



MYCOLOGY CRITIQUÉ

**Mycology Proficiency Testing Program
January 2004**

Wadsworth Center
New York State Department of Health



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Test Specimens and Grading Policy

Test Specimens

Minimal of two strains of each of the proposed mold specimens was examined for inclusion in the proficiency test event of January 2004. 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 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 colony morphology of all yeast strains was studied on corn meal agar with Tween 80 plates inoculated by Dalmau 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 80 percent agreement of 10 referee laboratories or 80 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 maximum score for each specimen is 20 based on the formula.

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

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 of the reference result for a particular organism against a single drug. If a result falls outside of this range, the lab gets a score of zero for that particular test component or set. The current testing format is based on the 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 maximum total score is $5 \times 20 = 100$. However, a lab that routinely does not perform tests with either of the two drugs is scored with the maximum score for a single isolate against one drug. Again, for five yeasts isolates, the total will be $20 \times 5 = 100$.

Mycology – General

	Specimen Key	Validated Specimen	Other Acceptable Answers
M-1	<i>Scytalidium</i> sp.	<i>Scytalidium</i> sp.	<i>Scytalidium dimidiatum</i> <i>Scytalidium hyalinum</i> <i>Scytalidium lignicola</i> <i>Aspergillus flavus</i> group
M-2	<i>Aspergillus flavus</i>	<i>Aspergillus flavus</i>	
M-3	<i>Aspergillus fumigatus</i>	<i>Aspergillus fumigatus</i>	
M-4	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	<i>Aspergillus niger</i> group
M-5	<i>Aspergillus nidulans</i>	<i>Aspergillus nidulans</i>	<i>Aspergillus nidulans</i> group <i>Emericella nidulans</i> <i>Exophiala castellanii</i> <i>Exophiala (Wangiella) dermatitidis</i> <i>Exophiala jeanselmei</i> <i>Exophiala moniliae</i> <i>Exophiala oligosperma</i>
Ed.sp.	<i>Exophiala spinifera</i>		

Mycology – Yeast Only

	Specimen Key	Validated Specimen	Other Acceptable Answers
Y-1	<i>Cryptococcus terreus</i>	<i>Cryptococcus terreus</i>	
Y-2	<i>Rhodotorula minuta</i>	<i>Rhodotorula minuta</i>	
Y-3	<i>Trichosporon mucoides</i>	(Not validated)	<i>Trichosporon asahii</i> <i>Trichosporon beigeli</i>
Y-4	<i>Trichosporon mucoides</i>	(Not validated)	<i>Trichosporon asahii</i> <i>Trichosporon beigeli</i>
Y-5	<i>Geotrichum candidum</i>	<i>Geotrichum candidum</i>	<i>Geotrichum penicillatum</i> <i>Geotrichum</i> sp.

Mycology – Antifungal Susceptibility Testing for Yeasts

	Specimen Key
S-1	<i>Candida tropicalis</i> ATCC 750
S-2	<i>Candida krusei</i> ATCC 6258
S-3	<i>Candida parapsilosis</i> ATCC 22019
S-4	<i>Candida parapsilosis</i> ATCC 90018
S-5	<i>Candida albicans</i> ATCC 90028
Ed.sp.	<i>Candida albicans</i> NYSDOH 40026

Mycology – General

	Correct Responses/ Total # Laboratories (%)	Referees (%)
M - 1 <i>Scytalidium</i> sp.	82/83 (99)	10/10 (100)
M - 2 <i>Aspergillus flavus</i>	83/83 (100)	10/10 (100)
M - 3 <i>Aspergillus fumigatus</i>	82/83 (99)	10/10 (100)
M - 4 <i>Aspergillus niger</i>	83/83 (100)	10/10 (100)
M - 5 <i>Aspergillus nidulans</i>	81/83 (98)	10/10 (100)

Mycology – Yeast Only

	Correct Responses/ Total # Laboratories (%)	Referees (%)
Y - 1 <i>Cryptococcus terreus</i>	61/61 (100)	10/10 (100)
Y - 2 <i>Rhodotorula minuta</i>	57/61 (93)	9/10 (90)
Y - 3 <i>Trichosporon mucoides</i> (Not validated)	48/61 (79)	7/10 (70)
Y - 4 <i>Trichosporon mucoides</i> (Not validated)	45/61 (74)	7/10 (70)
Y - 5 <i>Geotrichum candidum</i>	61/61 (100)	10/10 (100)

Mycology – Antifungal Susceptibility Testing for Yeasts

	Correct Responses/ Total # Laboratories (%)	
	Amphotericin B	Fluconazole
S - 1 <i>Candida tropicalis</i> ATCC 750	20/20 (100)	23/23 (100)
S - 2 <i>Candida krusei</i> ATCC 6258	20/20 (100)	23/23 (100)
S - 3 <i>Candida parapsilosis</i> ATCC 22019	20/20 (100)	23/23 (100)
S - 4 <i>Candida parapsilosis</i> ATCC 90018	20/20 (100)	21/23 (91)
S - 5 <i>Candida albicans</i> ATCC 90028	20/20 (100)	23/23 (100)

Mycology – General

Number of participating laboratories	83
Number of referee laboratories	10
Number of laboratories responding by deadline	83
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	82
Number of laboratories unsuccessfully completing this test	1

Mycology – Yeast Only

Number of participating laboratories	61
Number of referee laboratories	10
Number of laboratories responding by deadline	61
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	61
Number of laboratories unsuccessfully completing this test	0

Mycology – Antifungal Susceptibility Testing for Yeasts

Number of participating laboratories	23
Number of referee laboratories	3
Number of laboratories responding by deadline	23
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	23
Number of laboratories unsuccessfully completing this test	0

No. Laboratories Using Commercial Identification/Susceptibility System*

AMS Vitek system	17
API 20C AUX	46
IDS Rapid System	1
Microscan	1
Remel Uni-Yeast-Tek	6
Other	0

(*Includes multiple systems used by some labs)

M-1 *Scytalidium* sp.

Source: Toenail

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	82
Labs with incorrect ID:	1
(<i>Cryptococcus albidus</i>)	(1)

Clinical Significance: *Scytalidium* sp. is one of the most common toenail pathogens in tropical regions. Cases of subcutaneous or disseminated infections such as subcutaneous abscess, sinusitis, endophthalmitis, lymphadenitis, and fungemia in immunocompromised patients have also been reported.

Ecology: *Scytalidium* sp. is a cosmopolitan saprobe.

Laboratory Diagnosis:

1. **Culture** – *Scytalidium* sp. grew very rapidly. On Sabouraud's dextrose agar, at 25°C after 5 days, colony was white to gray-black, wooly texture on the surface (Figure 1, left panel). The reverse was pale white, brown to black (Figure 1, right panel).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed septate and branched hyphae. Oval to rectangular arthroconidia were unicellular or bicellular. Conidiophores were absent (Figure 2).
3. **Differentiation from other molds** – *Scytalidium* sp. was distinguished from *Aureobasidium* sp. by its wooly texture colonies instead of mucoid texture for *Aureobasidium* sp.
4. **In vitro susceptibility testing** – Generally, *Scytalidium* sp. is susceptible to amphotericin B, fluconazole, itraconazole, and ketoconazole, but resistant to 5 fluorocytosine (5 FC).
5. **Molecular tests** – PCR-restriction fragment length polymorphism (RFLP) ribotyping method was reported to rapidly discriminate dermatophytes, *Scytalidium* spp., and other fungi.

Further Reading:

1. Aamir, S., Aman, S., and Haroon, T. 2003. Mycetoma caused by *Scytalidium dimidiatum*. *Br. J. Dermatol.* 148: 174-176.
2. Dunn, J.J., Wolfe, M.J., Trachtenberg, J., Kriesel, J.D., Orlandi, R.R., and Carroll, K.C. 2004. Invasive fungal sinusitis caused by *Scytalidium dimidiatum* in a lung transplant recipient. *J. Clin. Microbiol.* 41: 5817-5819.
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6. Machouart-Dubach, M., Lacroix, C., de Chauvin, M.F., Le Gall, I., Giudicelli, C., Lorenzo, F., and Derouin, F. 2001. Rapid discrimination among dermatophytes, *Scytalidium* spp., and other fungi with a PCR-restriction fragment length polymorphism ribotyping method. *J. Clin. Microbiol.* 39: 685-690.
7. Ungpakorn, R., Lohapathan, S., and Reangchainam, S. 2004. Prevalence of foot diseases in outpatients attending the Institute of Dermatology, Bangkok, Thailand. *Clin. Exp. Dermatol.* 29: 87-90.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS2 region of rDNA. The sequence is deposited in GenBank under the accession numbers AY591349.

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ATCC 38906 (AY213688)          1                               50
NYSDOH 196-03 (AY594319)     GCATCGATGA AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
                               GCATCGATGA AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA

                               51                               100
TTCAGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCC TCGGTATTCC
TTCAGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCC TCGGTATTCC

                               101                              150
GAGGGGCATG CCTGTTCGAG CGTCATTACA ACCCTCAAGC TCTGCTTGGT
GAGGGGCATG CCTGTTCGAG CGTCATTACA ACCCTCAAGC TCTGCTTGGT

                               151                              200
GTTGGGCACC GCCCTTCCGC GGGCGCGCCT CAAAGACCTC GGCGGTGGCG
GTTGGGCACC GCCCTTCCGC GGGCGCGCCT CAAAGACCTC GGCGGTGGCG

                               201                              250
TCTTGCCTCG AGCGTAGTAG AAAACACCTC GCTTTGGAGC GCACGGCGCC
TCTTGCCTCG AGCGTAGTAG AAAACACCTC GCTTTGGAGC GCACGGCGCC

                               251                              300
GCCC GCCGGA CGAACCTTTT GAACTTTCTC AAGGTTGACC TCGGATCAGG
GCCC GCCGGA CGAACCTTTT GAACTTTCTC AAGGTTGACC TCGGATCAGG

                               301
TAGGGATACC CGCTGAACTT AAGCATATCA ATAAGCGGAG GA
TAGGGATACC CGCTGAACTT AAGCATATCA ATAAGCGGAG GA

```

Figure 3. Alignment of primary sequences of the ITS2 regions of *Scytalidium hyalinum* ATCC 38906 and PT specimen *Scytalidium* sp. NYSDOH 196-03. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Comments: All the participating laboratories except one correctly reported this specimen. *Aureobasidium* sp. can be easily differentiate from *Scytalidium* sp. by their colony texture.

Source: Bronchial wash

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	83

Clinical Significance: *Aspergillus flavus* causes pulmonary and disseminated infection in immunocompromised patients. It is the second most common species causing aspergillosis. Occasionally, this fungus can cause infection of sinus, eye, ear, and nails. *A. flavus* produces aflatoxins in certain foodstuff like peanuts that can cause mycotoxicosis.

Pathogenicity: *A. flavus* is angioinvasive, producing extensive damage to blood vessels, leading to infarction and necrosis.

Ecology: *A. flavus* is found worldwide on plants and organic materials.

Laboratory Diagnosis:

1. **Culture** – *A. flavus* grew very fast. On Sabouraud's dextrose agar, after 5 days at 25°C, the colony was yellow to green, powdery (Figure 4, left panel). The reverse was pale to yellowish (Figure 4, right panel).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed septate hyphae with rough, colorless conidiophores. Conidiophores end in vesicle, which was globose and the entire surface was covered (radiating) with one series or two series of sterigmata (uni- or biseriata). Conidia measuring 3 – 6 µm, produced from these sterigmata are round, rough walled and in chains (Figure 5).
3. **Differentiation from other *Aspergillus* species** – *A. flavus* was differentiated from other *Aspergilli* by yellow – green colonies, rough walled conidiophores, radiating conidial heads with uniseriate or biseriata sterigmata.
4. ***In vitro* susceptibility testing** – Susceptibility testing results of clinical isolates of *A. flavus* showed variable susceptibility to amphotericin B and to itraconazole. Voriconazole was reported to be more effective drug.
5. **Molecular tests** – A PCR based amplification of gene fragments – encoding alkaline proteases from *A. fumigatus* and *A. flavus* was used to detect these organisms in respiratory specimens. For molecular epidemiology, RAPD fingerprinting method has been used.

Comments: All participating laboratories correctly identified this specimen.

Further Reading:

1. Chi, C.Y., Fung, C.P., and Liu, C.Y. 2003. *Aspergillus flavus* epidural abscess and osteomyelitis in a diabetic patient. *J. Microbiol. Immunol. Infect.* 36: 145-148.
2. Myoken Y, Sugata T, Fujita Y, Kyo T, Fujihara M, Kohara T, Katsu M, and Mikami Y. 2003. Molecular epidemiology of invasive stomatitis due to *Aspergillus flavus* in patients with acute leukemia. *J. Oral. Patbol. Med.* 32: 215-218.
3. Nasa, G.L., Littera, R., Maccioni, A., Ledda, A., Vacca, A., and Contu, L. 2004. Voriconazole for the treatment of disseminated nodular cutaneous aspergillosis in a patient affected by acute myeloid leukemia. *Hematol. J.* 5: 178-180.
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5. Varanasi, N.L., Baskaran, I., Alangaden, G.J., Chandrasekar, P.H., and Manavathu, E.K. 2004. Novel effect of voriconazole on conidiation of *Aspergillus* species. *Int. J. Antimicrob. Agents.* 23: 72-79.
6. Willinger. B., Obradovic, A., Selitsch, B., Beck-Mannagetta, J., Buzina, W., Braun, H., Apfalter, P., Hirschl, A.M., Makristathis, A., and Rotter, M. 2003. Detection and identification of fungi from fungus balls of the maxillary sinus by molecular techniques. *J. Clin. Microbiol.* 41: 581-585.

M-3 *Aspergillus fumigatus*

Source: Sputum

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	82
Labs with incorrect ID:	1
(<i>Aspergillus versicolor</i>)	(1)

Clinical Significance: *Aspergillus fumigatus* is the most frequent etiologic agent of aspergillosis in humans. It causes pulmonary, sinus, cerebral, bone, ocular, cardiovascular, and other organ diseases especially in immunocompromised host.

Pathogenicity: *A. fumigatus* causes allergy (allergic broncho-pulmonary aspergillosis), local colonization (aspergilloma) and systemic infection (invasive aspergillosis). The fungus has a pronounced tendency to invade blood vessels (angioinvasion), which often causes fatal outcome.

Ecology: *A. fumigatus* is cosmopolitan in distribution; frequently isolated from compost, soil, and plant materials. Among various *Aspergilli*, *A. fumigatus* is the most common in nature with a wide range of temperature tolerance.

Laboratory Diagnosis:

1. **Culture** – *A. fumigatus* grew rapidly. On Sabouraud's dextrose agar, after 5 days at 25°C, the colony was blue – green, powdery (Figure 6, left panel), and pale yellow on reverse (Figure 6, right panel). It grew well at 45°C, which is used as a diagnostic feature for identification.
2. **Microscopic morphology** – Lactophenol cotton blue mount showed septate hyphae with smooth walled conidiophores. Conidiophore ends in vesicle, which was subglobose in shape and its upper half portion was covered (columnar) with one series of sterigmata (uniseriate). Conidia produced from these sterigmata were round, smooth, delicately rough walled and in chains (Figure 7).
3. **Differentiation from other *Aspergillus* species** – *A. fumigatus* was differentiated from other *Aspergillus* species by blue – green colonies, columnar conidial heads with uniseriate sterigmata and good growth at 45°C.
4. ***In vitro* susceptibility testing** – Susceptibility testing results indicated that isolates were variably susceptible to amphotericin B and to itraconazole, but more susceptible to caspofungin and newer azoles like voriconazole and posaconazole. Both tacrolimus (FK506) and cyclosporine (CY) exhibited a clear *in vitro* positive interaction with caspofungin against *A. fumigatus*.
5. **Molecular tests** – For the molecular epidemiology of *A. fumigatus* three typing methods have been used: multi – locus enzyme electrophoresis, random amplified polymorphic DNA, and sequence – specific DNA primers.

Comments: All participating laboratories except one correctly identified this important pathogen. *A. versicolor* has loosely radiated conidial head and covers most of vesicle compared to *A. fumigatus* with columnar conidial head. Hüll cells are often present in *A. versicolor*.

Further Reading:

1. Challier, S., Boyer, S., Abachin, E., and Berche, P. 2004. Development of a serum-based Taqman real-time PCR assay for diagnosis of invasive aspergillosis. *J. Clin. Microbiol.* 42: 844-846.
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6. Sallmann, S., Heilmann, A., Heinke, F., Kerkmann, M.L., Schuppler, M., Hahn, G., Gahr, M., Rosen-Wolff, A., Roesler, J. 2003. Caspofungin therapy for *Aspergillus* lung infection in a boy with chronic granulomatous disease. *Pediatr. Infect. Dis. J.* 22: 199-200.
7. Steinbach, W.J., Schell, W.A., Blankenship, J.R., Onyewu, C., Heitman, J., Perfect, J.R. 2004. *In vitro* interactions between antifungals and immunosuppressants against *Aspergillus fumigatus*. *Antimicrob. Agents Chemother.* 48: 1664-1669.
8. Stern, J.B., Girard, P., and Caliandro, R. 2004. Pleural diffusion of voriconazole in a patient with *Aspergillus fumigatus* empyema thoracis. *Antimicrob. Agents Chemother.* 48: 1065.
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Source: Ear

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	83

Clinical Significance: *Aspergillus niger* commonly causes ear infection, also implicated in pulmonary aspergilloma and rarely in primary cutaneous infection.

Ecology: *A. niger* is cosmopolitan in soil and on plants.

Laboratory Diagnosis:

1. **Culture** – *A. niger* was a fast growing fungus. On Sabouraud's dextrose agar, after 5 days at 25°C, the initial growth was white, becoming black later on giving "salt and pepper appearance" (Figure 8, left panel) and reverse turning pale yellow (Figure 8, right panel). Good growth was seen at 37°C.
2. **Microscopic morphology** – Lactophenol cotton blue mount showed septate hyphae with smooth-walled, simple conidiophores measuring up to 1 mm in length. Conidiophores end in vesicle, which was globose and entirely covered (radiating) with two series of sterigmata (biseriate). Conidia produced from these sterigmata were brown to black, round, rough walled, and in chains measuring 4 – 5 µm in diameter (Figure 9).
3. **Differentiation from other molds** – *A. niger* was differentiated from other *Aspergillus* species by its rapid growth, black colonies, microscopically biseriate, radiating heads with black, round, rough conidia.
4. **In vitro susceptibility testing** – Most clinical isolates were susceptible to amphotericin B and variably susceptible to itraconazole and resistant to fluconazole. Posaconazole, ravuconazole, and voriconazole exhibited promising activity against *A. niger*. *A. niger* was also particularly susceptible to caspofungin.
5. **Molecular tests** – PCR method has been described to differentiate various species in the *A. niger* aggregate.

Comments: All participating laboratories correctly identified this specimen.

Further Reading:

1. Aksoy, D.Y., Turker, A., Altundag, M.K., Abali, H., Durusu, M., Erman, M., Uner, A., Sungur, A.A., Unal, S., Uzun, O. 2003. Concomitant *Mycobacterium tuberculosis* and *Aspergillus niger* infection in a patient with acute myeloid leukemia. *Chemotherapy*. 49: 264-266.
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3. Kunel'skaia VIa, and Shadrin, G.B. 2004. Middle ear mycosis. *Vestn. Otorinolaringol.* 1: 57-60.
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M-5 *Aspergillus nidulans*

Source: Lung

Scoring:

Referee Labs with correct ID:	10
Labs with correct ID:	81
Labs with incorrect ID:	2
(<i>Aspergillus terreus</i>)	(1)
(<i>Aspergillus versicolor</i>)	(1)

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

Ecology: *A. nidulans* is cosmopolitan in soil.

Laboratory Diagnosis:
















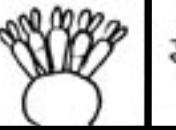











1. **Culture** – On Sabouraud's dextrose agar, after 7 days at 25°C, the colony was greenish-blue with whitish edge (Figure 10, left panel). Reverse appeared yellow to brownish (Figure 10, right panel).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed septate hyphae with brown, wavy conidiophores. Conidiophore terminated in vesicle, which was subglobose with its upper half covered by two series of sterigmata (biseriate). Conidia, measuring 5 – 7 µm in diameter, were round, smooth to rough walled. Round hülle cells and reddish color cleistothecia were also seen. Hülle cells were specialized structures made up of loose network of hyphae, having globose cells with thick walls that occur in certain groups of *Aspergilli*. Their characteristic shape provided a valuable diagnostic tool. Cleistothecia were sexual structures i.e. network of hyphae where mating between a and α compatible strains occurred. Ascospores (sexual spores) produced within these cleistothecia were lens shaped with equatorial crests (Figure 11). This cleistothecia or perfect state is known as *Emericella nidulans*.
3. **Differentiation from other *Aspergillus* species** – *A. nidulans* could be distinguished by its brown conidiophores, biseriate phialides and round hülle cells. Cleistothecia with lens shaped ascospores with equatorial crests were characteristic.
4. ***In vitro* susceptibility testing** – Most isolates were susceptible to amphotericin B and variably susceptible to itraconazole. Voriconazole was successful in clearing the infection.
5. **Molecular tests** – *A. nidulans* had a well-defined genetic system, which allows it to be used as model organism in basic and applied research.

Comments: One participating laboratory reported this organism as *A. versicolor* and one reported it as *A. terreus*. Microscopic features easily differentiate *A. nidulans* from *A. versicolor* and *A. terreus* by the presence of cleistothecia and ridged ascospores.

Further Reading:

1. Dotis, J. and Roilides, E. 2004. Osteomyelitis due to *Aspergillus* spp. in patients with chronic granulomatous disease: comparison of *Aspergillus nidulans* and *Aspergillus fumigatus*. *Int. J. Infect. Dis.* 8: 103-110.
2. Goldman, G.H. and Kafer, E. 2004. *Aspergillus nidulans* as a model system to characterize the DNA damage response in eukaryotes. *Fungal. Genet. Biol.* 41: 428-442.
3. Horre, R., Schumacher, G., Marklein, G., Kromer, B., Wardelmann, E., Gilges, S., De Hoog, G.S., Wahl, G., and Schaal, K.P. 2002. Case Report. Maxillary sinus infection due to *Emericella nidulans*. *Mycoses.* 45: 402-405.
4. Kontoyiannis, D.P., Lewis, R.E., May, G.S., Osherov, N., and Rinaldi, M.G. 2002. *Aspergillus nidulans* is frequently resistant to amphotericin B. *Mycoses.* 45: 406-407.
5. Rosen-Wolff, A., Koch, A., Friedrich, W., Hahn, G., Gahr, M., Roesler, J. 2004. Successful elimination of an invasive *Aspergillus nidulans* lung infection by voriconazole after failure of a combination of caspofungin and liposomal amphotericin B in a boy with chronic granulomatous disease. *Pediatr. Infect. Dis. J.* 23: 584-586.
6. Van 't Hek LG, Verweij PE, Weemaes CM, van Dalen R, Yntema JB, Meis JF. 1998. Successful treatment with voriconazole of invasive aspergillosis in chronic granulomatous disease. *Am. J. Respir. Crit. Care Med.* 157: 1694-1696.

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

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

Ed. Sp. **E***xophiala spinifera*

Source: Sputum

Scoring:

Referee Labs with correct ID:	52	
Labs with correct ID:	31	
Labs with incorrect ID:	31	
(<i>Phaeoannellomyces</i> sp.)	(14) (<i>Fonsecaea compacta</i>)	(1)
(<i>Phaeoannellomyces wernckii</i>)	(6) (<i>Fonsecaea pedrosoi</i>)	(1)
(Unidentified)	(5) (<i>Oidioderdrion</i> sp.)	(1)
(<i>Aureobasidium pullulans</i>)	(3)	

Clinical Significance: *Exophiala spinifera* is one of the most important dematiaceous human pathogens and causes mycetomas and subcutaneous phaeohyphomycoses

Ecology: *E. spinifera* is cosmopolitan and isolated from wood, soil, and water.

Laboratory Diagnosis:

1. **Culture** – *E. spinifera* grew slowly. On Sabouraud's dextrose agar, after 7 days at 25°C, the colony showed black, moist, and yeast-like growth initially, becoming woolly with age on the surface (Figure 12, left panel). The reverse was black (Figure 12, right panel).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed septate hyphae. Oval to cylindrical conidia accumulated at the tip of annellides. In annellidic conidiogenesis, tip papers, lengthens and acquires a ring of cell wall material as each conidium is released (Figure 13).
3. **Differentiation from other molds** – *E. spinifera* was characterized by annellidic conidiogenesis, which differentiated it from members of *Phialophora*. Variations in decomposition of casein and tyrosine, ability to grow in 15% sodium chloride, KNO₃ assimilation, and the maximum temperature of growth also helped in differentiation of *Exophiala* from *Phaeoannellomyces*.
4. **In vitro susceptibility testing** – *E. spinifera* is reported to be more susceptible to itraconazole than fluconazole.
5. **Molecular tests** – Pathogenic dematiaceous fungi and related taxa including *E. spinifera* could be identified based on large subunit ribosomal DNA D1/D2 domain sequence analysis.

Further Reading:

1. Abliz, P., Fukushima, K., Takizawa, K., and Nishimura, K. 2004. Identification of pathogenic dematiaceous fungi and related taxa based on large subunit ribosomal DNA D1/D2 domain sequence analysis. *FEMS Immunol. Med. Microbiol.* 40: 41-49.
2. Bossler, A.D., Richter, S.S., Chavez, A.J., Vogelgesang, S.A., Sutton, D.A., Grooters, A.M., Rinaldi, M.G., de Hoog, G.S., and Pfaller, M.A. 2003. *Exophiala oligosperma* causing olecranon bursitis. *J. Clin. Microbiol.* 41: 4779-4782.
3. Brandt, M.E. and Warnock, D.W. 2003. Epidemiology, clinical manifestations, and therapy of infections caused by dematiaceous fungi. *J. Chemother.* 15 Suppl 2:36-47.
4. Calista D, Leardini M, Arcangeli F. 2003. Subcutaneous *Exophiala jeanselmei* infection in a heart transplant patient. *Eur. J. Dermatol.* 13: 489.
5. De Monbrison, F., Piens, M.A., Ample, B., Euvrard, S., Cochat, P., and Picot, S. 2004. Two cases of subcutaneous phaeohyphomycosis due to *Exophiala jeanselmei*, in cardiac transplant and renal transplant patients. *Br. J. Dermatol.* 150: 597-598.
6. Hague, J. and Liotta, E. 2003. Subcutaneous phaeohyphomycosis caused by *Exophiala jeanselmei* in an immunocompromised host. *Cutis.* 72: 132-134.
7. Negroni, R., Helou, S.H., Petri, N., Robles, A.M., Arechavala, A., Bianchi, M.H. 2004. Case study: posaconazole treatment of disseminated phaeohyphomycosis due to *Exophiala spinifera*. *Clin. Infect. Dis.* 38: e15-20.
8. Vitale, R.G., De Hoog, G.S., and Verweij, P.E. 2003. *In vitro* activity of amphotericin B, itraconazole, terbinafine and 5-fluocytosine against *Exophiala spinifera* and evaluation of post-antifungal effects. *Med. Mycol.* 41: 301-307.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 and ITS2 rDNA. The sequences are deposited in GenBank under the accession numbers AY591350 and AY591351, respectively.

```

1
NH3-6 (AJ301704)      GTACAAGGTT TCCGTAGGTG AACCTGCGGA AGGAATCATT ACCGAGTTAG
NYSDOH 700-03 (AY591350)      TCCGTAGGTG AACCTGCGGA AGGA-TCATT ACCGAGTTAG

51
GGTCCTCAAA GGCCCGATCT CCCAACCCTT TGCTTATTAT ACCTTGTGTT
GGTCCTCAAA GGCCCGATCT CCCAACCCTT TGCTTATTAT ACCTTGTGTT

101
GCTTCGGTAG GTCTGGCTCG TCCTACCGGA GGACCGTAAA ACGTCCTCTG
GCTTCGGTAG GTCTGGCTCG TCCTACCGGA GGACCGTAAA ACGTCCTCTG

151
GAGAGTGCCT ACCGATAGCC CAAACCAAAA ACTCTTGAAC AAAACATGTA
GAGAGTGCCT ACCGATAGCC CAAACCAAAA ACTCTTGAAC AAAACATGTA

201
TTGTCTGAGT AAACAAAAAA ATTAAGCAA ACT
TTGTCTGAGT AAACAAAAAA ATTAAGCAA ACTTTCAAC AACGGATCTCT

251
TGGTTCTGGC ATCGATGAAG AACGCAGC

```

Figure 14. Alignment of primary sequences of the ITS1 regions of *Exophiala* sp. NH3-6 and PT specimen *Exophiala spinifera* NYSDOH 700-03. Gaps are indicated by dashes. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1
CBS667.76 (AY156964)  AACTTTCAAC AACGGATCTC TTGGTTCTGG CATCGATGAA GAACGCAGCG
NYSDOH 700-03 (AY591351)      G CATCGATGAA GAACGCAGCG

51
AAATGCGATA AGTAATGCGA ATTGCAGAAT TCTCGTGAGT CATCGAATCT
AAATGCGATA AGTAATGCGA ATTGCAGAAT TCCAGTGAGT CATCGAATCT

101
TTGAACGCAC ATTGCGCCCT TTGGTATTCC GAAGGGCATG CCTGTTTCGAG
TTGAACGCAC ATTGCGCCCT TTGGTATTCC GAAGGGCATG CCTGTTTCGAG

151
CGTCATTTTC ACCCCTCAAG CCCCCGGCTT GGTGTTGGAC GGTTTGGTCC
CGTCATTTTC ACCCCTCAAG CCCCCGGCTT GGTGTTGGAC GGTTTGGTTT

201
CGGGACCCCC CTGGACCCCT CCCAAAGACA ATGACGGCGG GCTGTGCGCAC
AGGTACCC- -TAGACCCCT CCTAAAGACA ATGACGGCGG -CTGTTGAAC

251
CCCCGGTACA CTGAGCATCT TCACGGAGCA CGTATCGGTA TTCAGGGTTCG
CCCCGGTACA CTGAGCTTCT TAAC TGAGCA CGTATCGGAT CTAGGGTTTG

301
ACGGCACCCG GTCT-CACAC CTATATCTTT CAAGGTTGAC CTCGGATCAA
GCGGCACCCG GTCTATACCT TTTTGTCTTA CAAGGTTGAC CTCGGATCAG

351
GTAGGAATAC CCGCTGAACT TAAGCATATC AATAAGCGGA GGA
GTAGGAATAC CCGCTGAACT TAAGCATATC AATAAGCGGA GGA

```

Figure 15. Alignment of primary sequences of the ITS2 regions of *Exophiala spinifera* CBS 667.76 and PT specimen *Exophiala spinifera* NYSDOH 700-03. Unmatched nucleotide bases are shaded. Gaps are indicated by dashes. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Comments: *Exophiala* species are usually included among the fungi called “black yeasts”. Conidia are typically borne at the tips of short annellides produced along the vegetative hyphae, which are often difficult to see and to determine. It is easy to confuse with *Phialophora*, which has conidia on phialides rather than annellides, as well as *Aureobasidium*, another genus of black yeast that produces conidia holoblastically on minute peg-like extensions of short hyphal branches or directly along the hyphae themselves. *Phaeoannellomyces werneckii* is able to grow on the media containing 15% NaCl, which differentiates it from *Exophiala* sp.

Source: Eye

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	61
Labs with incorrect ID:	0

Clinical Significance: *Cryptococcus terreus* is not a known pathogen; it is occasionally isolated as a contaminant in clinical laboratories.

Ecology: *C. terreus* is found common in soil.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar, after 7 days at 25°C, *C. terreus* colony was smooth, soft but not mucoid, white turning tan with age (Figure 16).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *C. terreus* showed round to ovoid cells either singly or in groups and no pseudohyphae (Figure 17).
3. **Differentiation from other yeasts** – Biochemically, *C. terreus* closely resembles *Cryptococcus albidus*. Both organisms are urea positive, assimilate nitrate, and do not grow on media containing cycloheximide, with maximum growth temperature of 35°C. However, *C. terreus* does not assimilate sucrose and melezitose, while *C. albidus* assimilates both. *C. terreus* was distinguished from *C. neoformans* is by its ability to assimilate nitrate.
4. **In vitro susceptibility testing** – No information available.
5. **Molecular tests** – No information available.

Further Reading:

1. De Minna, M.E. 1954. *Cryptococcus terreus* n. sp. from soil in New Zealand. *J. Gen. Microbiol.* 11: 195-197.
2. Fell, J.W. and StatzellTallman, A. 1992. Systematic placement of the basidiomycetous yeast *Cystofilobasidium lari* – *Marini* comb. Nov. as predicted by rRNA nucleotide sequence analysis. *Antonie van Leeuwenhoek.* 62: 209-213.
3. Spencer, J.F.T., Gorin, P.A.J., and Tulloch, A.P. 1970. *Torulopsis bombicola* sp. *Antonie van Leeuwenhoek.* 36: 129-133.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA. The sequences are deposited in GenBank under the accession numbers AY591343 and AY591344, respectively.

```

1                                                    50
CBS 1895 (AF444319)   TCCGTAGGTG AACCTGCGGA AGGATCATTG GTGAATTAAA CATGCTTGGT
NYSDOH 76M502 (AY591343) TCCGTAGGTG AACCTGCGGA AGGATCATTG GTGAATTAAA CATGCTTGGT

51                                                    100
GTCTTCGCTT CGGCAAGGCC CTTGCTTAAA TCACATCCTA ACACCTGTGA
GTCTTCGCTT CGGCAAGGCC CTTGCTTAAA TCACATCCTA ACACCTGTGA

101                                                    150
ACTGTAAGAC GTATGATGAG GTCTTTGGCC AAGTCATCGT CTGCCCATTT
ACTGTAAGAC GTATGATGAG GTCTTTGGCC AAGTCATCGT CTGCCCATTT

151                                                    200
TTAACAAACA ATTAATGTAA CAAACGTAGT CTTATTATAA CCTAATAAAA
TTAACAAACA ATTAATGTAA CAAACGTAGT CTTATTATAA CCTAATAAAA

201                                                    250
CTTTCAACAA CGGATCTCTT GGCTCTCGCA TCGATGAAGA ACGCAGCGAA
CTTTCAACAA CGGATCTCTT GGCTCTCGCA TCGATGAAGA ACGCAGCG

```

Figure 18. Alignment of primary sequences of the ITS1 regions of *Cryptococcus terreus* CBS 1895 and PT specimen *C. terreus* NYSDOH 76M502. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1                                                    50
CBS 1895 (AF444319)   CTTTCAACAA CGGATCTCTT GGCTCTCGCA TCGATGAAGA ACGCAGCGAA
NYSDOH 76M502 (AY591344) CTTTCAACAA CGGATCTCTT GGCTCTCGCA GCA TCGATGAAGA ACGCAGCGAA

51                                                    100
ATGCGATAAG TAATGTGAAT TGCAGAATTC AGTGAATCAT CGAATCTTTG
ATGCGATAAG TAATGTGAAT TGCAGAATTC AGTGAATCAT CGAATCTTTG

101                                                    150
AACGCACCTT GCGCTCTTTG GTATTCCGAA GAGCATGCCT GTTTGAGTGT
AACGCACCTT GCGCTCTTTG GTATTCCGAA GAGCATGCCT GTTTGAGTGT

151                                                    200
CATGAAAATA TCAACCTTGA CTTGGGTTTT GTGCTCTAGT CTTGGCTTGG
CATGAAAATA TCAACCTTGA CTTGGGTTTT GTGCTCTAGT CTTGGCTTGG

201                                                    250
AATTGGGTGC TTGCCGCTTT TACGAGCGGC TCACCTTAAA TGTATTAGCT
AATTGGGTGC TTGCCGCTTT TACGAGCGGC TCACCTTAAA TGTATTAGCT

251                                                    300
GGATCTGTCT TTGAGACTTG GTTTGACTION GCGTAATAAG TATTTGCTA
GGATCTGTCT TTGAGACTTG GTTTGACTION GCGTAATAAG TATTTGCTA

301                                                    350
AGGACATTCT TCGGAGGGGC CTCGTTTCTA GGACGCTTGT CCGCTTCCCA
AGGACATTCT TCGGAGGGGC CTCGTTTCTA GGACGCTTGT CCGCTTCCCA

351                                                    400
ATACAAGTTC CACCTCGTGG ACATGACTTT TTATTATCTG GCCTCAAATC
ATACAAGTTC CACCTCGTGG ACATGACTTT TTATTATCTG GCCTCAAATC

401
AGGTAGGACT ACCCGCTGAA CTTAAGCATA TCAATAAGCG GAGGA
AGGTAGGACT ACCCGCTGAA CTTAAGCATA TCAATAAGCG GAGGA

```

Figure 19. Alignment of primary sequences of the ITS2 regions of *Cryptococcus terreus* CBS 1895 and PT specimen *C. terreus* NYSDOH 76M502. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Comments: All participating laboratories reported this specimen correctly.

Source: Catheter

Scoring:	No. Labs
Referee Labs with correct ID:	9
Labs with correct ID:	57
Labs with incorrect ID:	4
(<i>Rhodotorula glutinis</i>)	(2)
(<i>Rhodotorula</i> sp.)	(2)

Clinical Significance: *Rhodotorula minuta* was reported as a causative agent of infections in humans with AIDS and leukemia. It was isolated from blood, sputum, throat swabs, and feces.

Ecology: *R. minuta* was found in water and on oat leaves.

Laboratory Diagnosis:

1. Culture – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was pink, smooth, and soft (Figure 20).
2. Microscopic morphology – On corn meal agar with Tween 80, *R. minuta* had no pseudohyphae, round blastoconidia were seen (Figure 21).
3. Differentiation from other yeasts – *R. minuta* did not assimilate maltose, which differentiated it from *R. glutinis* and *R. mucilaginosa*.
4. In vitro susceptibility testing – *R. minuta* was susceptible to amphotericin B, but resistant to azoles.
5. Molecular tests – No information available.

Further Reading:

1. Cutrona, A.F., Shah, M., Himes, M.S., and Miladore, M.A. 2002. *Rhodotorula minuta*: an unusual fungal infection in hip-joint prosthesis. *Am. J. Orthop.* 31: 137-140.
2. Garcia-Martos, P., Dominguez, I., Marin, P., Garcia-Agudo, R., Aoufi, S., and Mira, J. 2001. Antifungal susceptibility of emerging yeast pathogens. *Enferm. Infecc. Microbiol. Clin.* 19: 249-256.
3. Goldani, L.Z., Craven, D.E., and Sugar, A.M. 1995. Central venous catheter infection with *Rhodotorula minuta* in a patient with AIDS taking suppressive doses of fluconazole. *J. Med. Vet. Mycol.* 33: 267-270.
4. Pinna, A., Carta, F., Zanetti, S., Sanna, S., Sechi, L.A. 2001. Endogenous *Rhodotorula minuta* and *Candida albicans* endophthalmitis in an injecting drug user. *Br. J. Ophthalmol.* 85: 759.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 region of rDNA. The sequence is deposited in GenBank under the accession numbers AY591345.

```

1                                                    50
CBS 5706 (AF444627)   TCCGTAGGTG AACCTGCGGA AGGATCATT A ATGAATTTTA GGACGTTCTT
NYSDOH 76M553 (AY591345) TCCGTAGGTG AACCTGCGGA AGGATCATT A ATGAATTTTA GGACGTTCTT

51                                                    100
TTTAGAAGTC CGACCCTTTC ATTTTCTTAC ACTGTGCACA CACTTCTTTT
TTTAGAAGTC CGACCCTTTC ATTTTCTTAC ACCGTGCACA CACTTCTTTT

101                                                    150
TTACACACAC TTTTAACACC TTAGTATAAG AATGTAATAG TCTCTTAATT
TTACACACAC TTTTAACACC TTAGTATAAG AATGTAATAG TCTCTTAATT

151                                                    200
GAGCATAAAT AAAAAACAAA CTTTCAGCAA CGGATCTCTT GGCTCTCGCA
GAGCATAAAT AAAAAACAAA CTTTCAGCAA CGGATCTCTT GGCTCTCGCA

201                                                    250
TCGATGAAGA ACGCAGCGAA TTGCGATAAG TAATGTGAAT TGCAGAATTC
TCGATGAAGA ACGCAGC

```

Figure 22. Alignment of primary sequences of the ITS1 regions of *Rhodotorula minuta* CBS 5706 and PT specimen *R. minuta* NYSDOH 76M553. Unmatched nucleotide bases are shaded. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Comments: Two participating laboratories reported this specimen as *R. glutinis*, which is able to assimilate maltose but *R. minuta* does not. Another two laboratories did not identify this specimen to species level, which is not acceptable for this specimen.

Y-3

Source: Bronchoalveolar lavage

Scoring:	No. Labs
Referee Labs with correct ID:	7
Labs with correct ID:	48
Labs with incorrect ID:	13
(<i>Cryptococcus humicolus</i>)	(11)
(<i>Cryptococcus laurentii</i>)	(1)
(<i>Trichosporon</i> sp.)	(1)

Y-4

Source: CSF

Scoring:	No. Labs
Referee Labs with correct ID:	7
Labs with correct ID:	45
Labs with incorrect ID:	16
(<i>Cryptococcus humicolus</i>)	(10)
(<i>Cryptococcus laurentii</i>)	(3)
(<i>Blastoschizomyces capitatus</i>)	(2)
(<i>Trichosporon</i> sp.)	(1)

Clinical Significance: *Trichosporon mucoides* is the causative agent of white piedra, superficial infections and invasive trichosporonosis.

Ecology: *T. mucoides* is found in soil, water samples, and from vegetation.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was white to cream, yellowish, wrinkled (Figure 23).
2. **Microscopic morphology** – On corn meal agar with Tween 80, septate hyphae with arthroconidia were seen (Figure 24).
3. **Differentiation from other yeasts** – *T. mucoides* was a nonfermentative, urea positive, nitrate negative, cycloheximide resistant, and metabolically active for assimilation of a wide range of carbohydrates.
4. **In vitro susceptibility testing** – *T. mucoides* was resistant to amphotericin B but susceptible to various azoles such as fluconazole, ketocoanzole, and itraconazole.
5. **Molecular tests** – PCR assay of the ITS1 and ITS2 regions of ribosomal DNA was able to identify medically relevant *Trichosporon* species.

Further Reading:

1. Del Palacio, A., Perez, R.A., Albanil, R., Sotelo, T., and Kalter, D.C. 1990. Disseminated neonatal trichosporosis associated with hemophagocytic syndrome. *Pediatr. Infect. Dis. J.* 9: 520-522.
2. Garcia-Martos, P., Dominguez, I., Marin, P., Garcia-Agudo, R., Aoufi, S., and Mira, J. 2001. Antifungal susceptibility of emerging yeast pathogens. *Enferm. Infecc. Microbiol. Clin.* 19: 249-256.
3. Gokahmetoglu, S., Nedret Koc, A., Gunes, and T, Cetin, N. 2002. Case reports. *Trichosporon mucoides* infection in three premature newborns. *Mycoses.* 45: 123-125.
4. Nettles, R.E., Nichols, L.S., Bell-McGuinn, K., Pipeling, M.R., Scheel, P.J. Jr, and Merz, W.G. 2003. Successful treatment of *Trichosporon mucoides* infection with fluconazole in a heart and kidney transplant recipient. *Clin. Infect. Dis.* 36: E63-6.
5. Singh, N., Belen, O., Leger, M.M., and Campos, J.M. 2003. Cluster of *Trichosporon mucoides* in children associated with a faulty bronchoscope. *Pediatr. Infect. Dis. J.* 22: 609-12.
6. Sugita, T., Nishikawa, A., Ikeda, R., Shinoda, T. 1999. Identification of medically relevant *Trichosporon* species based on sequences of internal transcribed spacer regions and construction of a database for *Trichosporon* identification. *J. Clin. Microbiol.* 37: 1985-1993.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA. The sequences are deposited in GenBank under the accession numbers AY591346 and AY591347, respectively.

```

1                                                    50
wb305 (AF455482)   AAAAGTCGTA ACAAGGTTTC CGTAGGTGAA CCTGCGGAAG GATCATTAGT
UAMH 792 (AY591346)          TC CGTAGGTGAA CCTGCGGAAG GATCATTAGT

51                                                    100
GAATTGCTCT TTTGAGCGTTA AACTATATCC ATCTACACCT GTGAACTGTT
GAATTGCTCT TTTGAGCGTTA AACTATATCC ATCTACACCT GTGAACTGTT

101                                                    150
GATTGACTTC GGTCAATTAC TTTTACAAAC ATTGTGTAAT GAACGTCATG
GATTGACTTC GGTCAATTAC TTTTACAAAC ATTGTGTAAT GAACGTCATG

151                                                    200
TTATTATAAC AAAAATAACT TTCAACAACG GATCTCTTGG CTCTCGCATC
TTATTATAAC AAAAATAACT TTCAACAACG GATCTCTTGG CTCTCGCATC

201                                                    250
GATGAAGAAC GCAGCGAAAT GCGATAAGTA ATGTGAATTG CAGAATTCAG
GATGAAGAAC GCAGC

```

Figure 25. Alignment of primary sequences of the ITS1 regions of *Trichosporon mucoides* wb305 and PT specimen *T. mucoides* UAMH 792. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1                                                    50
UWFP-366 (AF335970) GCATCGATGA AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
UAMH 792 (AY591347) GCATCGATGA AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA

51                                                    100
TTCAGTGAAT CATCGAATCT TTGAACGCAA CTTGCGCTCT CTGGTATTCC
TTCAGTGAAT CATCGAATCT TTGAACGCAA CTTGCGCTCT CTGGTATTCC

101                                                    150
GGAGAGCATG CCTGTTTGAG TATCATGAAA TCTCAACCAT TAGGGTTTCT
GGAGAGCATG CCTGTTTGAG TATCATGAAA TCTCAACCAT TAGGGTTTCT

151                                                    200
TAATGGCTTG GATTTGGGCG CTGCCACTTG CCTGGCTCGC CTTAAAAGAG
TAATGGCTTG GATTTGGGCG CTGCCACTTG CCTGGCTCGC CTTAAAAGAG

201                                                    250
TTAGCGTATT AACTTGTCGA TCTGGCGTAA TAAGTTTCGC TGGTGTAGAC
TTAGCGTATT AACTTGTCGA TCTGGCGTAA TAAGTTTCGC TGGTGTAGAC

251                                                    300
TTGAGAAGTG CGCTTCTAAT CGTCTTCGGA CAATTCTTGA ACTCTGGTCT
TTGAGAAGTG CGCTTCTAAT CGTCTTCGGA CAATTCTTGA ACTCTGGTCT

301                                                    350
CAAATCAGGT AGGACTACCC GCTGAACTTA AGCATATCAA TAAGCGGAGG
CAAATCAGGT AGGACTACCC GCTGAACTTA AGCATATCAA TAAGCGGAGG

351
A
A

```

Figure 26. Alignment of primary sequences of the ITS2 regions of *Trichosporon mucoides* UWFP-366 and PT specimen *T. mucoides* UAMH 792. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Comments: Specimen Y-3 and Y-4 were the same isolates in this PT event. *C. humicolus* resembles *T. mucoides* morphologically and on assimilation patterns. However, all strains of *Trichosporon* produce arthroconidia while *C. humicolus* does not.

Source: Bronchial wash

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	61
Labs with incorrect ID:	0

Clinical Significance: *Geotrichum candidum* commonly causes pulmonary infections in immunocompromised patients. It also produces lesions in alimentary tract, vagina, and skin. *G. candidum* has also been reported to cause fungemia and disseminated infection.

Ecology: *G. candidum* is cosmopolitan in distribution. It has been isolated from air, water, plants, milk, and milk products. It is found as a commensal in pulmonary and gastrointestinal tract of humans.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar after 7 days at 25°C, colony grew rapidly, it was white to cream colored, flat with aerial mycelium, (Figure 27).
2. **Microscopic morphology** – On corn meal agar with Tween 80, true hyphae with arthroconidia were seen (Figure 28). Arthroconidia formation was by the fragmentation of hyphae, no disjunctor cells (empty cells between the arthroconidia) and no blastoconidia were formed.
3. **Differentiation from other yeasts** – *G. candidum* grew on the media containing cycloheximide, negative on urease reaction, grew sparingly at 37°C. It was differentiated from *Trichosporon* species by absence of blastoconidia, no growth at higher temperatures (40, 42, & 45°C). *Blastoschizomyces capitatus* could be differentiated from *G. candidum* by the lack of growth on a medium containing D-xylose as a carbon source and its growth at 45°C. *G. candidum* was differentiated from arthroconidia forming molds by its colony morphology. Microscopically *Arthrographis* and *Odiendron* had conidiophores while *Malbranchea* and *Coccidioides immitis* had disjunctor cells.
4. **In vitro susceptibility testing** – Limited studies suggested that most isolates were susceptible to amphotericin B and to azoles like fluconazole and itraconazole.
5. **Molecular tests** – Randomly amplified polymorphic DNA (RAPD) PCR had been used for the identification of *G. candidum* isolated from cheese. Using DNA/DNA reassociation techniques, de Hoog et al (1986 and 1990) found the relatedness between *G. candidum* and its teleomorph (sexual state) *Galactomyces geotrichum*.

Further Reading:

1. Andre, N., Coze, C., Gentet, J.C., Perez, R., and Bernard, J.L. 2004. *Geotrichum candidum* septicemia in a child with hepatoblastoma. *Pediatr. Infect. Dis. J.* 23: 86
2. Depagne, C., Louerat, C., Nesme, P. 2003. Herpes simplex and *Geotrichum candidum* pneumonia in a patient with moderate renal failure. *Rev. Pneumol. Clin.* 59: 297-300.
3. De Hoog, G.S., and Amberger, A.E. 1990. Electrophoretic protein patterns of *Geotrichum* and its teleomorphs. *Antonie Van Leeuwenboek.* 58: 101-105.
4. Pryce, T.M., Palladino, S., Kay, I.D., Coombs, G.W. 2003. Rapid identification of fungi by sequencing the ITS1 and ITS2 regions using an automated capillary electrophoresis system. *Med. Mycol.* 41: 369-81.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 region of rDNA. The sequence is deposited in GenBank under the accession numbers AY591348.

```

1
CBS 121.22 (AJ279451)      TTCTTGGTCA TTTAGAGGAA GTAAAAGTCG TAACAAGGTT TCCGTAGGTG
NYSDOH 302196 (AY591348)                                TCCGTAGGTG

51
AACCTGCGGA AGGATCATT TGAATTAATA ATATTTGTGA ATTTACCACA
AACCTGCGGA AGGATCATT TGAATTATTA ATATTTGTGA ATTTACCACA

101
ACAAACATCA ATCATACAAT CAATAATTAA AAAATTAAAA CTTTTAACAA
GCAAACATCA ATCATACAAT CAATAATTAA ATAATTAAAA CTTTTAACAA

151
TGGATCTCTT GGTTCTCGTA TCGATGAAGA ACGCAGCGAA ACGCGATATT
TGGATCTCTT GGTTCTCGCA TCGATGAAGA ACGCAGC
200

```

Figure 29. Alignment of primary sequences of the ITS1 regions of *Geotrichum candidum* CBS 121.22 and PT specimen *G. candidum* NYSDOH 302196. Unmatched nucleotide bases are shaded. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Comments: All the participating laboratories have correctly identified this specimen.

Introduction: Document M27-A2 published by the National Committee for Clinical Laboratory Standards (NCCLS) Subcommittee on Antifungal Susceptibility Testing is the current standard reference guide for determining the antifungal susceptibility testing of pathogenic yeasts. It includes two methods, broth microdilution and broth macrodilution. Various commercial systems are also being developed for antifungal susceptibility testing of yeasts, such as Sensititre YeastOne Colorimetric Panel and Etest. The disk diffusion testing method approved by NCCL as document M44-A is another good method for antifungal susceptibility testing of yeast, where the results could be read after 24 hr incubation rather than after 48 hr.

Materials & Methods: Twenty-one microbiology laboratories within the United States and one reference laboratory each from Canada and United Kingdom participated in this event. Two NCCLS quality control strains, *Candida krusei* ATCC 6258 (S-2), and *Candida parapsilosis* ATCC 22019 (S-3), three NCCLS reference strains, *Candida tropicalis* ATCC 750 (S-1), *Candida parapsilosis* ATCC 90018 (S-4), and *Candida albicans* ATCC 90028 (S-5), and an educational specimen *C. albicans* NYSDOH 40026 (S-edu.) were included in the January 28, 2004 antifungal proficiency testing event. These isolates except the educational specimen have been well characterized, and their MIC ranges against amphotericin B and fluconazole have been published. MICs within ± 2 dilutions of the reference result (range of MICs for a particular yeast described in NCCLS, M27-A2) are the acceptable results in this event.

Results: A total of 23 laboratories participated in this antifungal susceptibility testing event. The performances of all 23 laboratories were satisfactory. Of the 23 participating laboratories, 9 laboratories used the broth microdilution method, 12 laboratories used YeastOne Colorimetric microdilution method, and 2 laboratories used Etest. The supplementary information on antifungal susceptibility testing procedures is summarized in Table 1. The MIC results submitted by the 23 participants are illustrated in Figure 30. For amphotericin B, good performance was noted for all specimens irrespective of the methodology used by the laboratories. For fluconazole, good performance was seen for *C. tropicalis* ATCC 750 and *C. krusei* ATCC 6258. Overall agreement with the NCCLS reference ranges was 100% against amphotericin B and 98% against fluconazole for five tested isolates. Furthermore, after with the expansion of the reference range by ± 2 dilutions, 90% of answers for amphotericin B and 87% answers for fluconazole were within the NCCLS reference range. For the educational specimen *C. albicans* NYSDOH 40026, all participating laboratories reported relatively consistent range for amphotericin B. However, very wide range of MICs were reported for fluconazole with susceptible to resistant interpretations. For fluconazole susceptibility test, trailing phenomenon could typically be seen for some isolates of *C. albicans*.

Further Reading:

1. Arthington-Skaggs, B.A., Lee-Yang, W., Ciblak, M.A., Frade, J.P., Brandt, M.E., Hajjeh, R.A., Harrison, L.H., Sofair, A.N., Warnock, D.W.; and Candidemia Active Surveillance Group. 2002. Comparison of visual and spectrophotometric methods of broth microdilution MIC end point determination and evaluation of a sterol quantitation method for in vitro susceptibility testing of fluconazole and itraconazole against trailing and nontrailing *Candida* isolates. *Antimicrob. Agents Chemother.* 46: 2477-2481.
2. Barry, A.L., Pfaller, M.A., Rennie, R.P., Fuchs, P.C., and Brown, S.D. 2002. Precision and accuracy of fluconazole susceptibility testing by broth microdilution, Etest, and disk diffusion methods. *Antimicrob. Agents Chemother.* 46: 1781-1784.
3. Espinel-Ingroff, A., Pfaller, M., Messer, S.A., Knapp, C.C., Killian, S., Norris, H.A., and Ghannoum, M.A. 1999. Multicenter comparison of the sensititre YeastOne Colorimetric Antifungal Panel with the National Committee for Clinical Laboratory standards M27-A reference method for testing clinical isolates of common and emerging *Candida* spp., *Cryptococcus* spp., and other yeasts and yeast-like organisms. *J. Clin. Microbiol.* 37: 591-595.
4. Martin-Mazuelos, E., Gutierrez, M.J., Aller, A.I., Bernal, S., Martinez, M.A., Montero, O., and Quindos, G. 1999. A comparative evaluation of Etest and broth microdilution methods for fluconazole and itraconazole susceptibility testing of *Candida* spp. *J. Antimicrob. Chemother.* 43: 477-481.

*A*ntifungal susceptibility testing

Individual Isolates:

S-1 *Candida tropicalis* ATCC 750

Summary	NCCLS Reference Range	Expanded Range
Amphotericin B	0.5-2.0 µg/ml	0.25-4.0 µg/ml
Fluconazole	1.0-4.0 µg/ml	0.5-8.0 µg/ml

Nineteen laboratories reported values within the NCCLS reference range and 1 laboratory reported value within the expanded range for amphotericin B. Twenty-two laboratories reported values within the NCCLS reference range, 1 laboratory reported value within the expanded range for fluconazole.

S-2 *Candida krusei* ATCC 6258

Summary	NCCLS Reference range	Expanded Range
Amphotericin B	0.25-2.0 µg/ml	0.12-4.0 µg/ml
Fluconazole	16-64 µg/ml	8->64 µg/ml

All of the participating laboratories reported values within the NCCLS reference range for amphotericin B. Twenty-two laboratories reported values within the NCCLS reference range,

1 laboratory reported values within the expanded values for fluconazole.

S-3 *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

Nineteen laboratories reported values within the NCCLS reference range, 1 laboratory reported value within the expanded range for amphotericin B. Twenty-one laboratories reported values within the NCCLS reference range, 2 laboratories reported value within the expanded range for fluconazole.

S-4 *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

Seventeen laboratories reported values within the NCCLS reference range and 3 laboratories reported value within the expanded range for amphotericin B. Nineteen laboratories reported values within the NCCLS reference range, 2 laboratories reported value within the expanded range, 2 laboratories reported a MIC value higher than the expanded range for fluconazole.

S-5 *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

Fifteen laboratories reported values within the NCCLS reference range and 5 laboratories reported value within the expanded range for amphotericin B. Eighteen laboratories reported values within the NCCLS reference range, 5 laboratories

S-edu. *Candida albicans* NYSDOH 40026

Summary	Range reported by all participating laboratories
Amphotericin B	0.06-1.0 µg/ml
Fluconazole	0.25-512 µg/ml

Further Reading: (cont'd)

5. National Committee for Clinical Laboratory Standards. 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard – Second Edition. NCCLS document M27-A2 (ISBN 1-56238-469-4). National Committee for Clinical Laboratory Standards, Wayne, PA.

6. National Committee for Clinical Laboratory Standards. 2004. Method for antifungal disk diffusion susceptibility testing of yeasts; approved guideline. NCCLS document M44-A (ISBN 1-56238-532-1). National Committee for Clinical Laboratory Standards, Wayne, PA.

7. Posteraro, B., Romano, L., Sanguinetti, M., Masucci, L., Morace, G., and Fadda, G. 2000. Commercial systems for fluconazole susceptibility testing of yeasts: comparison with the broth microdilution method. *Diagn. Microbiol. Infect. Dis.* 38: 29-36.

8. Rex, J.H., Pfaller, M.A., Galgiani, J., Bartlett, M., Espinel-Ingroff, A., Ghannoum, M., Lancaster, M., Odds, F., Rinaldi, M., Walsh, T., and Barry, A. 1997. Development of interpretive breakpoints for antifungal susceptibility testing; Conceptual framework and analysis of *in vivo* and *in vitro* correlation data for fluconazole and itraconazole and *Candida* infections. *Clin. Infect. Dis.* 24: 235-247.

9. Rex, J.H., Pfaller, M.A., Walsh, T.J., Chaturvedi, V., Espinel-Ingroff, A., Ghannoum, M.A., Gosey, L.L., Odds, F.C., Rinaldi, M.G., Sheehan, D.J., and Warnock, D.W. 2001. Antifungal susceptibility testing: practical aspects and current challenges. *Clin. Microbiol. Rev.* 14: 643-58.

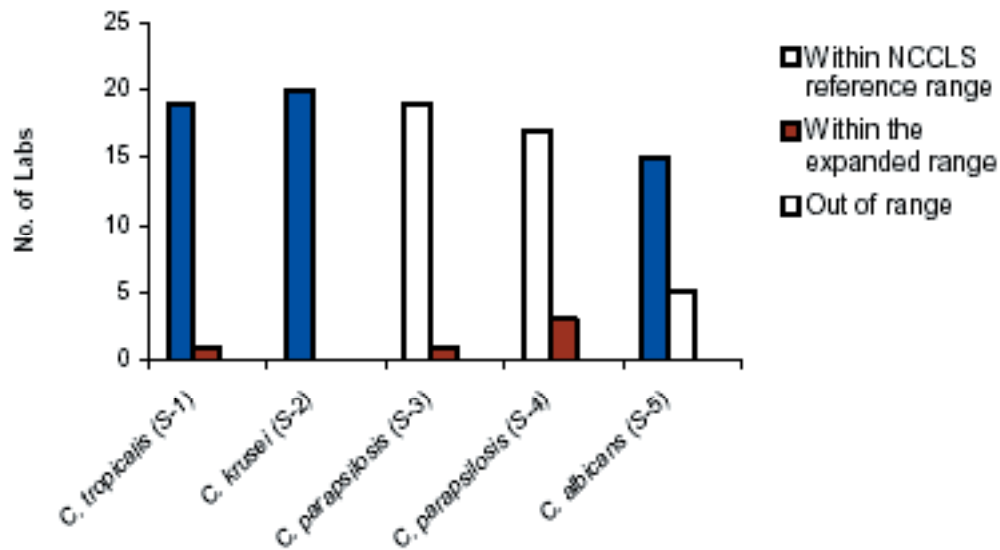
Table 1. Summary of supplementary information on antifungal susceptibility testing by participating laboratories

	No. Participant Labs
Test Method	
NCCLS broth microdilution	9
NCCLS broth macrodilution	0
Sensititre YeastOne Colorimetric	12
Etest	2
Medium employed	
RPMI 1640	11
RPMI 1640 w / alamar blue	2
Antibiotic medium 3	1
Sabouraud dextrose	3
YeastOne broth	6
Inoculum preparation *	
Spectrophotometric	13
MacFarland	15
Inoculum size (CFU/ml)	
0.5-2.5 × 10 ³	9
1.5-8 × 10 ³	10
0.5-1.0 × 10 ⁴	4
0.5-1.0 × 10 ⁵	0
Incubation temperature	
35°C	22
37°C	1
Incubation duration *	
24 hr	14
48 hr	11
Endpoint reading	
Visual	14
Spectrophotometric	0
Colorimetric	9
Scoring endpoint¹*	
100% inhibition	10
95% inhibition	1
80% inhibition	5
50% inhibition	3
Other (color change)	8
QC organism	
NCCLS recommended strains	23
Unknown	0

¹Most labs used 100% inhibition for amphotericin B and either 50% or 80% inhibition for fluconazole.

* More than one value reported by individual laboratories

A



B

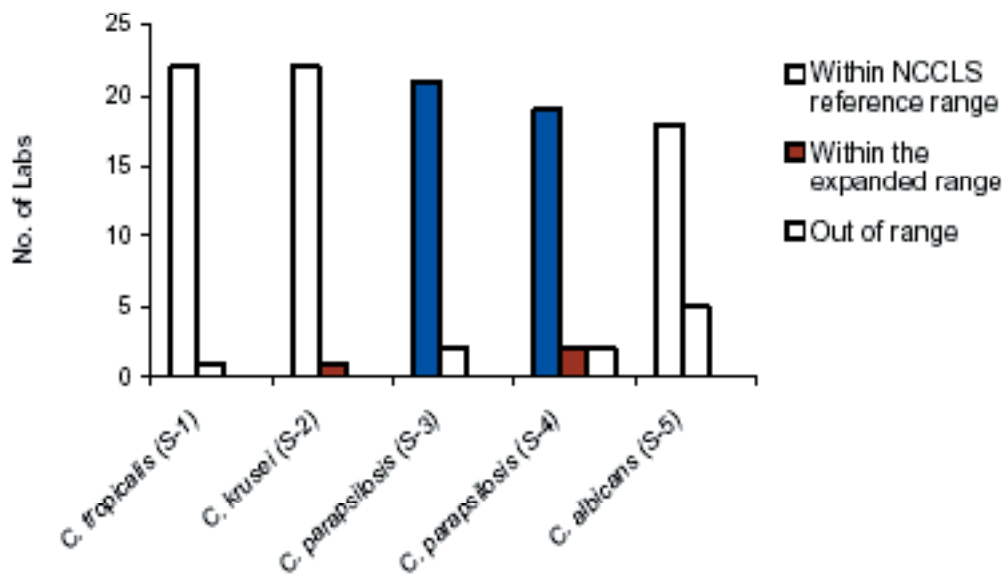


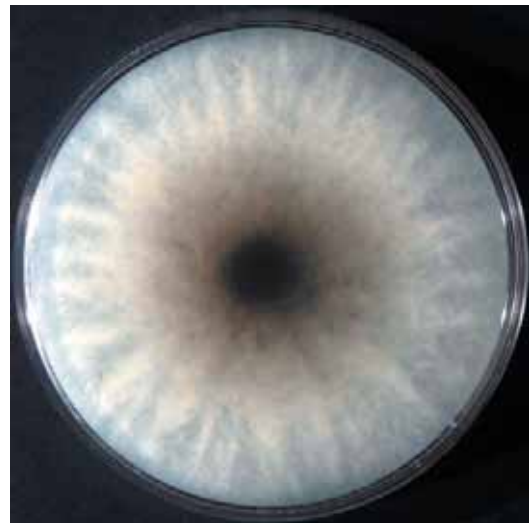
Figure 30. Summary of the results submitted by the participating laboratories for 5 isolates, for amphotericin B (A) and fluconazole (B).

M-1 *Scytalidium* sp.

Figure 1

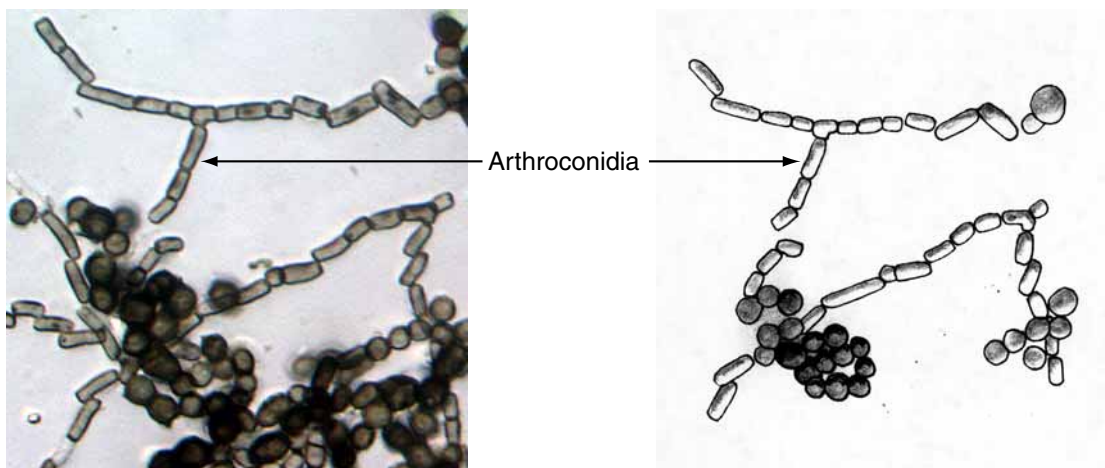


Five-day-old, pale yellow to brown, wooly texture colony of *Scytalidium* sp. on Sabouraud's dextrose agar.



The reverse shows pale white, brown to black.

Figure 2



Microscopic morphology of *Scytalidium* sp. showing rectangular arthroconidia (left; 400× magnification, right; line drawing not to scale).

Figure 4

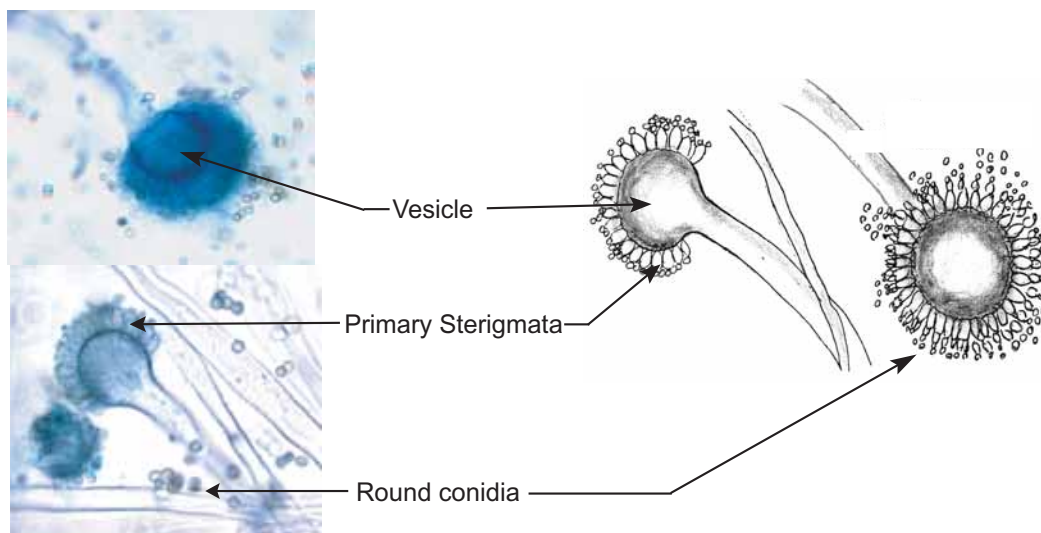


Five-day-old, yellow-green colony of *Aspergillus flavus* on Sabouraud's dextrose agar.



The reverse of the colony shows pale to yellowish.

Figure 5



Microscopic morphology of *Aspergillus flavus* depicting typical radiate heads with globose vesicle, uniseriate sterigmata, and round conidia (left; 400× magnification, right; line drawing not to scale).

Figure 6

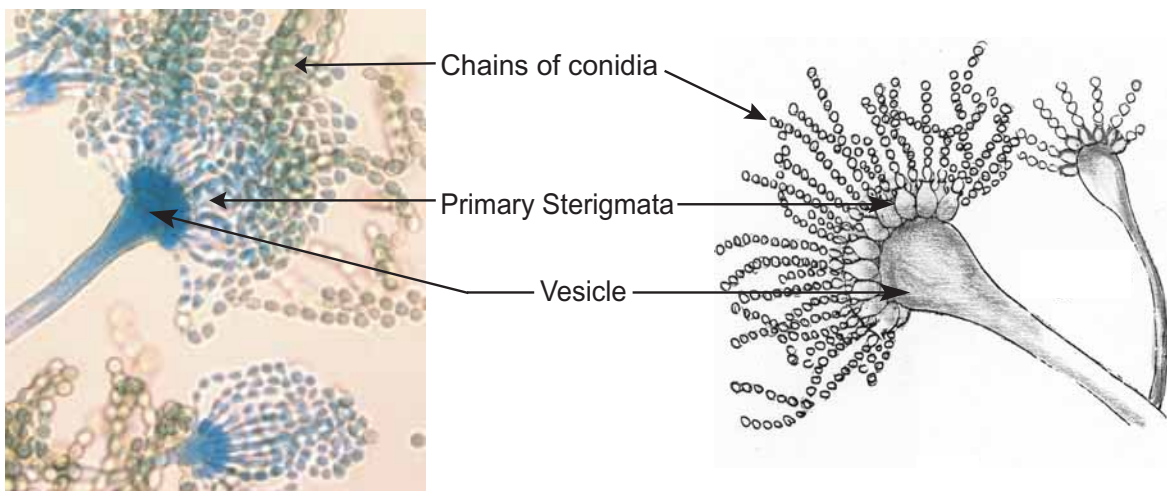


Five-day-old, blue-green, powdery colony of *Aspergillus fumigatus* on Sabouraud's dextrose agar.



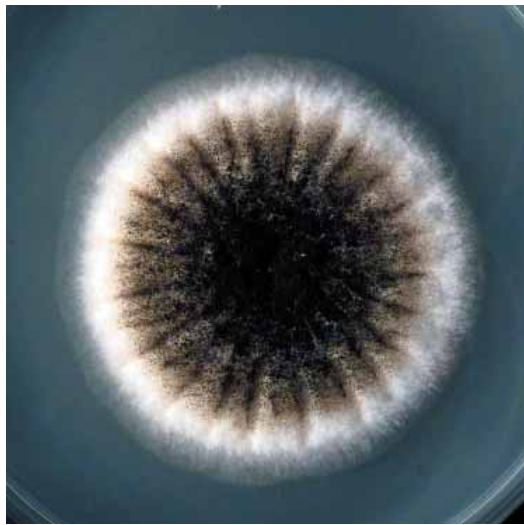
The reverse side of the colony is pale yellow.

Figure 7

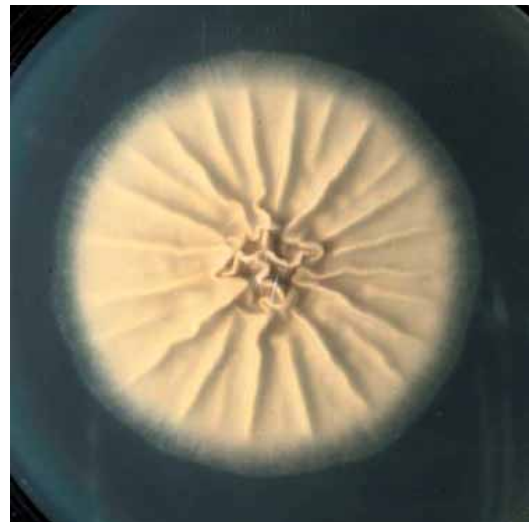


Microscopic morphology of *Aspergillus fumigatus* showing typical, columnar conidiophores consisting of subglobose vesicle with uniseriate sterigmata and large chains of round conidia (left; 400× magnification, right; line drawing not to scale).

Figure 8

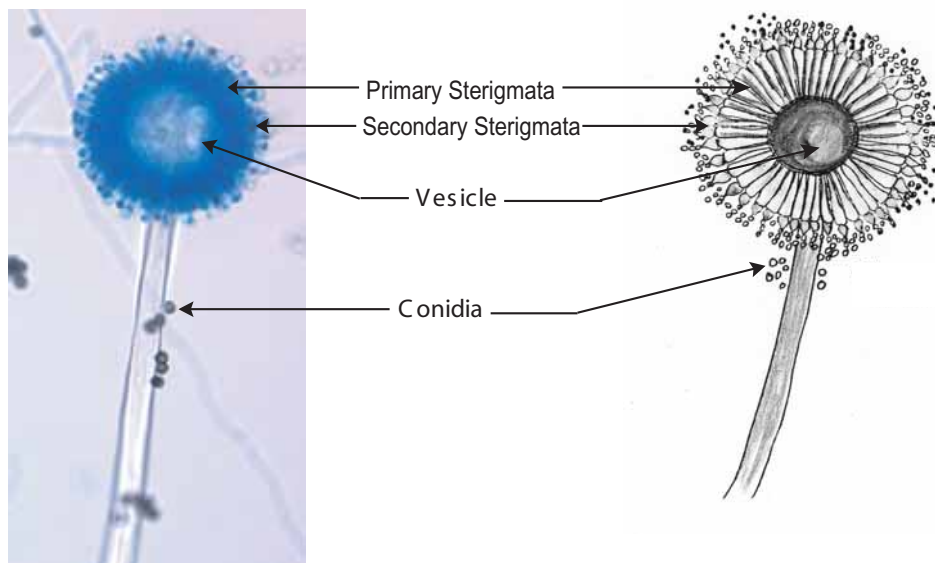


Five-day-old, black colony of *Aspergillus niger* on Sabouraud's dextrose agar. The colony showing typical "salt and pepper appearance", which results from darkly pigmented conidia borne in large numbers on conidiophores.



The reverse of the colony appears yellowish to pale.

Figure 9



Microscopic morphology of *Aspergillus niger* showing globose vesicle with biserial, radiating head and dark, round conidia (left; 400× magnification, right; line drawing not to scale).

Figure 10

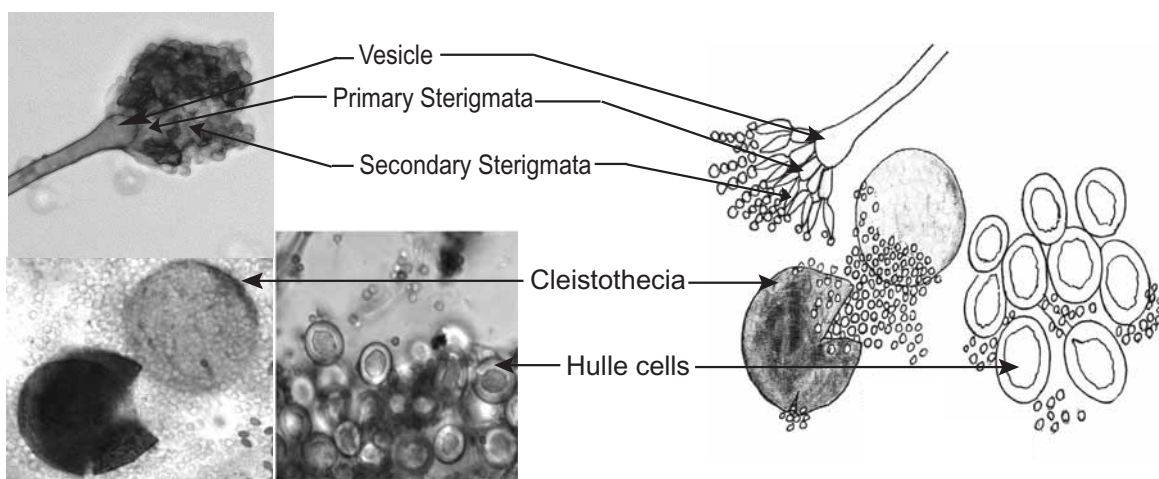


Five-day-old, green to blue colony of *Aspergillus nidulans* with whitish at the edge on Sabouraud's dextrose agar.



The reverse of the colony is yellow to brownish.

Figure 11

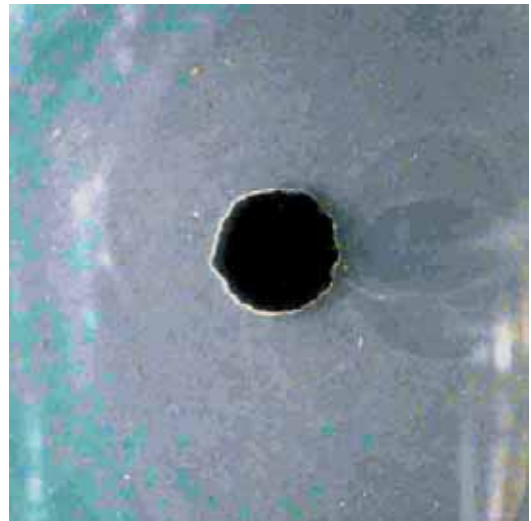


Microscopic morphology of *Aspergillus nidulans* showing subglobose vesicle with biserial, columnar head, cleistothecia with ascus and ascospores, and hülle cells (left; 400× magnification, right; line diagram not to scale).

Figure 12

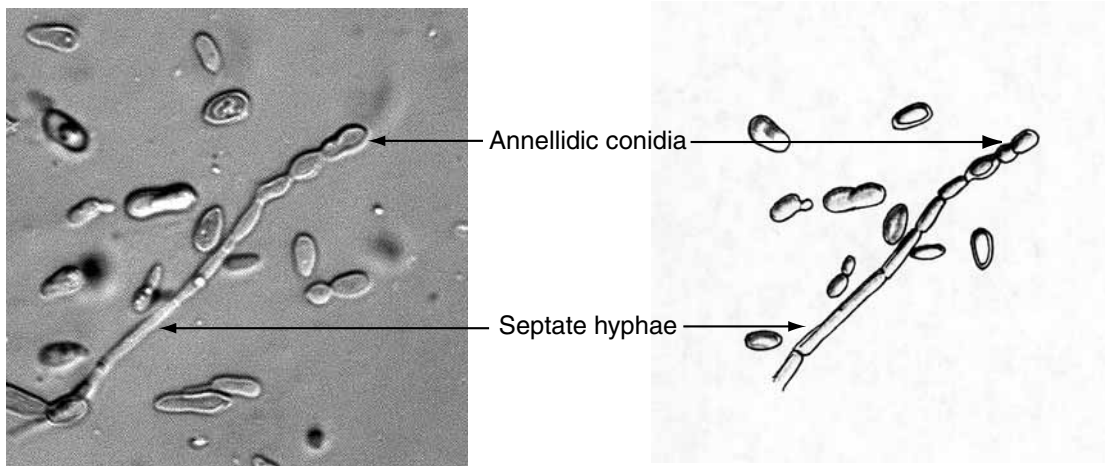


Five-day-old, black and mucoid colony of *Exophiala* sp. on Sabouraud's dextrose agar.



The reverse of the colony is black.

Figure 13



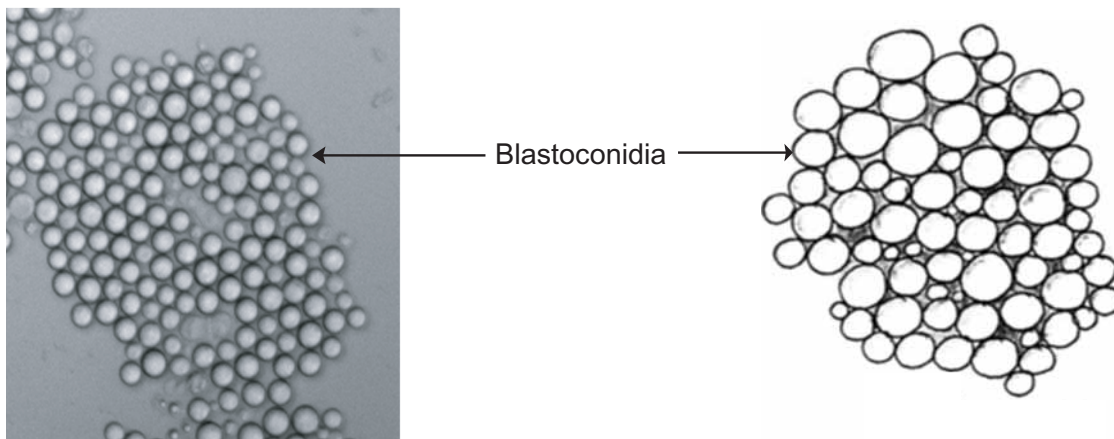
Microscopic morphology of *Exophiala* sp. on corn meal agar showing septate hyphae and annelidic conidiogenesis of conidia accumulated at the tip of annellides, oval to cylindrical shaped (left; 400× magnification, right; line drawing not to scale).

Figure 16



Seven-day-old, soft, smooth, and white colony of *Cryptococcus terreus* on Sabouraud's dextrose agar.

Figure 17



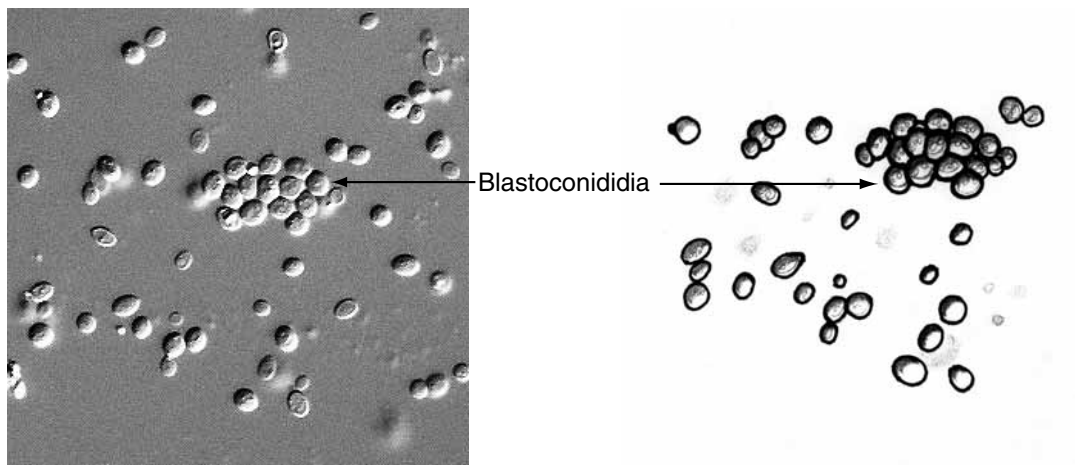
Microscopic morphology of *Cryptococcus terreus*. On corn meal agar with Tween 80 culture, round blastoconidia are seen (left; 400× magnification, right; line drawing not to scale).

Figure 20



Seven-day-old, soft, smooth, and pink colony of *Rhodotorula minuta* on Sabouraud's dextrose agar.

Figure 21



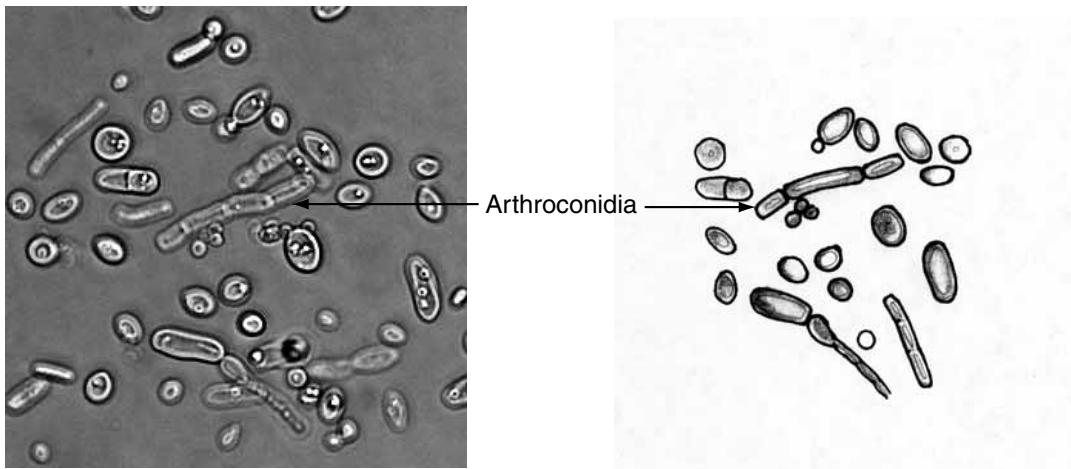
Microscopic morphology of *Rhodotorula minuta*. On corn meal agar with Tween 80 culture, round blastoconidia are seen (left; 400× magnification, right; line drawing not to scale).

Figure 23



Seven-day-old, white to cream, yellowish, wrinkled colony of *Trichosporon mucoides* on Sabouraud's dextrose agar.

Figure 24



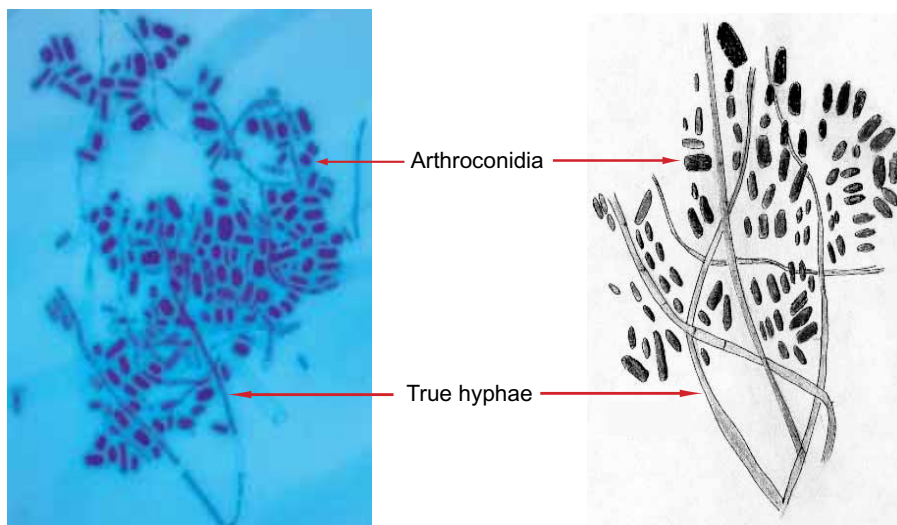
Microscopic morphology of *Trichosporon mucoides*, on corn meal agar with Tween 80 showing arthroconidia (left; 200 \times magnification, right; line drawing not to scale).

Figure 27



Seven-day-old, white, and mold like colony of *Geotrichum candidum* on Sabouraud's dextrose agar.

Figure 28



Microscopic morphology of *Geotrichum candidum* on corn meal agar with Tween 80 showing true hyphae and arthroconidia (left; 200× magnification, right; line drawing not to scale).

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New York State Department of Health

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