
(<i>Exophiala</i> species)	(1)

Clinical Significance: It is the causal agent of sporotrichosis, a subcutaneous infection at the site of implantation. It spreads in the body via lymphatic system. It is often called as “rose handler’s disease” because of the increased frequency in this group of individuals. Pulmonary and disseminated sporotrichosis are infrequently reported. Laboratory acquired infections have also been reported.

Ecology: Cosmopolitan, isolated from soil and decaying plant materials. Sphagnum moss and rose bushes are well known source of this organism.

Laboratory Diagnosis:

- Culture** – *Sporothrix schenckii* is a slow growing fungus; colonies measure up to 1 cm in a week. *S. schenckii* is a thermally dimorphic fungus. At 25° C, on Sabouraud’s dextrose agar, colonies are initially white, becoming black on the surface and on the reverse in a week, wrinkled and glabrous in texture (Fig.9). At 37° C, on enriched media like blood agar, brain heart infusion agar, colonies are cream to buff color, creamy in texture and grow in 2 weeks.
- Microscopic morphology** – Lactophenol cotton blue or Calcofluor mounts show thin, hyaline septate hyphae; conidiophores are slender and tapering. At the end of conidiophores, ovoid, hyaline conidia are formed sympodially (rosette formation) (Fig. 10). At 37° C, ovoid, single or multiple budding yeast cells are seen.
- Differentiation from other fungi** – *S. schenckii* is differentiated from other fungi by its slow growth, initially white colonies turning black, microscopically ovoid conidia produced sympodially (rosette formation). Nonpathogenic *Sporothrix* species does not convert to yeast phase at 37° C on enriched media. *Ophiostoma stenoceras*, a nonpathogenic organism microscopically resembles *Sporothrix*, but produces long necked perithecia after 2-3 weeks. *Exophiala* species produces annelloconidia, while *Phialophora* species produces phialoconidia, thus differentiating it from *S. schenckii*.
- Molecular tests** – Using universal fungal primers (ITS1 and ITS4) directed towards the rRNA gene, PCR amplification was performed, then the amplicons were detected by enzyme immunoassay (EIA) colorimetrically, hybridization using specific oligonucleotide probes were used to identify dimorphic and yeast-like pathogens (6). Mitochondrial DNA analysis of *Sporothrix schenckii* for epidemiology purposes has been done in various continents (2, 3, 4). Karyotyping by pulse field gel electrophoresis (PFGE) of clinical isolates demonstrated 6-8 chromosomes and genome size of 28 Mbp (8).
- In vitro susceptibility testing** – Susceptibility testing results indicate that isolates are susceptible to amphotericin B, itraconazole, and ketoconazole, but less susceptible to fluconazole (5,7).

Comments: Two of the participating labs reported this organism as *Acremonium* species or *Exophiala jeanselmei* based on the microscopic morphology. *Acremonium* species produces long phialides along with round to oval conidia, and *Exophiala jeanselmei* produces conidiophores bearing single or chains of annelloconidia at the tip. However, *S. schenckii* produces initial white colonies turning black, microscopically sympodially formed oval conidia, and yeasts at 37° C on enriched media.

Further reading:

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Source: Lung

Clinical Significance: *Stachybotrys* does not cause infections in humans. However, inhalation or absorption of the toxins is associated with respiratory symptoms in infants (1,5, 6).

Ecology: Cosmopolitan, found in indoor habitats; on moist areas of wallpaper, carpet, sheet rock, papers, etc.

Laboratory Diagnosis:

1. **Culture** – *Stachybotrys* species is a moderately rapid growing fungus; colonies measure up to 3 cm in 4 days. At 25^o C, on Sabouraud's dextrose agar, colonies are buff to black in color, with orange-black reverse, powdery in texture (Fig.11).
2. **Microscopic morphology** – Lactophenol cotton blue or Calcofluor mounts show hyaline septate hyphae: conidiophores hyaline to black, simple. Conidiophores end in phialides, which are in groups of 3-10 at the tip of conidiophores, conidia are black, unicellular, formed in slimy masses at the tip of phialides (Fig. 12).
3. **Differentiation from other fungi** – *Stachybotrys* species is differentiated from other fungi by its moderately rapid growth; surface and reverse of colonies are black, and conidia are produced in slimy masses. *Memnoniella* species produces conidia in chains, unlike *Stachybotrys*.
4. **Molecular tests** – A fungus specific PCR assay was developed using only one primer set for detecting indoor fungi (7). Method for quantification of *S. chartarum* conidia using real-time, fluorescence probe based detection of PCR products was developed (4). Using sequences for the 18 S rRNA gene of *S. chartarum*, primers were designed for specific identification and quantification of *S. chartarum* (2).
5. **In vitro susceptibility testing** – N/A

Further reading:

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Y-1 *Candida famata*

Source: Blood

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	60
Labs with incorrect ID:	10
<i>(Candida guilliermondii)</i>	<i>(10)</i>

Clinical Significance: An infrequent causal agent of nosocomial fungemia in immunosuppressed patients. Also, rare causative agent of ocular infections, arthritis, and peritonitis.

Epidemiology: Cosmopolitan, found in plants, soil and dairy products.

Laboratory Diagnosis:

1. **Culture** – At 25^o C, on Sabouraud's dextrose agar, colonies are white to yellowish, soft, smooth to slightly wrinkled in 3 –5 days (Fig. 13).
2. **Microscopic morphology** – On corn meal agar with Tween – 80, round to oval blastoconidia with no or rudimentary pseudohyphae but with longer incubation (more than a week), primitive or well-developed pseudohyphae are seen (Fig. 14).
3. **Differentiation from other yeasts** – *Candida famata* ferments glucose, sucrose and trehalose, grows at 37^o C, and grows on media containing cycloheximide. It forms primitive to well developed pseudohyphae on corn meal agar or Dalmau plate, when incubated longer, which differentiates it from *C. guilliermondii*. It does not produce true hyphae, which differentiates it from *C. ciferrii*. It does not grow at 45^o C, differentiating from *C. lusitaniae*.
4. **Molecular tests** – Primers for large ribosomal subunit DNA sequences were used in PCR to differentiate between *C. famata* and *C. guilliermondii* (5). The amplification of 340 bp of the large rDNA led to rapid and specific identification of *C. famata* (6). RAPD-PCR analysis was applied to identify *C. famata* in dairy product (1).
5. **In vitro susceptibility testing** – Almost all clinical isolates are susceptible to amphotericin B, 5 FC, and azoles such as fluconazole, itraconazole, and ketoconazole (2, 3).

Comments: Ten of the participating labs identified this isolate as *C. guilliermondii*. In the API 20C AUX yeast identification system *C. famata* and *C. guilliermondii* are assigned the following biocode, such as 6356173, 6356371, 6356372, 6356373, 6366373, 6373373, 6376173, and 6376333. However, *C. famata* infrequently assimilates melezitose and raffinose (60%), while *C. guilliermondii* assimilates these two carbohydrates frequently (90%). Additionally, morphology on corn meal agar or Dalmau plate differentiates these two organisms. *C. famata*, when incubated for longer time on corn meal agar, produces rudimentary to well differentiated pseudohyphae, while *C. guilliermondii* produces few to moderate pseudohyphae.

Further Reading:

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Source: Peritoneum

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	69
Labs with incorrect ID:	1
<i>(Trichosporon asahii)</i>	<i>(1)</i>

Clinical Significance: An infrequent causal agent of fungemia in indwelling catheter patients. Also, reported to cause infection in burn patients who are treated with topical nystatin (4).

Ecology: Cosmopolitan, found in dairy products.

Laboratory Diagnosis:

1. **Culture** – At 25^o C, colonies on Sabouraud's dextrose agar are white to cream, wrinkle in 2 – 3 days (Fig. 15).
2. **Microscopic morphology** – On corn meal agar with Tween 80, branched pseudohyphae with chains of elongated blastoconidia are seen (Fig. 16).
3. **Differentiation from other yeasts** – *Candida rugosa* ferments only glucose, does not grow on media containing cycloheximide, variable growth at 42^o C, and is urea and nitrate negative. Microscopically, it forms branched pseudohyphae that differentiates it from *C. lusitanae* and *C. parapsilosis*. It does not form true hyphae, differentiating it from *Trichosporon beigeli*.
4. **Molecular tests** – PCR assay of the ITS1 and ITS2 region of ribosomal DNA was developed to identify *Candida rugosa* in clinical specimens. A repetitive sequence-based PCR technique was developed to characterize the genotypic relatedness among *C. rugosa* isolates (5). Karyotyping by PFGE was developed as a typing tool for discrimination among strains of *C. rugosa* (3).
5. **In vitro susceptibility testing** – Clinical isolates are susceptible to 5FC, and various azoles like itraconazole, ketoconazole and fluconazole. It is less susceptible to polyene antifungals like amphotericin B and nystatin (2, 4, 8).

Comments: One participating lab reported this isolate as *Trichosporon asahii* probably based on corn meal agar morphology. *C. rugosa* produces branched pseudohyphae, but not true hyphae. In API 20C AUX yeast identification system, separate biocodes are generated for *C. rugosa* and *T. asahii*.

Further Reading:

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Source: Catheter

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	67
Labs with incorrect ID:	3
(<i>Blastoschizomyces capitatus</i>)	(1)
(<i>Candida lambica</i>)	(1)
(<i>Candida zeylanoides</i>)	(1)

Clinical Significance: A frequent causal agent of nosocomial fungemia in immunosuppressed patients. It also causes disseminated disease including endocarditis, peritonitis, vaginitis, urinary tract infections, and sinusitis (1, 6, 8).

Ecology: Cosmopolitan, found in air, dairy products and on man.

Laboratory Diagnosis:

- Culture** – At 25° C, on Sabouraud's dextrose agar, colonies are dry but soft, cream to buff, glassy surface, wrinkled in 2-3 days (Fig. 17).
- Microscopic morphology** – On corn meal agar with Tween 80, long, branched pseudohyphae with elongated blastoconidia are seen (Fig. 18).
- Differentiation from other yeasts** – *Candida krusei* ferments glucose, but not sucrose or cellobiose, and does not grow on the media containing cycloheximide. It does not assimilate sucrose, differentiating it from *C. parapsilosis* and *C. lusitaniae*. It grows well at 42° C, differentiating it from *C. lambica*. It does not produce arthroconidia, thus differentiating it from *B. capitatus*. CHROMagar Candida has been used for rapid, presumptive identification of *C. krusei* (10).
- Molecular tests** – DNA probes have been designed from the ITS regions and were incorporated into a reverse hybridization line probe assay for the detection of ITS PCR products for identification of fungal pathogens (7). Panfungal PCR and multiplex liquid hybridization were developed for the detection of clinically important yeasts in tissue specimens (4). PFGE, RFLP and RAPD procedures were used for DNA fingerprinting and electrophoretic karyotyping of oral *C. krusei* isolates (2, 3).
- In vitro susceptibility testing** – Clinical isolates are susceptible to amphotericin B and flucytosine. *C. krusei* is innately resistant to fluconazole and variably resistant to other azoles such as itraconazole and ketoconazole (5, 8).

Comments: Three of the participating labs reported this isolate as *B. capitatus*, *C. lambica*, or *C. zeylanoides*. *B. capitatus* produces arthroconidia on corn meal agar, *C. lambica* does not grow at 42° C, and in API 20C AUX biocode for *C. zeylanoides* is distinct from *C. krusei*.

Further Reading:

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Y-4 *Cryptococcus uniguttulatus*

Source: CSF

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	70

Clinical Significance: *Cryptococcus uniguttulatus* has not been reported as a causative agent of infections in humans till recently. However, a case of ventriculitis due *C. uniguttulatus* has just been documented (4).

Ecology: Found on leaves and in soil.

Laboratory Diagnosis:

1. Culture – At 25⁰ C, on Sabouraud's dextrose agar colonies are smooth, dull, soft, cream colored, in 3 days (Fig.19).
2. Microscopic morphology – On cornmeal agar with Tween 80, round blastoconidia are seen (Fig. 20). No pseudo- or true hyphae are formed.
3. Differentiation from other yeasts- *C. uniguttulatus* does not ferment any carbohydrate, does not grow at 37⁰ C or on the media containing cycloheximide. It produces urease enzyme. It does not form brown colonies on caffeic seed agar, thus differentiating from *C. neoformans*. It does not assimilate nitrate, differentiating from *C. albidus*. *C. laurentii* assimilates lactose and dulcitol, but *C. uniguttulatus* does not assimilate these carbohydrates.
4. Molecular tests – N/A
5. In vitro susceptibility testing – A single clinical isolate was susceptible to amphotericin B and itraconazole (4).

Comments: All of the participating labs correctly identified this isolate.

Further Reading:

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4. McCurdy, L. H., and J. D. Morrow. 2001. Ventriculitis due to *Cryptococcus uniguttulatus*. South Med J. 94: 65-6.

Y-5 *Candida lambica*

Source: Lung biopsy

Scoring: **No. Labs**

Referee Labs with correct ID: 0

Labs with correct ID: 16

Labs with incorrect ID: 54

<i>(Blastoschizomyces capitatus)</i>	(7)	<i>(Candida species)</i>	(2)
<i>(Candida glabrata)</i>	(3)	<i>(Candida zeylanoides)</i>	(4)
<i>(Candida inconspicua)</i>	(6)	<i>(Geotrichum capitatum)</i>	(1)
<i>(Candida krusei)</i>	(5)	<i>(Hansenula anomala)</i>	(2)
<i>(Candida lipolytica)</i>	(1)	<i>(Saccharomyces cerevisiae)</i>	(2)
<i>(Candida norvegensis)</i>	(20)	<i>(Sporobolomyces salmonicolor)</i>	(1)

Clinical Significance: *Candida lambica* has not been reported as a causative agent of infections in humans. However, it is often isolated from blood, sputum, and urine as a contaminant. A case report due to *Candida lambica* has recently been reported (8).

Ecology: Found in dairy products, water, and fruits.

Laboratory Diagnosis:

- Culture** – At 25° C, on Sabouraud's dextrose agar, colonies are smooth to wrinkled, cream-colored, and soft in 3 days (Fig. 21).
- Microscopic morphology** – On corn meal agar with Tween 80, long, branched pseudohyphae with oval to elongated blastoconidia are seen (Fig. 22).
- Differentiation from other yeasts** – *Candida lambica* ferments glucose, does not grow on media containing cycloheximide. It does not assimilate sucrose, which differentiates it from *C. parapsilosis* and *C. lusitanae*. It does not grow at 42° C, which differentiates it from *C. krusei*.
- Molecular tests** – Restriction analysis of PCR amplified ITS2 region of rDNA was developed for rapid and reliable identification of yeasts (1, 2). PCR-reverse cross blot hybridization assay was developed for direct detection and identification of yeasts in clinical samples (7).
- In vitro susceptibility testing** – Few isolates studied are susceptible to amphotericin B, flucytosine and azoles (5, 6).

Comments: This specimen was not validated. Of the participating labs, only 16 labs correctly identified this isolate. In the API 20C AUX and Vitek 1 and 2 yeast identification database, this yeast is not listed. *Candida lambica*, *Candida norvegensis*, *Candida krusei*/*inconspicua*, *Candida lipolytica*, *Candida glabrata*, *Geotrichum candidum*, and *Blastoschizomyces capitatus* are assigned same biocode in these commercial systems. Additional tests have been recommended by the manufacturer to differentiate among these organisms. Based on the first or preferred assigned biocode, these organisms are misidentified if the additional tests are not done. Four of the participating labs identified this isolate as *C. zeylanoides*, which grows on the media containing cycloheximide, while *C. lambica* does not grow on the media containing cycloheximide. Two of the participating labs reported this isolate as *Hansenula anomala*, which produces ascospores on Malt or V8 agar, while *C. lambica* does not produce any ascospores. Two of the participating labs identified this isolate as *Saccharomyces cerevisiae*, which does not form any pseudohyphae on the corn meal agar, while *C. lambica* produces prolific pseudohyphae. One lab reported this isolate as *Sporobolomyces salmonicolor*, which is a pink colored yeast, producing satellite colonies (ballistoconidia formation), while *C. lambica* does not produce satellite colonies.

Further Reading:

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Source: Sputum

Clinical Significance: Causal agent of cutaneous lesions, peritonitis, olecranon bursitis and disseminated disease in immunosuppressed patients.

Ecology: Cosmopolitan.

Laboratory Diagnosis:

1. **Culture** – At 25^o C, on Sabouraud's dextrose agar, colonies are dull white, and smooth in 2-5 days (Fig. 23).
2. **Microscopic morphology** – On corn meal agar with Tween 80, round, immature sporangia of various sizes and mature sporangia filled with sporangiospores are seen (Fig. 24). No blastoconidia or pseudo- and true hyphae are formed.
3. **Differentiation from other yeasts** – *Prototheca zopfii* does not grow on media containing cycloheximide, and grows well at 37^o C. It does not produce any zone of inhibition to 50 µg clotrimazole disk at 37^o C, which differentiates it from *Prototheca wickerhamii* (1).
4. **Molecular tests** – Sequence analysis of the mitochondrial small subunit rRNA from *P. wickerhamii* showed higher homology with mitochondrial sequence from plants (5).
5. **In vitro susceptibility testing** – Most of the clinical isolates are susceptible to amphotericin B, variably susceptible to itraconazole and ketconazole, but resistance to flucytosine and fluconazole (2).

Further Reading:

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Antifungal Susceptibility Testing

Introduction

The National Committee for Clinical Laboratory Standards (NCCLS) Subcommittee on Antifungal Susceptibility Testing developed M27-A Standards that describe a method for determining the antifungal susceptibility testing of pathogenic yeasts (2). Various commercial systems are also being developed for antifungal susceptibility testing of yeasts. Currently, antifungal susceptibility tests are mostly performed at reference laboratories, but many clinical laboratories have expressed interest in this testing (1).

Materials & Methods

The isolates included in January 30, 2002 event were two NCCLS quality control strains and three NCCLS reference strains (3, 4). These isolates have been well characterized, and their MICs range have been published (3, 4). Fourteen microbiology laboratories within United States and one reference lab each from Canada and United Kingdom participated in this testing event. Acceptable results were MICs within ± 2 dilution from the reference result (range of MICs for a particular yeasts are described in NCCLS, M27-A, 2).

Results

A total of 14 labs participated in this antifungal susceptibility testing event on January 30, 2002. The performance of only one lab was unsatisfactory. Of the 14 participating laboratories, 10 labs used broth microdilution methods, 3 labs used Etest, and 1 lab used broth macrodilution. Of the 10 labs, 5 labs used commercially prepared YeastOne Colorimetric microdilution method, while the other 5 labs performed testing according to NCCLS M27-A guidelines. The supplementary information on antifungal susceptibility testing procedures is summarized in Table 1. The MIC results submitted by the 14 participants are illustrated in Fig. 1. For amphotericin B, good performance was noted for *C. krusei*, and *C. albicans* irrespective of the methodology used by the laboratories. Similarly, for fluconazole good performance was seen for *C. krusei*, *C. parapsilosis*, and *C. albicans*. Overall agreement with the NCCLS reference ranges was 93% against amphotericin B and 99% against fluconazole for all five isolates, with the expansion of the reference range by one dilution. Overall, the level of performance closely matches the agreement observed among laboratories participating in the ungraded antifungal susceptibility testing survey of the College of American Pathologists (1).

Further Reading:

1. College of American Pathologists. 2001. Antifungal Susceptibility Testing. F-B Survey: 8-11.
2. National Committee for Clinical Laboratory Standards. 1997. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard NCCLS document M27-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
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4. Rex, J. H., M. A. Pfaller, T. J. Walsh, V. Chaturvedi, A. Espinel-Ingroff, M. A. Ghannoum, L. L. Gosey, F. C. Odds, M. G. Rinaldi, D. J. Sheehan, and D. W. Warnock. 2001. Antifungal susceptibility testing: practical aspects and current challenges. Clin Microbiol Rev. 14: 643-58.

Individual Isolates

S-1 *Candida krusei* ATCC 6258

Summary	NCCLS Reference Range	Expanded Range
Amphotericin B	0.5-2.0 µg/ml	0.25-4.0 µg/ml
Fluconazole	16-64 µg/ml	8->64 µg/ml

All the participating labs reported values within the expanded range, for both the drugs.

S-2 *Candida albicans* ATCC 24433

Summary	NCCLS Reference range	Expanded Range
Amphotericin B	0.25-1.0 µg/ml	0.12-2.0 µg/ml
Fluconazole	0.25-1.0 µg/ml	0.12-2.0 µg/ml

All the participating labs reported values within the expanded range for amphotericin B, while one lab reported lower MIC value than the expanded range for fluconazole.

S-3 *Candida albicans* ATCC 90028

Summary	NCCLS Reference range	Expanded Range
Amphotericin B	0.5-2.0 µg/ml	0.25-4.0 µg/ml
Fluconazole	0.25-1.0 µg/ml	0.12-2.0 µg/ml

All the participating labs reported values within the expanded range for fluconazole, while two labs reported lower MIC values than the expanded range for amphotericin B.

S-4 *Candida parapsilosis* ATCC 22019

Summary	NCCLS Reference range	Expanded range
Amphotericin B	0.25-1.0 µg/ml	0.12-2.0 µg/ml
Fluconazole	2.0-8.0 µg/ml	1.0-16.0 µg/ml

All the participating labs reported values within the expanded range for fluconazole, while one lab reported lower MIC value than the expanded range for amphotericin B.

S-5 *Candida parapsilosis* ATCC 90018

Summary	NCCLS Reference range	Expanded range
Amphotericin B	0.5-2.0 µg/ml	0.25-4.0 µg/ml
Fluconazole	0.25-1.0 µg/ml	0.12-2.0 µg/ml

All the participating labs reported values within the expanded range for fluconazole, while two labs reported lower MIC values than the expanded range for amphotericin B.

Antifungal Susceptibility Testing

Table 1. Summary of supplementary information on antifungal susceptibility testing by the participating laboratories

Test Method	No. Participants Labs
NCCLS broth microdilution	5
NCCLS broth macrodilution	1
Sensititre YeastOne Colorimetric	5
Etest	3
Media employed	
RPMI 1640	9
RPMI 1640 w/ alamar blue	4
Casitone agar	1
Inoculum preparation	
Spectrophotometric	7
MacFarland	7
Inoculum size	
0.5-2.5 X 10 ³	9
1.5-8 X 10 ³	5
Incubation temperature	
35° C	12
37° C	2
Incubation duration	
24 hour	5
48 hour	9
Endpoint reading	
Visual	8
Spectrophotometric	1
Colorimetric	5
Scoring endpoint*	
100% inhibition	7
80% inhibition	6
50% inhibition	3
other (color change)	5
QC organism	
NCCLS recommended strains	13
Unknown	1

*Most labs used 100% inhibition for amphotericin B and either 80 or 50% for azoles.

Antifungal Susceptibility Testing

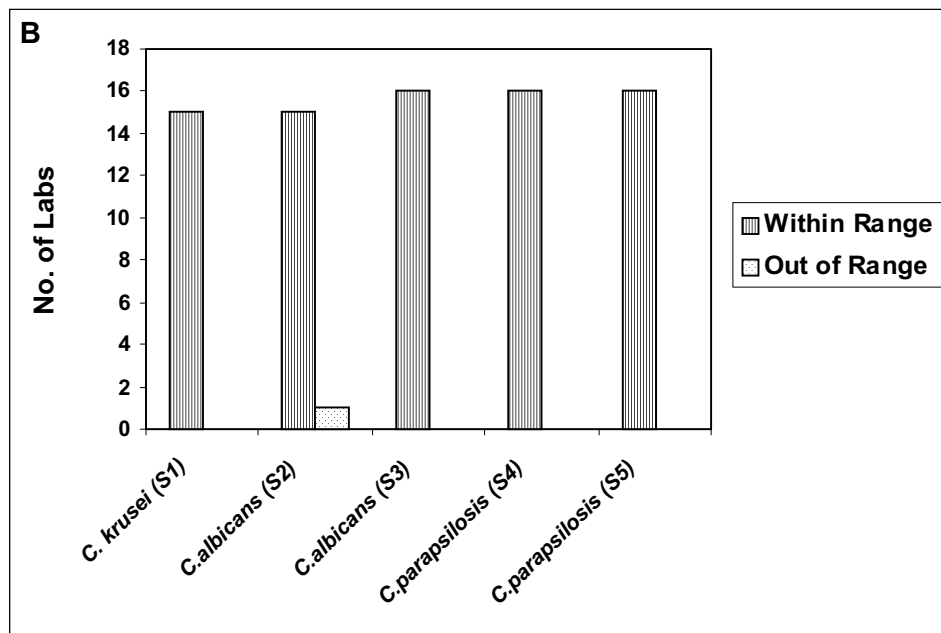
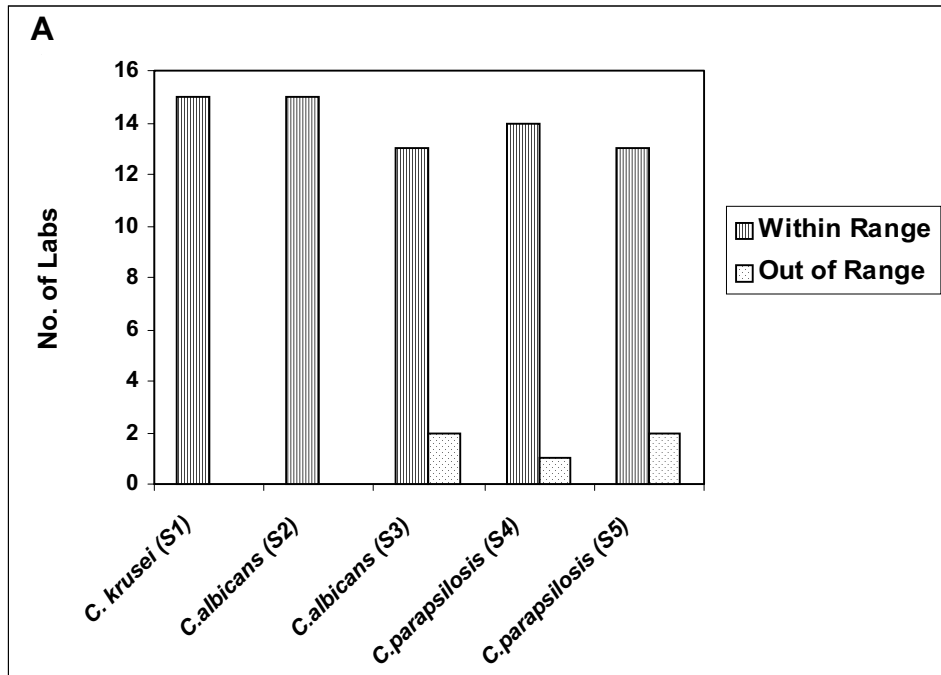


Figure S. Summary of the results submitted by the participating labs for 5 isolates for amphotericin B (A) and fluconazole (B).

M-1 *Scedosporium prolificans*



Figure 1. One week old, brownish-gray to black colony of *S. prolificans* on Sabouraud's dextrose agar.

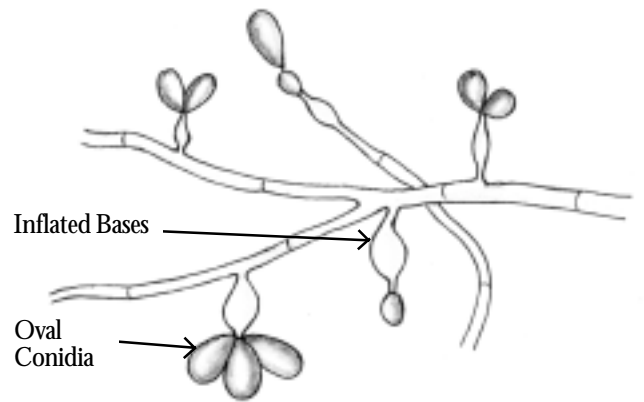
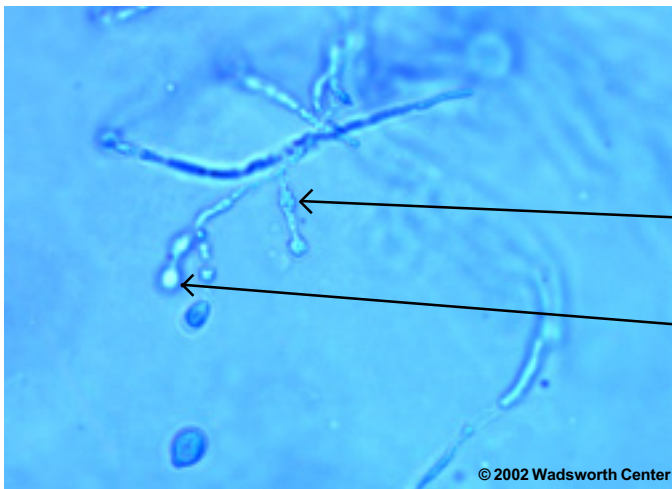


Figure 2. Microscopic morphology of *Scedosporium prolificans*. Hyphae, swollen conidiophores with annellations, truncated oval conidia are seen (left; 400X magnification, right; line drawing not to scale).



Figure 3. Three day old, yellowish orange colony of *Epicoccum* species on Sabouraud's dextrose agar.

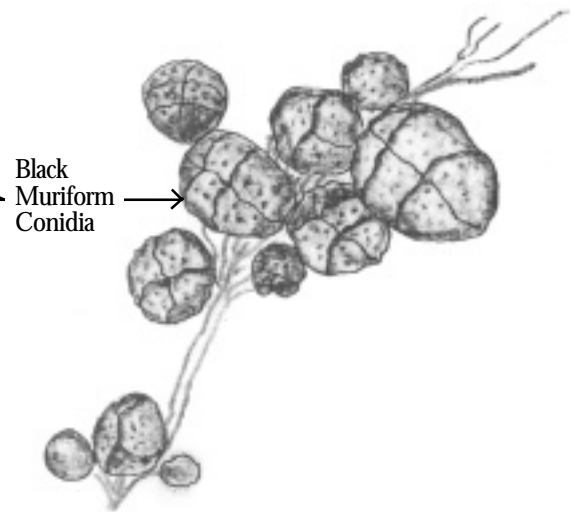
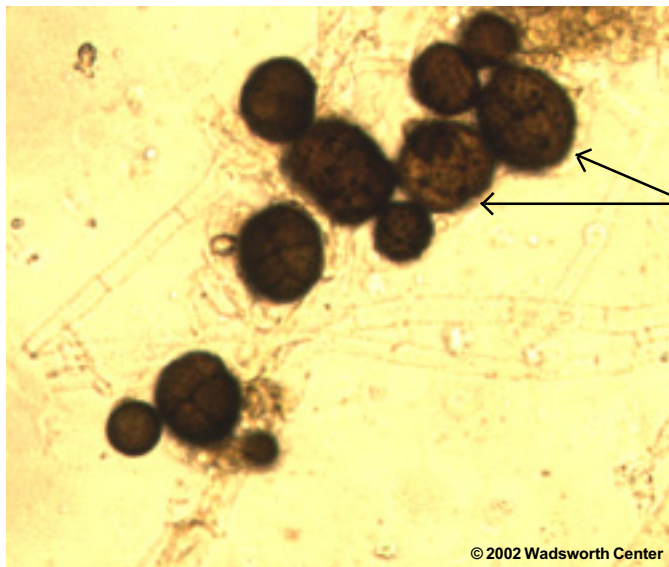


Figure 4. Microscopic morphology of *Epicoccum* species. Hyphae with brownish black muriform conidia formed along the sporodochia are seen (left; 400X magnification, right; line drawing not to scale).

M-3 *Curvularia* species

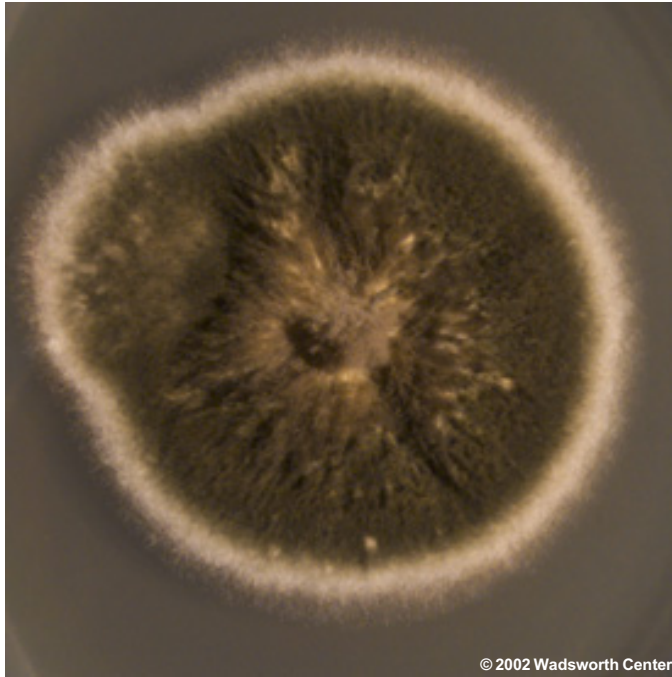


Figure 5. Three day old, woolly, brownish black colony of *Curvularia* species on Sabouraud's dextrose agar.

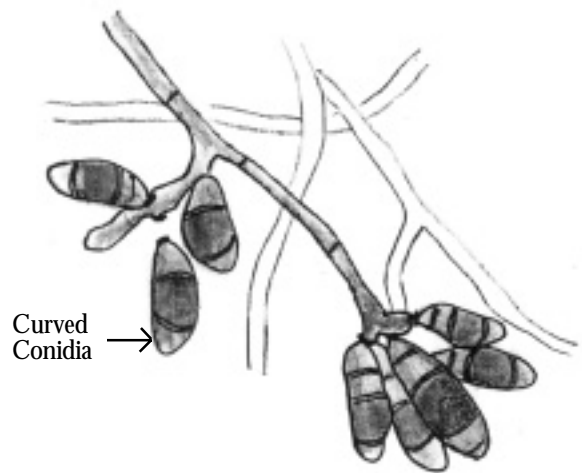


Figure 6. Microscopic morphology of *Curvularia* species. Hyphae and poroconidia formed sympodially, slightly curved with transverse septations are seen (left; 400X magnification, right; line drawing not to scale).

M - 4 *Aureobasidium pullulans*



Figure 7. Three day old, initially white turning black in sectors, creamy colony of *Aureobasidium pullulans* on Sabouraud's dextrose agar.

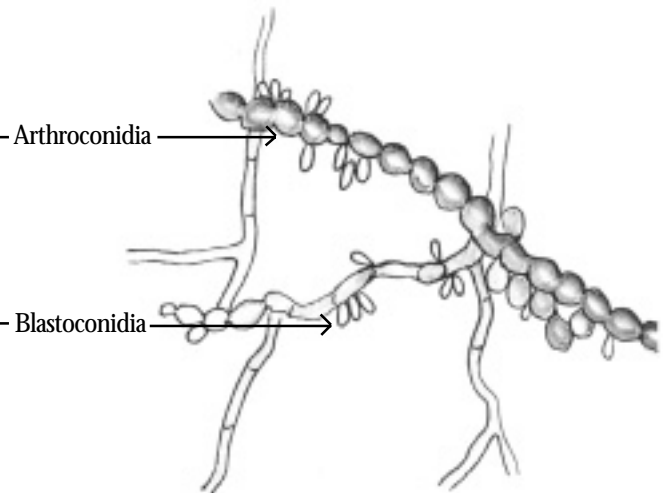
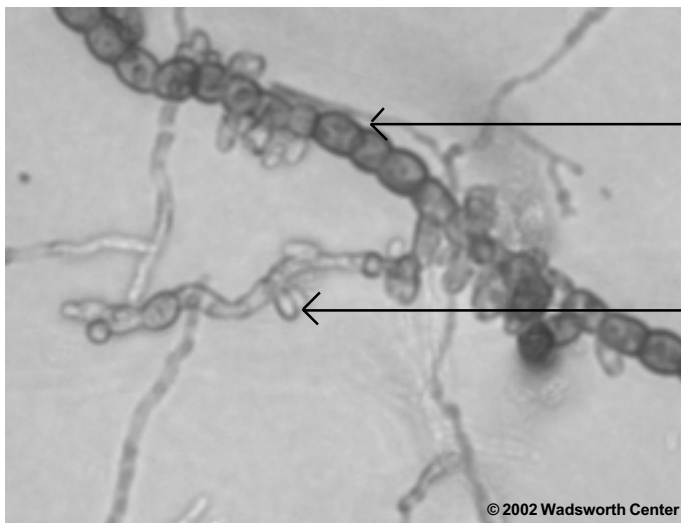


Figure 8. Microscopic morphology of *Aureobasidium pullulans*. Hyphae and blastoconidia produced synchronously, dark arthroconidia and chlamydoconidia are seen (left; 400X magnification, right; line drawing not to scale).



Figure 9. Two-week-old, initially white turning black wrinkled colony of *Sporothrix schenckii* on Sabouraud's dextrose agar.

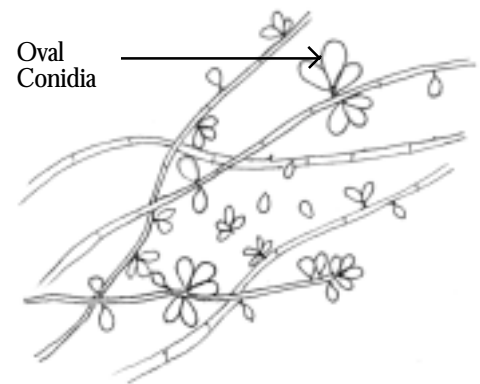
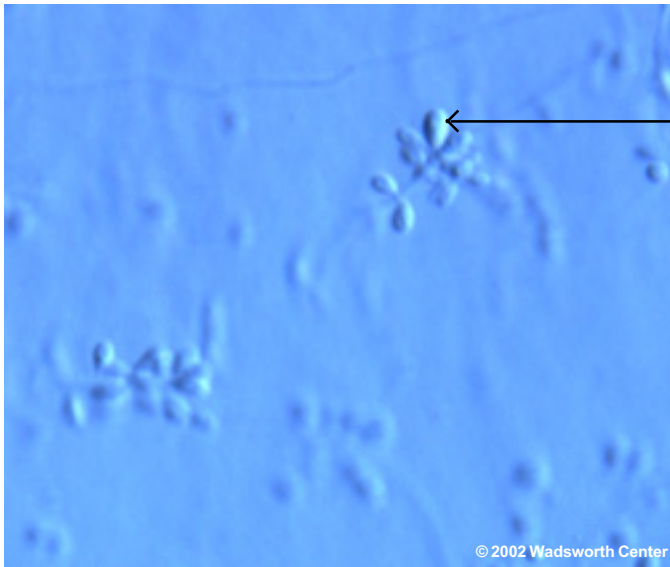


Figure 10. Microscopic morphology of *Sporothrix schenckii*. Hyphae and slender conidiophores with ovoid conidia formed sympodially (rosette formation) are seen (left; 200X magnification, right; line drawing not to scale).

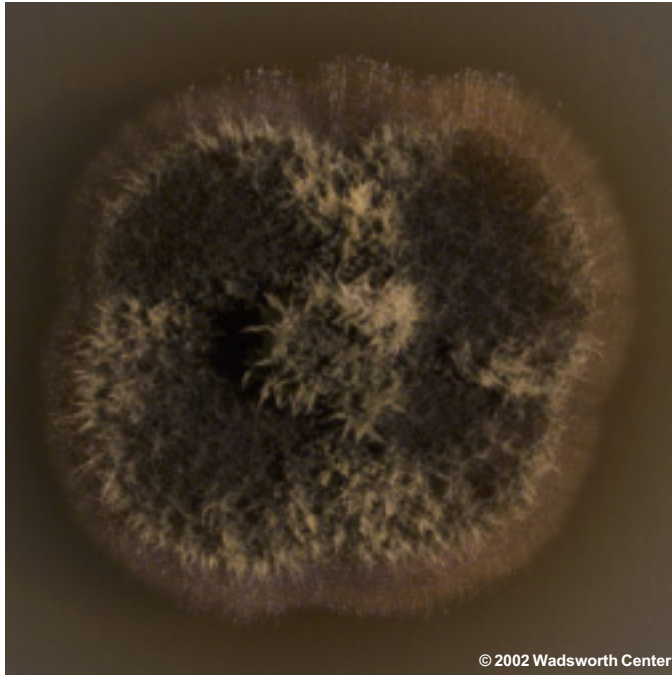


Figure 11. Five-day-old, black, powdery colony of *Stachybotrys* species on Sabouraud's dextrose agar.

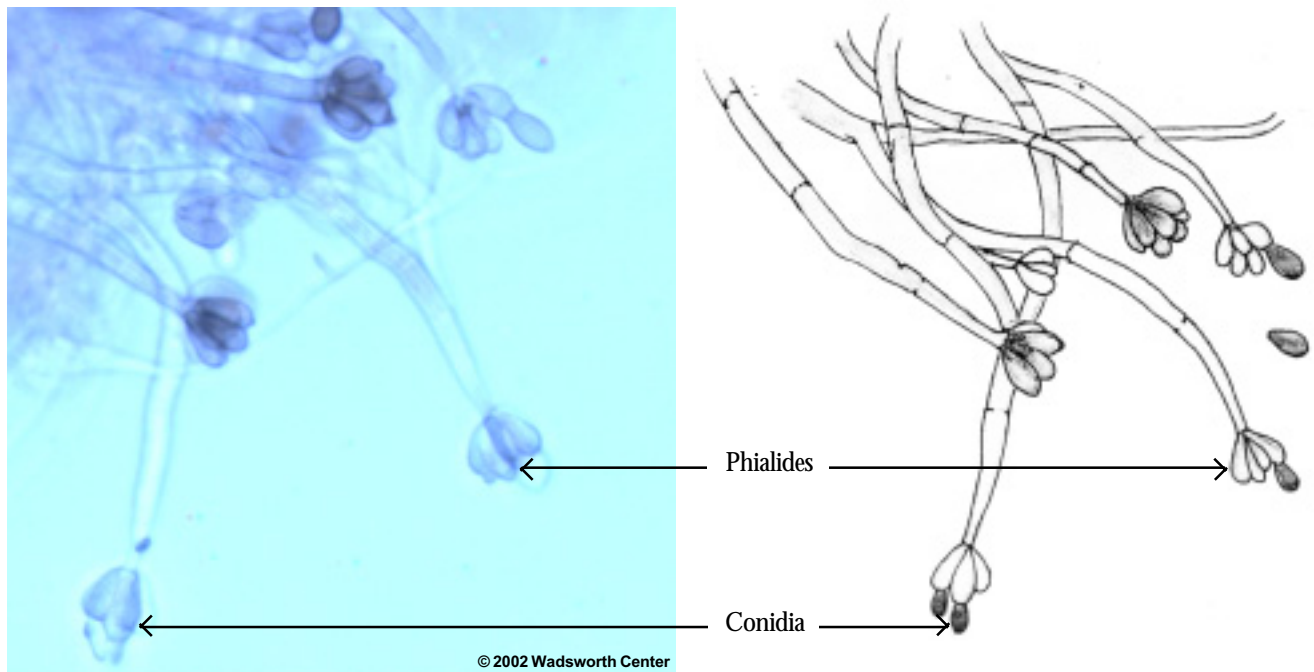


Figure 12. Microscopic morphology of *Stachybotrys* species. Hyphae and simple conidiophores with phialides in-groups and unicellular conidia are seen (left; 200X magnification, right; line drawing not to scale).



Figure 13. Three-day-old, soft, smooth colony of *Candida famata* on Sabouraud's dextrose agar.

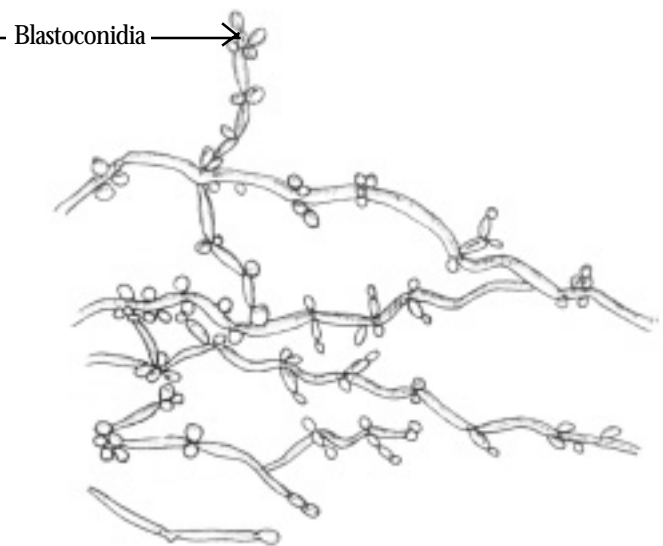
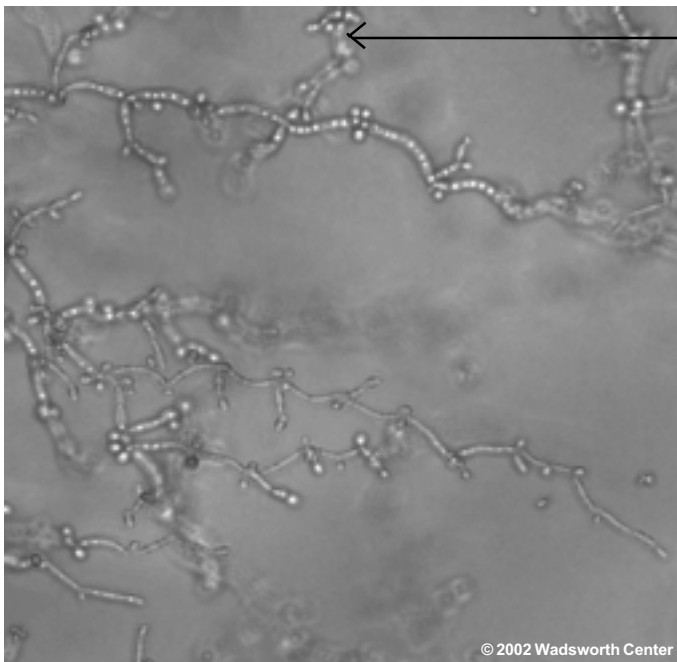


Figure 14. Microscopic morphology of *Candida famata*. On corn meal agar culture, incubated for week, well-developed pseudohyphae with oval blastoconidia are seen (left; 200X magnification, right; line drawing not to scale).

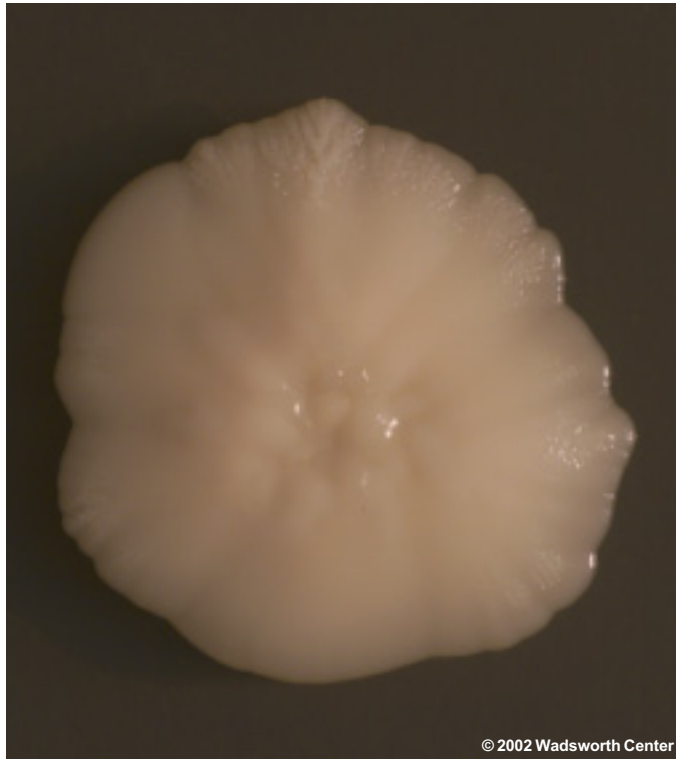


Figure 15. Three-day-old, cream colored, wrinkled colony of *Candida rugosa* on Sabouraud's dextrose agar.

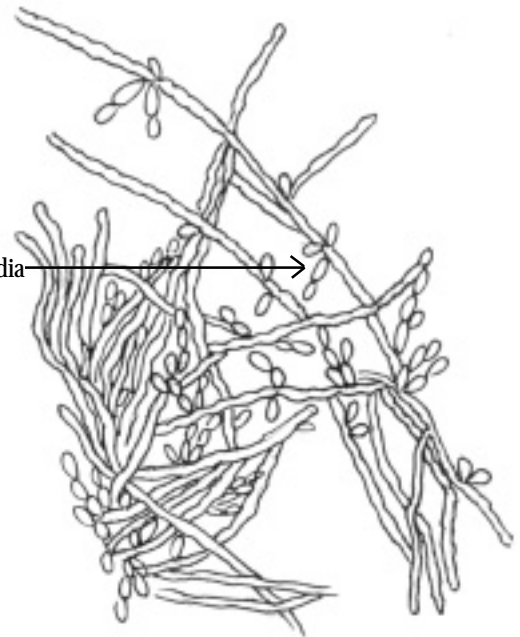
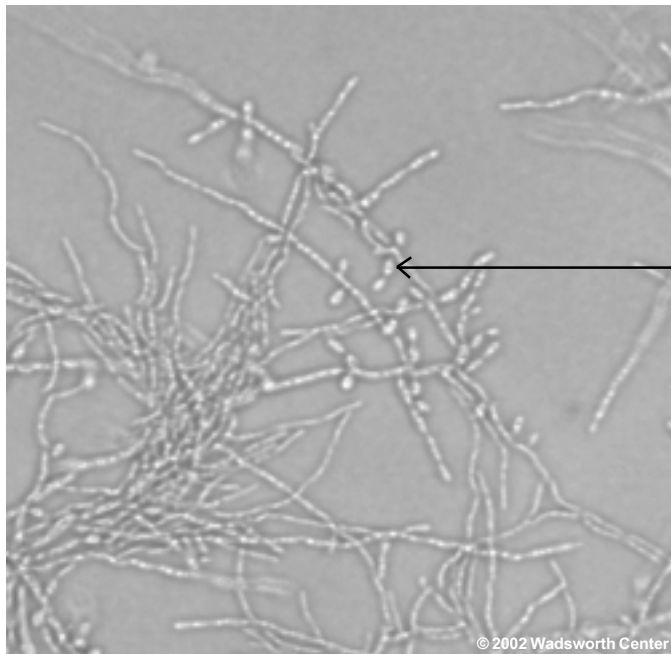


Figure 16. Microscopic morphology of *Candida rugosa*. On corn meal agar culture, branched pseudohyphae with elongated blastoconidia are seen (left; 200X magnification, right; line drawing not to scale).



Figure 17. Three-day-old, dry but soft wrinkled colony of *Candida krusei* on Sabouraud's dextrose agar.

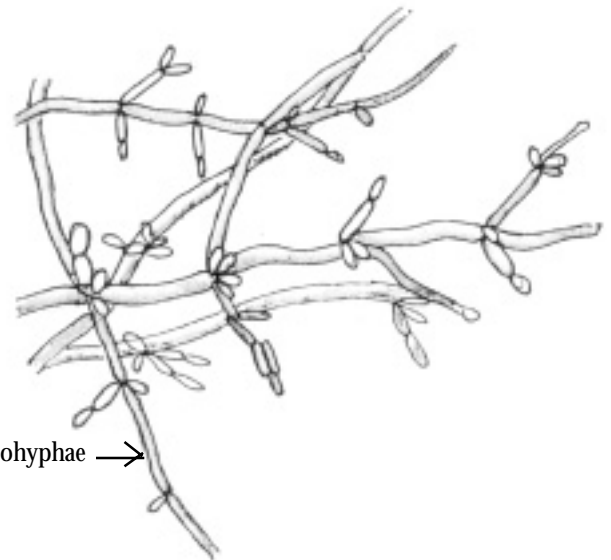
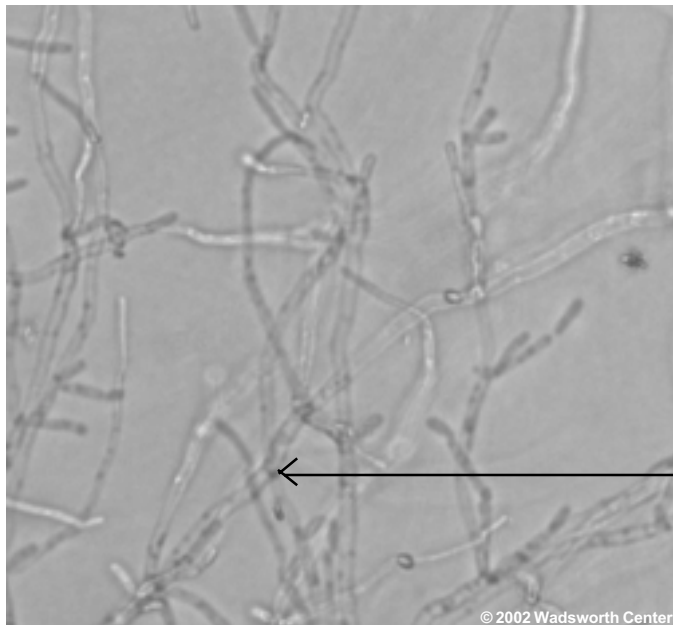


Figure 18. Microscopic morphology of *Candida krusei*. On corn meal agar culture, long, branched pseudohyphae with oval blastoconidia are seen (left; 200X magnification, right; line drawing not to scale).



Figure 19. Three-day-old, soft smooth colony of *Cryptococcus uniguttulatus* on Sabouraud's dextrose agar.

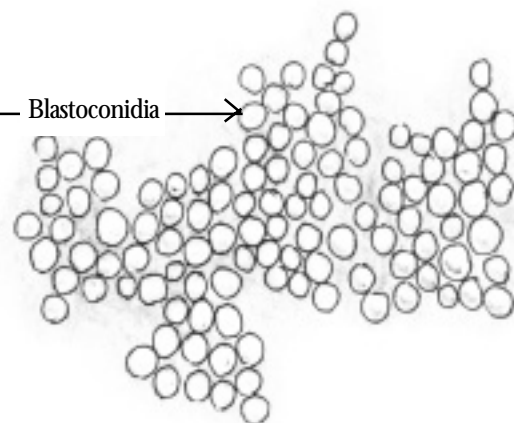
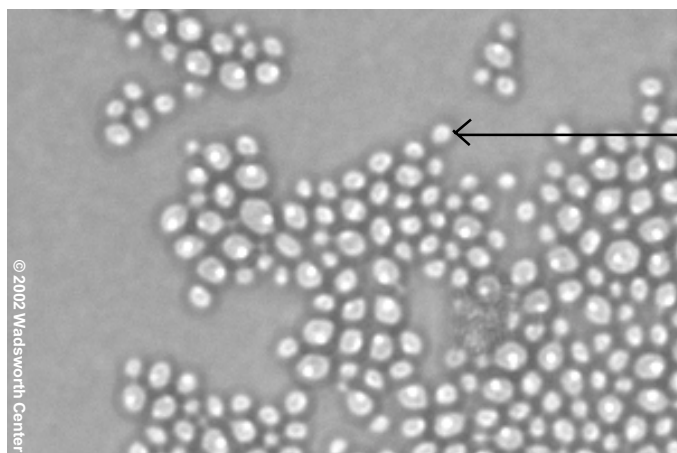


Figure 20. Microscopic morphology of *Cryptococcus uniguttulatus*. On corn meal agar culture, round blastoconidia are seen (left; 200X magnification, right; line drawing not to scale).



Figure 21. Three-day-old, soft wrinkled colony of *Candida lambica* on Sabouraud's dextrose agar.

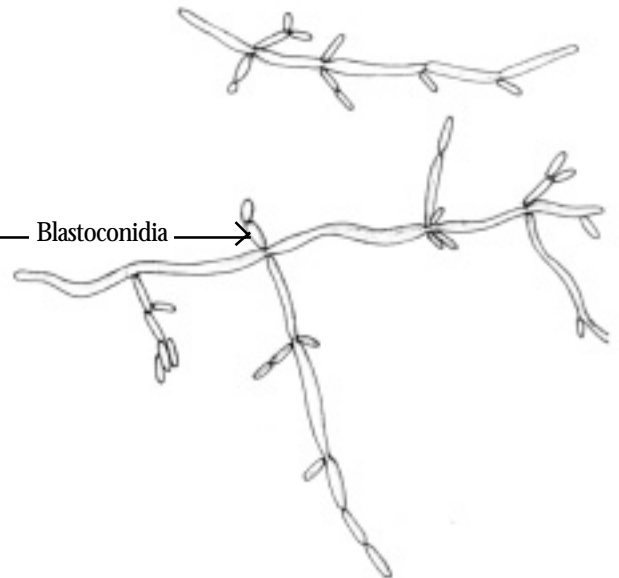


Figure 22. Microscopic morphology of *Candida lambica*. On corn meal agar culture, long, branched pseudohyphae with oval blastoconidia are seen (left; 200X magnification, right; line drawing not to scale).



Figure 23. Five-day-old, soft smooth colony of *Prototheca zopfii* on Sabouraud's dextrose agar.

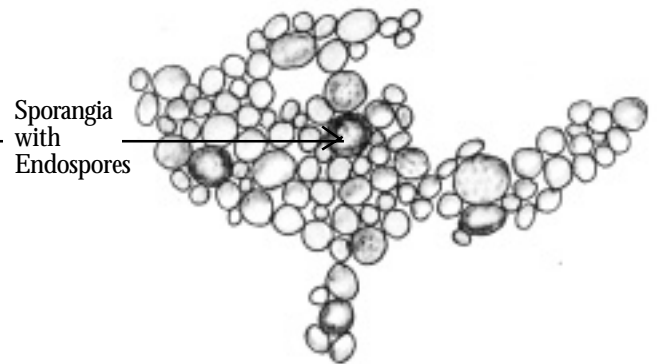
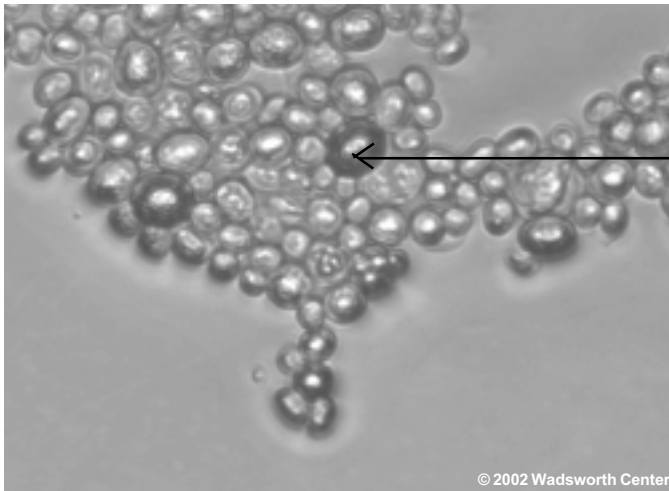


Figure 24. Microscopic morphology of *Prototheca zopfii*. On corn meal agar culture, round, immature sporangia and mature sporangia with endospores are seen (left; 200X magnification, right; line drawing not to scale).

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W a d s w o r t h C e n t e r

N e w Y o r k S t a t e D e p a r t m e n t o f H e a l t h

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