

A detailed microscopic image showing a dense network of thin, branching, and sometimes septate hyphae, characteristic of a filamentous fungus. The hyphae are light brown to tan in color and are set against a darker, textured background.

**MYCOLOGY** CRITIQUÉ

**Mycology Proficiency Testing Program  
October 2003**

**Wadsworth Center**  
New York State Department of Health



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# *Test Specimens and Grading Policy*

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## Test Specimens

A minimum of two strains of each of the proposed yeast specimens were examined for inclusion in the proficiency test event of October 2003. The colony morphology of all yeast strains was studied on Sabouraud dextrose agar. The microscopic morphologic features were examined 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 grading formula used for each specimen is:

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

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 laboratory 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 laboratory 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  $20 \times 5 = 100$ .

# Answer Key

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## Mycology – General

	Specimen Key	Validated Specimen	Other Acceptable Answers
M-1	<i>Aspergillus terreus</i>	<i>Aspergillus terreus</i>	
M-2	<i>Absidia corymbifera</i>	<i>Absidia corymbifera</i>	<i>Absidia</i> sp.
M-3	<i>Chaetomium</i> sp.	(Not validated)	
M-4	<i>Emmonsia parva</i>	<i>Emmonsia parva</i>	<i>Emmonsia crescens</i> <i>Emmonsia</i> sp.
M-5	<i>Bipolaris</i> sp.	<i>Bipolaris</i> sp.	<i>Bipolaris spicifera</i>

## Mycology – Yeast Only

	Specimen Key	Validated Specimen	Other Acceptable Answers
Y-1	<i>Candida krusei</i>	<i>Candida krusei</i>	<i>Candida krusei/inconspicua</i>
Y-2	<i>Candida lambica</i>	<i>Candida lambica</i>	
Y-3	<i>Candida rugosa</i>	<i>Candida rugosa</i>	
Y-4	<i>Rhodotorula glutinis</i>	(Not validated)	
Y-5	<i>Blastoschizomyces capitatus</i>	<i>Blastoschizomyces capitatus</i>	<i>Geotrichum capitatum</i> <i>Trichosporon capitatum</i>

## Mycology – Antifungal Susceptibility Testing for Yeasts

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

Mycology – General

	Correct Responses/ Total # Labs (%)	Referees (%)
M - 1 <i>Aspergillus terreus</i>	81/83 (98)	10/10 (100)
M - 2 <i>Absidia corymbifera</i>	73/83 (88)	9/10 (90)
M - 3 <i>Chaetomium</i> sp. (Not validated)	55/83 (66)	8/10 (80)
M - 4 <i>Emmonsia parva</i>	68/83 (82)	9/10 (90)
M - 5 <i>Bipolaris</i> sp.	77/83 (93)	9/10 (90)

Mycology – Yeast Only

	Correct Responses/ Total # Labs (%)	Referees (%)
Y - 1 <i>Candida krusei</i>	60/60 (100)	10/10 (100)
Y - 2 <i>Candida lambica</i>	57/60 (95)	10/10 (100)
Y - 3 <i>Candida rugosa</i>	60/60 (100)	10/10 (100)
Y - 4 <i>Rhodotorula glutinis</i> (Not validated)	30/60 (50)	7/10 (70)
Y - 5 <i>Blastoschizomyces capitatus</i>	54/60 (90)	10/10 (100)

Mycology – Antifungal Susceptibility Testing for Yeasts

	Correct Responses/ Total # Labs (%)	Correct Responses/ Total # Labs (%)
	Amphotericin B	Fluconazole
S- 1 <i>Candida parapsilosis</i> ATCC 22019	18/19 (95)	22/23 (96)
S- 2 <i>Candida tropicalis</i> ATCC 750	19/19 (100)	21/23 (91)
S- 3 <i>Candida parapsilosis</i> ATCC 90018	19/19 (100)	20/23 (87)
S- 4 <i>Candida albicans</i> ATCC 90028	19/19 (100)	22/23 (96)
S- 5 <i>Candida krusei</i> ATCC 6258	19/19 (100)	23/23 (100)

**Mycology – General Yeast Only**

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	75
Number of laboratories unsuccessfully completing this test	8

**Mycology – Yeast Only**

Number of participating laboratories	60
Number of referee laboratories	10
Number of laboratories responding by deadline	60
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	58
Number of laboratories unsuccessfully completing this test	2

**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	21
Number of laboratories unsuccessfully completing this test	2

**No. Laboratories Using Commercial Identification/Susceptibility System\***

AMS Vitek system	26
API 20C AUX	46
IDS Rapid System	4
Microscan	1
Remel Uni-Yeast-Tek	7
Other	1

(\*Includes multiple systems used by some labs)

Source: Bronchial wash

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	81
Labs with incorrect ID:	2
( <i>Aspergillus flavus</i> )	(1)
( <i>Aspergillus versicolor</i> )	(1)

**Clinical Significance:** *Aspergillus terreus* is an uncommon pathogen that causes allergic or invasive bronchopulmonary aspergillosis. It also causes cutaneous, ophthalmic, pulmonary, and disseminated infections. Keratitis, arthritis, spondylodiscitis, and suppurative otitis are also reported to be caused by *A. terreus*.

**Ecology:** *A. terreus* is worldwide distribution and often isolated from stored crops.

**Laboratory Diagnosis:**



















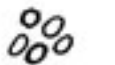
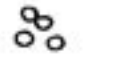
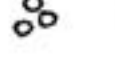
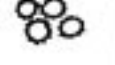

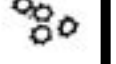



1. **Culture** – *A. terreus* grew rapidly. The colony was cinnamon to brown powdery on the surface on Sabouraud’s dextrose agar, at 25°C after 7 days (Figure 1, left panel). The reverse was pale yellow to brown (Figure 1, right panel).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed hyaline hyphae with solitary aleurioconidia attached (Figure 2, top panel) and phialides biseriata and conidial head in the form of compact column (Figure 2, bottom panel). Conidia were in chains, round, and smooth walled.
3. **Differentiation from other molds** – *A. terreus* forms cinnamon to brown colony which differentiates it from *A. fumigatus* (dark greenish to gray colony), *A. niger* (black colony), *A. flavus* (yellow to green colony), *A. versicolor* (yellow, tan, to green colored colony) *A. nidulans* (usually green colony), *A. glaucus* (green colony), and *A. clavatus* (blue-green colony). *A. terreus* phialides are biseriata, but *A. fumigatus*, *A. glaucus*, and *A. clavatus* are uniseriate. *A. flavus* phialides are both uniseriate and biseriata. Cleistothecia usually present in *A. nidulans* and *A. glaucus* but not in *A. terreus*. Conidial head is loosely radiate and covers most of vesicle for *A. versicolor* but is more compact and phialides limited mainly to the upper part of the vesicle in *A. terreus*. Please refer the attached Table on page 8.
4. **In vitro susceptibility testing** – *A. terreus* is resistant to fluconazole and 5 fluorocytosine (5 FC) but susceptible to itraconazole and ketoconazole. Some isolates are susceptible to amphotericin B.
5. **Molecular tests** – Molecular typing with the random amplified polymorphic DNA (RAPD) technique was used to discriminate between patient isolates. Nested PCR with a mixture of specific primers to DNA topoisomerase II gene was reported to identify pathogenic *Aspergillus* species including *A. terreus*.

**Comments:** All participating laboratories except two correctly identified this specimen.

**Further Reading:**

1. Baddley, J.W., Pappas, P.G., Smith, A.C., and Moser, S.A. 2003. Epidemiology of *Aspergillus terreus* at a university hospital. *J. Clin. Microbiol.* 41: 5525-5529.
2. Kanbe, T., Yamaki, K., and Kikuchi, A. 2002. Identification of the pathogenic *Aspergillus* species by nested PCR using a mixture of specific primers to DNA topoisomerase II gene. *Microbiol. Immunol.* 46: 841-848.
3. Khoury, H., Poh, C.F., Williams, M., Lavoie, J.C., and Nevill, T.J. 2003. Acute myelogenous leukemia complicated by acute necrotizing ulcerative gingivitis due to *Aspergillus terreus*. *Leuk. Lymphoma.* 44: 709-713.
4. Martin-Mazuelos, E., Peman, J., Valverde, A., Chaves, M., Serrano, M.C., and Canton, E. 2003. Comparison of the Sensititre YeastOne colorimetric antifungal panel and Etest with the NCCLS M38-A method to determine the activity of amphotericin B and itraconazole against clinical isolates of *Aspergillus* spp. *J. Antimicrob. Chemother.* 52: 365-370.
5. Walsh, T.J., Petraitis, V., Petraitiene, R., Field-Ridley, A., Sutton, D., Ghannoum, M., Sein, T., Schaufele, R., Peter, J., Bacher, J., Casler, H., Armstrong, D., Espinel-Ingroff, A., Rinaldi, M.G., Lyman, C.A. 2003. Experimental pulmonary aspergillosis due to *Aspergillus terreus*: pathogenesis and treatment of an emerging fungal pathogen resistant to amphotericin B. *J. Infect. Dis.* 188: 305-319.

**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>
<b>Colony</b>	Yellow - green	Blue - green	Dark- green	Black	Tan - buff	Pale - green
<b>Conidiophores</b>						
<b>Vesicle</b>						
<b>Sterigmata</b>						
<b>Conidia</b>						
<b>Other Structures</b>						

Source: Ear swab

Scoring:	No. Labs
Referee Labs with correct ID:	9
Laboratories with correct ID:	73
Laboratories with incorrect ID:	10
( <i>Mucor circinelloides</i> )	(4)
( <i>Mucor</i> sp.)	(4)
( <i>Rhizopus</i> sp.)	(2)

**Clinical Significance:** Infections with *Absidia corymbifera* usually occur in immunocompromised hosts but infections in immunocompetent hosts have also been reported.

**Ecology:** *A. corymbifera* is predominantly found in soil, compost, and decaying vegetation.

**Laboratory Diagnosis:**

1. **Culture** – *A. corymbifera* grew very fast. On Sabouraud’s dextrose agar, after 7 days at 25°C, the colony was gray with wooly texture (Figure 3, left panel). The reverse was pale to yellowish (Figure 3, right panel).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed branched sporangiophores with a funnel-shaped swelling (apophysis) under the pyriform sporangium (Figure 4). Rhizoid is present but rare.
3. **Differentiation from other zygomycetes** – *A. corymbifera* has very typical funnel-shaped apophysis which distinguish it from *Mucor*, *Rhizomucor*, and *Rhizopus*. *A. corymbifera* assimilates lactose and nitrate but not ethanol, while *Rhizopus* does the opposite.
4. **In vitro susceptibility testing** – *A. corymbifera* is resistant to fluconazole and 5 fluorocytosine but susceptible to amphotericin B.
5. **Molecular tests** – No information available.

**Further Reading:**

1. Buchta, V., Kalous, P., Otcenasek, M., and Vanova, M. 2003. Primary cutaneous *Absidia corymbifera* infection in a premature newborn. *Infection*. 31: 57-59.
2. Cloughley, R., Kelehan, J., Corbett-Feeney, G., Murray, M., Callaghan, J., Regan, P., and Cormican, M. 2002. Soft tissue infection with *Absidia corymbifera* in a patient with idiopathic aplastic anemia. *J. Clin. Microbiol.* 40: 725-727.
3. Ibanez, R., Serrano-Heranz, R., Sanchez-Zaballos, I., Carbonero, P., and Lopez, G. 1997. Disseminated mucormycosis due to *Absidia corymbifera* in a patient with inflammatory bowel disease. *Clin. Microbiol. Infect.* 3: 268-270.
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5. Paterson, P.J., Marshall, S.R., Shaw, B., Kendra, J.R., Ethel, M., Kibbler, C.C., Prentice, H.G., and Potter, M. 2000. Fatal invasive cerebral *Absidia corymbifera* infection following bone marrow transplantation. *Bone Marrow Transplant.* 26: 701-703.
6. Thami, G.P., Kaur, S., Bawa, A.S., Chander, J., Mohan, H., and Bedi, M.S. 2003. Post-surgical zygomycotic necrotizing subcutaneous infection caused by *Absidia corymbifera*. *Clin. Exp. Dermatol.* 28: 251-253.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS2 region of rDNA. The sequences are deposited in GenBank under the accession numbers AY533554.

```

1
ATCC 46773 (AF117938) AAAGTGCAT AATTATTGCG ACTTGCATTC
NYSDOH 273A (AY533554) GCATCGATGA AGAACGCAGC AAAGTGCAT AATTATTGCG ACTTGCATTC

51
ATAGTGAATC ATCGAGTTCT TGAACGCATC TTGCGCCTAG TAGTCAATCT
ATAGTGAATC ATCGAGTTCT TGAACGCATC TTGCGCCTAG TAGTCAATCT

101
ACTAGGCACA GTTGTTCAG TATCTGCATC CACCAATCAA CTTAACCTTT
ACTAGGCACA GTTGTTCAG TATCTGCATC CACCAATCAA CTTAACCTTT

151
TGTGTTGAGT TGGAAGTGGG CTTCTAGTTG ATGGCATTTA GTTGCTGTCA
TGTGTTGAGT TGGAAGTGGG CTTCTAGTTG ATGGCATTTA GTTGCTGTCA

201
TGGCCTTAAA TCAATGTCCT AGGTGTTAGA ACATCTAACA CCGGATGGAA
TGGCCTTAAA TCAATGTCCT AGGTGTTAGA ACATCTAACA CCGGATGGAA

251
ACTTTAGAGC GCTTTAAGAG CAGCTTGGTT AGTGAGTTCA ATAATTCCAA
ACTTTAGAGC GCTTTAAGAG CAGCTTGGTT AGTGAGTTCA ATAATTCCAA

301
GCATTAAGTC TTTAATGAA CTAGCTTTTC TATCTATGGG AACTACTTGG
GCATTAAGTC TTTAATGAA CTAGCTTTTC TATCTATGGG AACTACTTGG

351
GAGAAATCCA AGTAACCTTT AAAGTGCAT TTAGATCTGA AATCAACTGA
GAGAAATCCA AGTAACCTTT AAAGTGCAT TTAGATCTGA AATCAACTGA

401
GACCACCCGC TGAACTTAA
GACCACCCGC TGAACTTAA GCATATCAATA AGCGGAGGA

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Figure 5. Alignment of primary sequences of the ITS2 regions of *A. corymbifera* ATCC 46773 and PT specimen *A. corymbifera* NYSDOH 273A. Unmatched nucleotide bases are shaded. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

**Comments:** Ten participating laboratories misidentified this specimen as *Mucor* or *Rhizopus* species. Pyriform (pear-shaped) sporangium terminating in an funnel-shaped apophysis is the typical microscopic structure used to differentiate *A. corymbifera* from *Mucor* and *Rhizopus* species. Additional growth tests might help differentiation too.

Source: Toenail

Scoring:	No. Labs
Referee Labs with correct ID:	8
Laboratories with correct ID:	55
Laboratories with incorrect ID:	28

<i>(Chrysosporium</i> sp.)	(5)	<i>(Arthrographis</i> sp.)	(1)
<i>(Scytalidium hyalinum)</i>	(5)	<i>(Microsporium audouinii)</i>	(1)
<i>(Scytalidium</i> sp.)	(5)	<i>(Pithomyces</i> sp.)	(1)
<i>(Malbranchea</i> sp.)	(3)	<i>(Scytalidium lignicola)</i>	(1)
<i>(Sterile hyphae)</i>	(2)	<i>(Trichophyton mentagrophytes)</i>	(1)
<i>(Trichophyton terrestre)</i>	(2)	(Unidentified)	(1)

**Clinical Significance:** *Chaetomium* sp. is an unusual etiological agent of human infections, occasionally reported as an agent in phaeohyphomycosis.

**Ecology:** *Chaetomium* sp. is mainly found in soil and on plant debris.

#### Laboratory Diagnosis:

- Culture** – *Chaetomium* sp. grew rapidly. On Sabouraud's dextrose agar, after 7 days at 25°C, the colony was white to gray, yellowish, wooly surface (Figure 6, left panel), and pale yellow on reverse (Figure 6, right panel).
- Microscopic morphology** – Lactophenol cotton blue mount showed round, oval, or flask-shaped perithecia (best seen on potato dextrose agar) with long, brown setae. Ascospores, oval to lemon shaped, emerged from the ostiole (opening) of the perithecium (Figure 7).
- Differentiation from other molds** – *Chaetomium* sp. is differentiated from other molds by its very typical perithecium, which is a large round or pear-shaped structure with a small rounded opening called ostiole (which differentiates it from cleistothecium) and containing asci and ascospores. The ascospores are unicellular and most commonly lemon-shaped.
- In vitro susceptibility testing** – *Chaetomium* sp. is susceptible to ravuconazole, voriconazole, and albaconazole, but resistant to micafungin in general.
- Molecular tests** – Oligonucleotide fingerprinting of rRNA genes (OFRG) was reported to identify *Alternaria*, *Ascobolus*, *Chaetomium*, *Cryptococcus*, and *Rhizoctonia* clades (Valinsky et al., 2002).

#### Further Reading:

- Barron, M.A., Sutton, D.A., Veve, R., Guarro, J., Rinaldi, M., Thompson, E., Cagnoni, P.J., Moultny, K., and Madinger, N.E. 2003. Invasive mycotic infections caused by *Chaetomium perlucidum*, a new agent of cerebral phaeohyphomycosis. *J. Clin. Microbiol.* 41: 5302-5307.
- Serena, C., Ortoneda, M., Capilla, J., Pastor, F.J., Sutton, D.A., Rinaldi, M.G., and Guarro, J. 2003. *In vitro* activities of new antifungal agents against *Chaetomium* spp. and inoculum standardization. *Antimicrob. Agents. Chemother.* 47: 3161-3164.
- Teixeira, A.B., Trabasso, P., Moretti-Branchini, M.L., Aoki, F.H., Vigorito, A.C., Miyaji, M., Mikami, Y., Takada, M., and Schreiber, A.Z. 2003. Phaeohyphomycosis caused by *Chaetomium globosum* in an allogeneic bone marrow transplant recipient. *Mycopathologia.* 156: 309-312.
- Tomsikova, A. 2002. Causative agents of nosocomial mycoses. *Folia Microbiol (Praha).* 47: 105-112.
- Valinsky, L., Della Vedova, G., Jiang, T., and Borneman, J. 2002. Oligonucleotide fingerprinting of rRNA genes for analysis of fungal community composition. *Appl. Environ. Microbiol.* 68: 5999-6004.

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 AY533555 and AY533556, respectively.

```

1                                                    50
6/97-38 (AJ279468)    CATTTAGAGG AAGTAAAGTC GTAACAAGGT CTCCGTTGGT GAACCAGCGG
NYSDOH 1244-00 (AY533555)    TCCGTAGGT GAACCTGCGG

51                                                    100
AGGGATCATT ACAGAGTTGC AAAACTCCCT AAACCATTGT GAACGTTACC
AGGGATCATT ACAGAGTTGC AAAACTCCCT AAACCATTGT GAACGTTACC

101                                                    150
TAAACCGTTG CTTCGGCGGG CGGCCCGGG GTTTACCCCC CGGGCGCCCC
TAAACCGTTG CTTCGGCGGG CGGCCCGGG GTTTACCCCC CGGGCGCCCC

151                                                    200
TGGGCCCCAC CGCGGGCGCC CGCCGGAGGT CACCAAAACTC TTGATAATTT
TGGGCCCCAC CGCGGGCGCC CGCCGGAGGT CACCAAAACTC TTGATAATTT

201                                                    250
ATGGCCTCTC TGAGTCTTCT GTACTGAATA AGTCAAAACT TTCAACAACG
ATGGCCTCTC TGAGTCTTCT GTACTGAATA AGTCAAAACT TTCAACAACG

251                                                    300
GATCTCTTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT GCGATAAGTA
GATCTCTTGG TTCTGGCATC GATGAAGAAC GCAGC

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Figure 8. Alignment of primary sequences of the ITS1 regions of *Chaetomium* sp. 6/97-38 and PT specimen *Chaetomium* sp. NYSDOH 1244-00. Unmatched nucleotide bases are shaded. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1                                                    50
6/97-38 (AJ279468)    GATCTCTTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT GCGATAAGTA
NYSDOH 1244-00 (AY533556)    GCATC GATGAAGAAC GCAGCGAAAT GCGATAAGTA

51                                                    100
ATGTGAATTG CAGAATTCAG TGAATCATCG AATCTTTGAA CGCACATTGC
ATGTGAATTG CAGAATTCAG TGAATCATCG AATCTTTGAA CGCACATTGC

101                                                    150
GCCCCGCCAGT ATTCTGGCGG GCATGCCTGT TCGAGCGTCA TTTCAACCAT
GCCCCGCCAGT ATTCTGGCGG GCATGCCTGT TCGAGCGTCA TTTCAACCAT

151                                                    200
CAAGCCCCGG GCTTGTGTTG GGGACCTGCG GCTGCCGAG GCCCTGAAAA
CAAGCCCCGG GCTTGTGTTG GGGACCTGCG GCTGCCGAG GCCCTGAAAA

201                                                    250
GCAGTGGCGG GCTCGCTGTC ACACCGAGCG TAGTAGCATA CATCTCGCTC
GCAGTGGCGG GCTCGCTGTC ACACCGAGCG TAGTAGCATA CATCTCGCTC

251                                                    300
TGGGCGTGCT GCGGGTCCG GCCGTTAAAC CACCTTTTAA CCCAAGGTTG
TGGGCGTGCT GCGGGTCCG GCCGTTAAAC CACCTTTTAA CCCAAGGTTG

301                                                    350
ACCTCGGATC AGGTAGGAAG ACCCGCTGAA CTTAAGCATA TCAATAAGC
ACCTCGGATC AGGTAGGAAG ACCCGCTGAA CTTAAGCATA TCAATAAGCG

351
GAGGA

```

Figure 9. Alignment of primary sequences of the ITS2 regions of *Chaetomium* sp. 6/97-38 and PT specimen *Chaetomium* sp. NYSDOH 1244-00. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

**Comments:** This specimen was not validated in the current test event. It is possible that many laboratories did not see the typical perithecia structure of *Chaetomium* that could help in identification.

Source: Bronchoalveolar lavage

Scoring:	No. Labs
Referee Labs with correct ID:	9
Labs with correct ID:	68
Labs with incorrect ID:	15
( <i>Chrysosporium</i> sp.)	(8)
( <i>Beauveria</i> sp.)	(3)
( <i>Blastomyces dermatitidis</i> )	(2)
( <i>Aspergillus flavus</i> )	(1)
( <i>Trichophyton mentagrophytes</i> )	(1)

**Clinical Significance:** *Emmonsia parva* is an uncommon agent of the pulmonary disease termed adiaspiromycosis.

**Ecology:** *E. parva* is found in soil and on plant materials worldwide.

**Laboratory Diagnosis:**

- Culture** – *E. parva* grew at a moderate rate. On Sabouraud’s dextrose agar, after 7 days at 25°C, the colony showed white color and velvety surface (Figure 10, left panel). Reverse appeared yellowish to pale (Figure 10, right panel).
- Microscopic morphology** – Lactophenol cotton blue mount showed hyaline septate hyphae with simple, or sometimes branched conidiophores and large adiaspores (large, thick-walled cells that enlarge significantly in diameter at temperatures of 37°C and above) (Figure 11). Conidia form directly on short stalks along the sides of the hyphae or 2 to 3 conidia on short narrow denticles of conidiophores.
- Differentiation from other molds** – *E. parva* is different from *Chrysosporium* species by its positive growth on the medium with cycloheximide at 25°C. and no chlamydoconidia. *E. parva* is different from *Blastomyces dermatitidis* by forming adiaspores at 40°C and range from 10-25 µm and not converting to a yeast phase at 37°C (St-Germain and Summerbell, 1996). (However, according to Hoog et al., 2000, yeast cells can be formed at 37°C for *Emmonsia*.) Unlike *Sporotrichum*, *E. parva* does not produce large chlamydoconidia at 25°C. The outlined characteristics of these species are summarized in the table on the next page.
- In vitro susceptibility testing** – No information available.
- Molecular tests** – Large-subunit ribosomal and internal transcribed spacer region DNA sequences were reported to be used for differentiation phylogenetically closed species. Isolates may give false-positive results with the *B. dermatitidis* DNA probe, exoantigen, and direct immunofluorescent-antigen tests.

**Further Reading:**

- dos Santos, V.M. 1999. Culture development and morphological diagnosis of *Emmonsia crescens* in armadillos. *Rev. Soc. Bras. Med. Trop.* 32: 307.
- Peterson, S.W., and Sigler, L. 1998. Molecular genetic variation in *Emmonsia crescens* and *Emmonsia parva*, etiologic agents of adiaspiromycosis, and their phylogenetic relationship to *Blastomyces dermatitidis* (*Ajellomyces dermatitidis*) and other systemic fungal pathogens. *J. Clin. Microbiol.* 36: 2918-2925.
- Thomas, De Montpreville V., Huerre, M., and Dulmet, E. 1999. Adiaspiromycosis, 2 cases of incidental finding. *Ann. Pathol.* 19: 513-515.
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- Wagoner, M.D., Badr, I.A., and Hidayat, A.A. 1999. *Chrysosporium parvum* keratomycosis. *Cornea.* 18: 616-620.
- Wellinghausen, N., Kern, W.V., Haase, G., Rozdzinski, E., Kern, P., Marre, R., Essig, A., Hetzel, J., and Hetzel, M. 2003. Chronic granulomatous lung infection caused by the dimorphic fungus *Emmonsia* sp. *Int. J. Med. Microbiol.* 293: 441-445.

## Differentiation of *Emmonsia* species from some related fungi

Characteristic	<i>Emmonsia parva</i> var. <i>parva</i> and var. <i>crecens</i>	<i>Chrysosporium</i> species/ <i>Sporotrichum pruinosum</i>	<i>Blastomyces dermatitidis</i>
Growth on cycloheximide medium (25°C)	Positive	No growth	Positive
Chlamydoconidia (25°C)	No Chlamydoconidia	Chlamydoconidia measuring more than 20 µm in diameter	No Chlamydoconidia
Chlamydoconicia / adiaspores (37°C)	Globose, thick walled chlamydoconida (10-20 µm); adiaspores (40-200 µm)	Globose, pyriform chlamydoconida, > 20 µm in diameter	Yeast form with broadbase budding (8-30 µm)
Specific DNA probe for <i>B. dermatitidis</i>	Negative (Possible false positive)	Negative	Positive

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 AY533557 and AY533558, respectively.

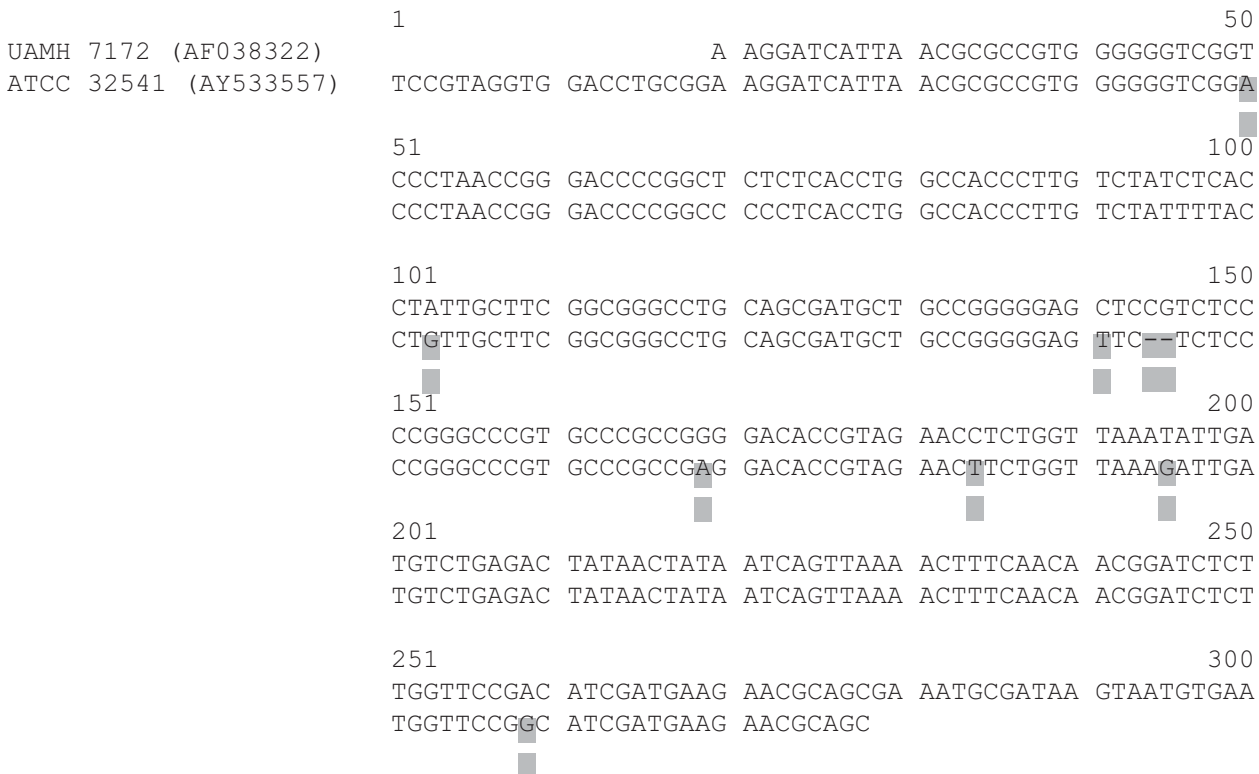


Figure 12. Alignment of primary sequences of the ITS1 regions of *E. parva* UAMH 7172 and PT specimen *E. parva* ATCC 32541. Unmatched nucleotide bases are shaded. Gaps are indicated by dashes. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1
UAMH 4489 (AF038327) TATAATCAGT TAAAACTTTC AACAACGGAT CTCTTGGTTC CGA CATCGAT
ATCC 32541 (AY533558) GCATCGAT

51
GAAGAACGCA GCGAAATGCG ATAAGTAATG TGAATTGCAG AATTCCGTGA
GAAGAACGCA GCGAAATGCG ATAAGTAATG TGAATTGCAG AATTCCGTGA

101
ATCATCGAAT CTTTGAACGC ACATTGCGCC CCCTGGTATT CCGGGGGGCA
ATCATCGAAT CTTTGAACGC ACATTGCGCC CCCTGGTATT CCGGGGGGCA

151
TGCCTGTCCG AGCGTCATTG CAACCCTCAA GCCCGGCTTG TGTGTTGGGC
TGCCTGTCCG AGCGTCATTG CAACCCTCAA GCCCGGCTTG TGTGTTGGGC

201
CTCTCGTCCCC CCGTGGACGT GCCCGAAATG CAGCGGCGGC GTCGTGTTCC
TCTCTCGTCCCC CCGTGGACGT GCCCGAAATG CAGCGGCGGC GTCGTGTTCC

251
GGTGCCCGAG CGTATGGGGC TTTGTACACC GCTCTAGAGG CCCGGCCGGC
GGTGCCCGAG CGTATGGGGC TTTGTACACC GCTCTAGAGG CCCGGCCGGC

301
TCCGGCCCCA TCTCAACCCC TTCGAGGGAG GCGGGTCTTC GGACCGGTCT
TCCGGCCCCA TCTAAACCCC C---GGGGTG GAGGGTCTCC GGACCCCT

351
CCCCA-CCAG GTTGACCTCG GATCAGGTAG GGATACCCGC TGAACTTAAG
CCCCAACCCAG GTTGACCTCG GATCAGGTAG GGATACCCGC TGAACTTAAG

401
CATATCAATA AGCGGAGGAA AAGAAACCAA CAGGGATTGC CTCAGTAACG
CATATCAATA AGCGGAGGA

```

Figure 13. Alignment of primary sequences of the ITS2 regions of *E. parva* UAMH 4489 and PT specimen *E. parva* ATCC 32541. 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:** This specimen was confused with *Chrysosporium* sp by a number of laboratories. In the old literature, *Emmonsia parva* var. *crescens* and *Emmonsia parva* var. *parva* also referred to as *Chrysosporium parvum* var. *parvum* and *Chrysosporium parvum* var. *crescens*. The isolate sent in this event was *E. parva* with positive growth on media containing cycloheximide, which distinguishes it from *Chrysosporium* species with known not to grow on media containing cycloheximide. *Beauveria* is distinct because its clusters of swollen conidiogenous cells with a zigzag apices. *Trichophyton mentagrophytes* can be differentiated by it has rounded conidia borne in dense clusters, spiral hyphae, and club shaped multiseptated macroconidia.

## M-5 *Bipolaris* sp.

Source: Skin lesion

No. Labs

Referee Labs with correct ID: 9

Labs with correct ID: 77

Labs with incorrect ID: 6

(*Scytalidium* sp.) (2)

(*Basidiobolus ranarum*) (1)

(*Nigrospora* sp.) (1)

(*Scytalidium lignicola*) (1)

(*Sterile hyphae*) (1)

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

**Ecology:** *Bipolaris* sp. is widespread on grass and other plant materials, also found in soil and air.

### Laboratory Diagnosis:

1. **Culture** – *Bipolaris* sp. was a fast grower. On Sabouraud's dextrose agar, after 7 days at 25°C, the colony was whitish becoming gray, black color on the surface and downy texture (Figure 14, left panel). Reverse was black with brownish edges (Figure 14, right panel).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed dark septate hyphae. Elongated conidiophores bent at the point where each conidium was formed, which produced a zigzag appearance. This is called sympodial geniculate growth – conidiogenous structure that continues to increase in length by forming a new growing point just below each new terminal conidium, often resulting in a bent appearance. The conidia were brown, thick walled, with 3-5 septations (Figure 15). There was slightly protruding hilum at one end of the conidia attached to the conidiophore.
3. **Differentiation from other molds** – *Bipolaris* sp. can be distinguished from *Exserohilum* and *Drechslera* by the shape and size of conidia. *Bipolaris* conidia with hilum (a scar at the base of the conidium where it was attached to the conidiogenous cell) slightly protrudes, average size 8×26 μm, 3-5 septa, but conidia of *Exserohilum* with hilum strongly protrudes, average size 14×90 μm, 5-12 septa, and conidia of *Drechslera* lack of protruded hilum, average size 16×65 μm, 3-5 septa.
4. **In vitro susceptibility testing** – *Bipolaris* sp. is susceptible to amphotericin B, itraconazole, ketoconazole, and miconazole but resistant to 5 fluorocytosine.
5. **Molecular tests** – No information available.

### Further Reading:

1. Bava, A.J., Fayad, A., Cespedes, C., and Sandoval, M. 2003. Fungal peritonitis caused by *Bipolaris spicifera*. *Med. Mycol.* 41: 529-531.
2. Buzina, W., Braun, H., Schimpl, K., and Stammberger, H. 2003. *Bipolaris spicifera* causes fungus balls of the sinuses and triggers polypoid chronic rhinosinusitis in an immunocompetent patient. *J. Clin. Microbiol.* 41: 4885-4887.
3. Espinel-Ingroff, A. 2001. *In vitro* fungicidal activities of voriconazole, itraconazole, and amphotericin B against opportunistic moniliaceous and dematiaceous fungi. *J. Clin. Microbiol.* 39: 954-958.
4. Latham, R.H. 2000. *Bipolaris spicifera* meningitis complicating a neurosurgical procedure. *Scand. J. Infect. Dis.* 32: 102-103.
5. Moore, M.L., Collins, G.R., Hawk, B.J., and Russell, T.S. 2003. Disseminated *Bipolaris spicifera* in a neonate. *J. Perinatol.* 21: 399-401.
6. Shin, E.J., Guertler, N., Kim, E., and Lalwani, A.K. 2003. Screening of middle ear effusion for the common sinus pathogen *Bipolaris*. *Eur. Arch. Otorhinolaryngol.* 260: 78-80.

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 AY533559 and AY533560, respectively.

```

1
(AY253918) GTACAGGTCT CCGTAGGTGA ACCTGCGGAG GGATCATTAC ACAATAAAAT
NYSDOH DMD441 (AY533559) T CCGTAGGTGA ACCTGCGGAG GGATCATTAC ACAATAAAAT

51
ACGAAGGCCG TTCGCGGCTG GACTATTTAT TACCCTTGTC TTTTGCGCAC
ACGAAGGCCG TTCGCGGCTG GACTATTTAT TACCCTTGTC TTTTGCGCAC

101
TTGTTGTTTC CTGGGCGGGT TCGCTCGCCA CCAGGACCAC AATATAAACC
TTGTTGTTTC CTGGGCGGGT TCGCTCGCCA CCAGGACCAC AATATAAACC

151
TTTTTTATGC AGTTGCAATC AGCGTCAGTA TAACAAATGT AAATCATTTA
TTTTTTATGC AGTTGCAATC AGCGTCAGTA TAACAAATGT AAATCATTTA

201
CAACTTTCAA CAACGGATCT CTTGGTTCTG GCATCGATGA AGAACGCAGC
CAACTTTCAA CAACGGATCT CTTGGTTCTG GCATCGATGA AGAACGCAGC

```

Figure 16. Alignment of primary sequences of the ITS1 regions of *B. spicifera* and PT specimen *B. spicifera* NYSDOH DMD441. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1
(AY253918) GAAATGCGAT ACGTAGTGTG AATTGCAGAA TTCAGTGAAT CATCGAATCT
NYSDOH DMD441 (AY533560) TGCGAT ACGTAGTGTG AATTGCAGAA TTCAGTGAAT CATCGAATCT

51
TTGAACGCAC ATTGCGCCCT TTGGTATTCC AAAGGGCATG CCTGTTCGAG
TTGAACGCAC ATTGCGCCCT TTGGTATTCC AAAGGGCATG CCTGTTCGAG

101
CGTCATTTGT ACCCTCAAGC TTTGCTTGGT GTTGGGCGTT TTTGTCTTTG
CGTCATTTGT ACCCTCAAGC TTTGCTTGGT GTTGGGCGTT TTTGTCTTTG

151
GCCCCGCAAA GACTCGCCTT AAAATGATTG GCAGCCGGCC TACTGGTTTC
GCCCCGCAAA GACTCGCCTT AAAATGATTG GCAGCCGGCC TACTGGTTTC

201
GCAGCGCAGC ACATTTTTGC GCTTGCAATC AGCAAAAGAG GACGGCAATC
GCAGCGCAGC ACATTTTTGC GCTTGCAATC AGCAAAAGAG GACGGCAATC

251
CATCAAGACT CTTTCTCACG TTTGACCTCG GATCAGGTAG GGATACCCGC
CATCAAGACT CTTTCTCACG TTTGACCTCG GATCAGGTAG GGATACCCGC

301
TGAACTTAAG C
TGAACTTAAG CATATCAATA AGCGGAGGA

```

Figure 17. Alignment of primary sequences of the ITS2 regions of *B. spicifera* and PT specimen *B. spicifera* NYSDOH DMD441. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

**Comments:** Most participating laboratories correctly identified this specimen. Other species identified included *Scytalidium* spp., which lack conidiophores; arthroconidia hyaline or pale brown, unicellular or bicellular, oval or ellipsoidal. *Nigrospora* sp. has short and inflated conidiophores, conidia black, unicellular. *Basidiobolus ranarum* form sporangiophores with inflated tip producing ejectable, unispored sporangioles and zygospores with conjugation beak.

Source: Catheter

Scoring	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	60
Labs with incorrect ID:	0

**Clinical Significance:** *Candida krusei* is a frequent causal agent of nosocomial fungemia in immunosuppressed patients. It also causes disseminated disease including endocarditis, peritonitis, vaginitis, urinary tract infections, and sinusitis.

**Ecology:** *C. krusei* is cosmopolitan, found in air, on human, and dairy products.

### Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar, after 7 days at 25°C, *C. krusei* colony was dry but soft, cream to buff, glassy and wrinkled (Figure 18).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *C. krusei* showed branched pseudohyphae with elongated blastoconidia (Figure 19).
3. **Differentiation from other yeasts** – *C. 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. lusitanae*. It grows well at 42°C, differentiating it from *C. lambica*. It does not produce arthroconidia, thus differentiating it from *Blastoschizomyces capitatus*.
4. **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. **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. Panfungal PCR and multiplex liquid hybridization were developed for the detection of clinically important yeasts in tissue specimens. PFGE, RFLP, and RAPD procedures were used for DNA fingerprinting and electrophoretic karyotyping of oral *C. krusei* isolates.

### Further Reading:

1. Abbas, J., Bodey, G.P., Hanna, H.A., Mardani, M., Girgawy, E., Abi-said, D., Whimbey, E., Hachem, R., and Raad, I. 2000. *Candida krusei* fungemia. An escalating serious infection in immunocompromised patients. *Arch. Intern. Med.* 160: 2659-2664.
2. Cresti, S., Posteraro, B., Sanguinetti, M., Guglielmetti, P., Rossolini, G.M., Morace, G., and Fadda, G. 1999. Molecular typing of *Candida* spp. By random amplification of polymorphic DNA and analysis of restriction fragment length polymorphism of ribosomal DNA repeats. *New Microbiol.* 22: 41-52.
3. Dassanayake, R.S., Samaranyake, Y.H., and Samaranyake, L.P. 2000. Genomic diversity of oral *Candida krusei* isolates as revealed by DNA fingerprinting and electrophoretic karyotyping. *APMIS.* 108: 697-704.
4. Hendolin, P.H., Paulin, L., Koukila-Kahkola, P., Anttila, V.J., Malmberg, H., Richardson, M., and Ylikoski, J. 2000. Panfungal PCR and multiplex liquid hybridization for detection of fungi in tissue specimens. *J. Clin. Microbiol.* 38: 4186-4192.
5. Katiyar, S.K., and Edlind, T.D. 2001. Identification and expression of multidrug resistance-related ABC transporter genes in *Candida krusei*. *Med. Mycol.* 39; 109-116.
6. Krcmery, V., Jr., Spanik, S., Kunova, A., Trupl, J., Grausova, S., Krupova, I., Mateicka, F., Pichnova, E., Grey, E., and Sabo, A. 1999. Nosocomial *Candida krusei* fungemia in cancer patients: report of 10 cases and review. *J. Chemother.* 11: 131-136.
7. Martin, C., Roberts, D., van Der Weide, M., Rossau, R., Jannes, G., Smith, T., and Maher, M. 2000. Development of a PCR-based line probe assay for identification of fungal pathogens. *J. Clin. Microbiol.* 38: 3735-3742.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 regions of rDNA. The sequences are deposited in GenBank under the accession numbers AY533547.

```

1
(AF411417) TCCGTAGGTG AACCTGCGGA AGGATCATT CTGTGATTTA GTACTACACT 50
ATCC 6258   CCGCAGGTT CACCTACGGA AGGATCATT CTGTGATTTA GTACTACACT
(AY533547)

51
GCGTGAGCGG AACGAAAACA ACAACACCTA AAATGTGGAA TATAGCATAT 100
GCGTGAGCGG AACGAAAACA ACAACACCTA AAATGTGGAA TATAGCATAT

101
AGTCGACAAG AGAAATCTAC GAAAAACAAA CAAAACCTTTC AACAAACGGAT 150
AGTCGACAAG AGAAATCTAC GAAAAACAAA CAAAACCTTTC AACAAACGGAT

151
CTCTTGGTTC TCGCATCGAT GAAGAGCGCA GC
CTCTTGGTTC TCGCATCGAT GAAGAACGCA GC

```

Figure 20. Alignment of primary sequences of the ITS1 regions of *C. krusei* and PT specimen *C. krusei* ATCC 6258. 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 correctly identified this specimen.

**Further Reading: (contd.)**

8. Safdar, A., van Rhee, F., Henslee-Downey, J.P., Singhal, S., and Mehta, J. 2001. *Candida glabrata* and *Candida krusei* fungemia after high-risk allogeneic marrow transplantation: no adverse effect of low-dose fluconazole prophylaxis on incidence and outcome. *Bone Marrow Transplant.* 28; 873-878.

Shemer, R., Weissman, Z.,

9. Hashman, N., and Kornitzer, D. 2001. A highly polymorphic degenerate microsatellite for molecular strain typing of *Candida krusei*. *Microbiol.* 147: 2021-2028.

10. Willinger, B. and Manafi, M. 1999. Evaluation of CHROMagar *Candida* for rapid screening of clinical specimens for *Candida* species. *Mycoses.* 42: 61-65.

## Y-2 *Candida lambica*

Source: Lung Biopsy

Scoring	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	57
Labs with incorrect ID:	3
( <i>Candida boidinii</i> )	(2)
( <i>Candida zeylanoides</i> )	(1)

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

**Ecology:** *C. lambica* was found in dairy products, water, and fruits.

### Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar after 7 days at 25°C, colonies were smooth to wrinkled, cream colored, and soft (Figure 21).
2. **Microscopic morphology** – On corn meal agar with Tween 80, long, branched pseudohyphae with oval to elongated blastoconidia are seen (Figure 22).
3. **Differentiation from other yeasts** – *C. 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*.
4. **In vitro susceptibility testing** – Few isolates studied are susceptible to amphotericin B, flucytosine, and azoles.
5. **Molecular tests** – Restriction analysis of PCR amplified ITS2 region of rDNA was developed for rapid and reliable identification of yeasts. PCR-reverse cross blot hybridization assay was developed for direct detection and identification of yeasts in clinical samples.

### Further Reading:

1. Chen, Y.C., Eisner, J.D., Kattar, M.M., Rassoulian-Barrett, S.L., Lefe, K., Bui, U., Limaye, A.P., and Cookson, B.T. 2001. Polymorphic internal transcribed spacer region 1 DNA sequences identify medically important yeasts. *J. Clin. Microbiol.* 39: 4042-4051.
2. Graf, B., Adam, T., Zill, E., and Gobel, U.B. 2000. Evaluation of the VITEK 2 system for rapid identification of yeasts and yeast-like organisms. *J. Clin. Microbiol.* 38: 1782-1785.
3. Kruger, W.H., Froger, N., Russmann, B., Renges, H., Kabisch, H., and Zander, A.R. 1998. Treatment of mycotic infections after haemopoietic progenitor cell transplantation with liposomal amphotericin B. *Bone Marrow Transplant.* 22: S10-13.
4. Pfaller, M.A., Diekema, D.J., Messer, S.A., Boyken, L., Hollis, R.J., Jones, R.N., and International Fungal Surveillance Participant Group. 2003. *In vitro* activities of voriconazole, posaconazole, and four licensed systemic antifungal agents against *Candida* species infrequently isolated from blood. *J. Clin. Microbiol.* 41: 78-83.
5. Trowbridge, J., Ludmer, L.M., Riddle, V.D., Levy, and C.S., Barth, W.F. 1999. *Candida lambica* polyarthritis in a patient with chronic alcoholism. *J. Rheumatol.* 26: 1846-1848.

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 AY533548 and AY533549, respectively.

```

1                                                    50
ATCC 24750 (AF336843)   TCCGTAGGTG AACCTGCGGA AGGATCATT A CTGTGATTTA TACACCACAC
NYSDOH 647-89 (AY533548) TCCGTAGGTG AACCTGCGGA AGGATCATT A CTGTGATTTA TCACCACAC

51                                                    100
TGC GTGGGCG ACACGAAACA CCGAAACCGA ACGCACGCCG TCAAGCAAGA
TGC GTGGGCG ACACGAAACA CCGAAACCGA ACGCACGCCG TCAAGCAAGA

101                                                    150
AATCCACAAA ACTTTCAACA ACGGATCTCT TGGTTCTCGC ATCGATGAAG
AATCCACAAA ACTTTCAACA ACGGATCTCT TGGTTCTCGC ATCGATGAAG

151
AACGCAGC
AACGCAGC

```

Figure 23. Alignment of primary sequences of the ITS1 regions of *C. lambica* ATCC 24750 and PT specimen *C. lambica* NYSDOH 647-89. Unmatched nucleotide bases are shaded. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1                                                    50
UWFP-346 (AF218998)   GCATCGATGA AGAACGCAGC GAAATGCGAT ACCTAGTGTG AATTGCAGCC
NYSDOH 647-89 (AY533549) GCATCGATGA AGAACGCAGC GAAATGCGAT ACCTAGTGTG AATTGCAGCC

51                                                    100
ATCGTGAATC ATCGAGTTCT TGAACGCACA TTGCGCCCGC TGGTATTCCG
ATCGTGAATC ATCGAGTTCT TGAACGCACA TTGCGCCCGC TGGTATTCCG

101                                                    150
GCGGGCATGC CTGTCTGAGC GTCGTTTCCT TCTTGAGAGC GTGCTTCAGA
GCGGGCATGC CTGTCTGAGC GTCGTTTCCT TCTTGAGAGC GAGCTTCAGA

151                                                    200
CCTGGCGGGC TGTCTCTCGG GACGGCGCGC CCAAAGCGAG GGGCCTTCTG
CCTGGCGGGC TGTCTCTCGG GACGGCGCGC CCAAAGCGAG GGGCCTTCTG

201                                                    250
CGCGAACTAG ACTGTGCGCG CGGGGCGGCC GGCGAACTTA TTACCAAGCT
CGCGAACTAG ACTGTGCGCG CGGGGCGGCC GGCGAACTTA T-ACCAAGCT

251                                                    300
CGACCTCAGA TCAGGCAGGA GTACCCGCTG AACTTAAGCA TATCAATAAG
CGACCTCAGA TCAGGCAGGA GTACCCGCTG AACTTAAGCA TATCAATAAG

301
CGGAGGA
CGGAGGA

```

Figure 24. Alignment of primary sequences of the ITS2 regions of *C. lambica* UWFP-346 and PT specimen *C. lambica* NYSDOH 647-89. 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:** This specimen was sent out earlier as a graded test specimen in January 2002 PT event and only 23% participating laboratories reported correct identification. However, in the current event 95% participating laboratories correctly identified this specimen. Two laboratories reported this isolate as *C. boidinii*, which can grow on media without thiamine but *C. lambica* can not. Only one laboratory reported this isolate as *C. zeylanoides*, which grows on the media containing cycloheximide, while *C. lambica* does not.

Source: Saliva

Scoring	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	60

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

**Ecology:** *C. rugosa* is cosmopolitan, especially on dairy products.

**Laboratory Diagnosis:**

1. **Culture** – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was white to cream, wrinkled (Figure 25).
2. **Microscopic morphology** – On corn meal agar with Tween 80, branched pseudohyphae with chains of elongated blastoconidia are seen (Figure 26).
3. **Differentiation from other yeasts** – *C. rugosa* ferments only glucose, does not grow on media containing cycloheximide, variable growth at 42°C, and is urea and nitrate negative. Microscopically, it forms branched pseudohyphae that differentiates it from *C. lusitaniae* and *C. parapsilosis*. It does not form true hyphae, differentiating it from *Trichosporon beigeli*.
4. **In vitro susceptibility testing** – Clinical isolates are susceptible to 5-flucytosine, and various azoles such as fluconazole, ketocoanzole, and itraconazole. It is less susceptible to polyene antifungals like amphotericin B and nystatin.
5. **Molecular tests** – PCR assay of the ITS1 and ITS2 regions of ribosomal DNA was developed to identify *C. rugosa* in clinical specimens. A repetitive sequence-based PCR technique was developed to characterize the genotypic relatedness among *C. rugosa* isolates. Karyotyping by PFGE was developed as a typing tool for discrimination among strains of *C. rugosa*.

**Further Reading:**

1. Dib, J.C., Dube, M., Kelly, C., Rinaldi, M.G., and Patterson, J.E. 1996. Evaluation of pulsed-field gel electrophoresis as a typing system for *Candida rugosa*: comparison of karyotype and restriction fragment length polymorphisms. *J. Clin. Microbiol.* 34: 1494-1496.
2. Dube, M.P., Heseltine, P.N., Rinaldi, M.G., Evans, S., and Zawacki, B. 1994. Fungemia and colonization with nystatin-resistant *Candida rugosa* in a burn unit. *Clin. Infect. Dis.* 18: 77-82.
3. Krcmery, V. and Barnes, A.J. 2002. Non-albicans *Candida* spp. causing fungaemia: pathogenicity and antifungal resistance. *J. Hosp. Infect.* 50: 243-2460.
4. Lopes Colombo, A., Azevedo Melo, A.S., Crespo Rosas, R.F., Salomao, R., Briones, M., Hollis, R.J., Messer, S.A., and Pfaller, M.A. 2003. Outbreak of *Candida rugosa* candidemia: an emerging pathogen that may be refractory to amphotericin B therapy. *Diagn. Microbiol. Infect. Dis.* 46: 253-257.
5. Redkar, R.J., Dube, M.P., McCleskey, F.K., Rinaldi, M.G., and del Vecchio, V.G. 1996. DNA fingerprinting of *Candida rugosa* via repetitive sequence-based PCR. *J. Clin. Microbiol.* 34: 1677-1681.
6. Reinhardt, J.F., Ruane, P.J., Walker, L.J., and George, W.L. 1985. Intravenous catheter-associated fungemia due to *Candida rugosa*. *J. Clin. Microbiol.* 22: 1056-1057.
7. Shenoy, S., Samuga, M., Urs, S., Anuradha, K.M., Kurian, M.M., Augustine, A., Anand, A.R., and Prasad, A. 1996. Intravenous catheter-related *Candida rugosa* fungaemia. *Trop. Doct.* 26: 31.

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 AY533550 and AY533551, respectively.

```

1
ATCC 10571 (AF335927) TCCGTAGGTG AACCTGCGGA AGGATCATT CAACAACAAT ACAACTTTGT
NRRL 95 (AY533550) TCCGTAGGTG AACCTGCGGA AGGATCATT CAACAACAAT ACAACTTTGT

51
GTCTGAACAA TAACTTCAAG TACCGATCAT CAAATTGTTA AAACAAAAC
GTCTGAACAA TAACTTCAAG TACCGATCAT CAAATTGTTA AAACAAAAC

101
TTCAACAACG GATCTCTAGG TTCTCGCATC GATGAAGAAC GCAGC
TTCAACAACG GATCTCTAGG TTCTCGCATC GATGAAGAAC GCAGC

```

Figure 27. Alignment of primary sequences of the ITS1 regions of *C. rugosa* ATCC 10571 and PT specimen *C. rugosa* NRRL 95. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1
ATCC 10571T (AF218971) GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAGTACG AAACGCAAGT
NRRL 95 (AY533551) GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAGTACG AAACGCAAGT

51
CGTGAATCAT CGAATCTTTG AACGCACATT GCGCTGTGTG GCATTCCGCA
CGTGAATCAT CGAATCTTTG AACGCACATT GCGCTGTGTG GCATTCCGCA

101
CAGCATGCCT GTTTGAGCAA TATTTCTCTC TCGCAAGGTG TTGGGCACCA
CAGCATGCCT GTTTGAGCAA TATTTCTCTC TCGCAAGGTG TTGGGCACCA

151
CGCCGGCAGG CGTCTGCCCC AAACGCGACC GTCTAAAACA GTTAAGCTTG
CGCCGGCAGG CGTCTGCCCC AAACGCGACC GTCTAAAACA GTTAAGCTTG

201
TTACAGACTC ACGATCTTAT TCTCAAATCA GGTAGGACTA CCCGCTGAAC
TTACAGACTC ACGATCTTAT TCTCAAATCA GGTAGGACTA CCCGCTGAAC

251
TTAAGCATAT CAATAAGCGG AGGA
TTAAGCATAT CAATAAGCGG AGGA

```

Figure 28. Alignment of primary sequences of the ITS2 regions of *C. rugosa* ATCC 10571T and PT specimen *C. rugosa* NRRL 95. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

**Comments:** All the participating laboratories reported this specimen correctly in current PT event.

## Y-4 *Rhodotorula glutinis*

Source: CSF

Scoring	No. Labs
Referee Labs with correct ID:	7
Labs with correct ID:	30
Labs with incorrect ID:	30
( <i>Rhodotorula mucilaginosa</i> )	(25)
( <i>Rhodotorula</i> sp.)	(4)
( <i>Sporobolomyces salmonicolor</i> )	(1)

**Clinical Significance:** *Rhodotorula glutinis* is not a common human pathogen. A few cases of infection were reported.

**Ecology:** *R. glutinis* is worldwide in distribution, found in water, soil, and fruits.

### Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar after 7 days at 25°C, colony is pink to red, surface smooth to wrinkled, fluid or mucoid (Figure 29).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *R. glutinis* has no pseudohyphae with either single or small clusters of round blastoconidia (Figure 30).
3. **Differentiation from other yeasts** – *R. glutinis* does not assimilate lactose and melibiose. It is nitrate positive which differentiated it from *R. mucilaginosa*.
4. **In vitro susceptibility testing** – No information available.
5. **Molecular tests** – RAPD patterns were used for identification.

### Further Reading:

1. Asim, M., Meigh, J.A., and Farr, M.J. 1999. Fungal peritonitis caused by *Candida parapsilosis* and subsequently *Rhodotorula glutinis* in a CAPD patient. *Perit. Dial. Int.* 19: 183.
2. Colombo, A.L., Dantas, L.S., Abramczyk, M.L., Cypriano, M., Fischman, O., Iazzetti, A.V., Petrilli, A.S., and Selijan, M.P. 1997. *Rhodotorula glutinis* Fungemia: a Case Report and Literature Review. *Braz. J. Infect. Dis.* 1: 204-207.
3. Dorey, M.W., Brownstein, S., Kertes, P.J., Gilberg, S.M., and Toye, B. 2002. *Rhodotorula glutinis* endophthalmitis. *Can. J. Ophthalmol.* 37: 416-418.
4. Fanci, R., Pecile, P., Martinez, R.L., Fabbri, A., and Nicoletti, P. 1997. Amphotericin B treatment of fungemia due to unusual pathogens in neutropenic patients: report of two cases. *J. Chemother.* 9: 427-430.
5. Hsueh, P.R., Teng, L.J., Ho, S.W., and Luh, K.T. 2003. Catheter-related sepsis due to *Rhodotorula glutinis*. *J. Clin. Microbiol.* 41: 857-859.
- Lanzafame, M., De Checchi, G., Parinello, A., Trevenzoli, M., and Cattelan, A.M. 2001. *Rhodotorula glutinis*-related meningitis. *J. Clin. Microbiol.* 39: 410.

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 AY533552 and AY533553, respectively.

```

1                                                    50
CBS 6085 (AF444502)   TCCGTAGGTG AACCTGCGGA AGGATCATTG GTGAATCTAG GACGTCCAAC
NYSDOH 475-98 (AY533552) TCCGTAGGTG AACCTGCGGA AGGATCATTG GTGAATCTAG GACGTCCAAC
51                                                    100
TTAACTTGGA GTCCGAACTC TCACTTTCTA ACCCTGTGCA TCTGTTTTAA
TTAACTTGGA GTCCGAACTC TCACTTTCTA ACCCTGTGCA TCTGTTTTAA
101                                                    150
AATTGGCTAG TAGCTCTTCG GAGTGAACCA CCATTTTTCA CTTATACAAA
AATTGGCTAG TAGCTCTTCG GAGTGAACCA CCATTTTTCA CTTATACAAA
151                                                    200
CACAAAGTCT ATGAATGTAA GCAAATTTAT AACAAAACAA AACTTTCAAC
CACAAAGTCT ATGAATGTAA GCAAATTTAT AACAAAACAA AACTTTCAAC
201                                                    250
AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG AAATGCGATA
AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG

```

Figure 31. Alignment of primary sequences of the ITS1 regions of *R. glutinis* CBS 6085 and PT specimen *R. glutinis* NYSDOH 475-98. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1                                                    50
CBS 9084 (AF444661)   AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG AAATGCGATA
NYSDOH 475-98 (AY533553)          G CATCGATGAA GAACGCAGCG AAATGCGATA
51                                                    100
CGTAATGTGA ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACC
CGTAATGTGA ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACC
101                                                    150
TTGCGCTCCT TGGTATTCCG AGGAGCATGC CTGTTTGAGT GTCATGAAAT
TTGCGCTCCT TGGTATTCCG AGGAGCATGC CTGTTTGAGT GTCATGAAAT
151                                                    200
CTTCAACCCA CCTCTTTCTT AGTGAATCTG GTGGTGCTTG GTTTCTGAGC
CTTCAACCCA CCTCTTTCTT AGTGAATCTG GTGGTGCTTG GTTTCTGAGC
201                                                    250
GCTGCTCTGC TTCGGCTTAG CTCGTTTCGTA ATGCATTAGC ATCCGCAACC
GCTGCTCTGC TTCGGCTTAG CTCGTTTCGTA ATGCATTAGC ATCCGCAACC
251                                                    300
GAAACTTCGG ATTGACTTGG CGTAATAGAC TATTCGCTGA GGATTCCAGA
GAAACTTCGG ATTGACTTGG CGTAATAGAC TATTCGCTGA GGATTCCAGA
301                                                    350
CTTGTTCTGG AGCCGAGTTG GGTAAAGGA AGCTTCTAAT CCTAAAGTCT
CTTGTTCTGG AGCCGAGTTG GGTAAAGGA AGCTTCTAAT CCTAAAGTCT
351                                                    400
ATTTTTTGAT TAGATCTCAA ATCAGGTAGG ACTACCCGCT GAACTTAAGC
ATTTTTTGAT TAGATCTCAA ATCAGGTAGG ACTACCCGCT GAACTTAAGC
401
ATATCAATAA GCGGAGGA
ATATCAATAA GCGGAGGA

```

Figure 32. Alignment of primary sequences of the ITS2 regions of *R. glutinis* CBS 9084 and PT specimen *R. glutinis* NYSDOH 475-98. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

**Comments:** This specimen was not validated in the current test event. Twenty-five out of sixty participating laboratories reported it as *R. mucilaginosa*, which is nitrate negative but *R. glutinis* is nitrate positive. One laboratory reported it as *Sporobolomyces salmonicolor*, which can produce various amounts of true and pseudohyphae but *R. glutinis* usually has no pseudohyphae.

## Y-5 *Blastoschizomyces capitatus*

Source: Bone lesions

Scoring	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	54
Labs with incorrect ID:	6
( <i>Geotrichum candidum</i> )	(3)
( <i>Candida norvegensis</i> )	(2)
(Unidentified)	(1)

**Clinical Significance:** *Blastoschizomyces capitatus* is an opportunistic pathogen in neutropenic patients.

**Ecology:** *B. capitatus* is frequently isolated from sputum, and it is a minor component of the normal skin flora. The fungus is also found in wood pulp and poultry feces.

### Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was smooth to wrinkled, raised, and hyaline (Figure 33).
2. **Microscopic morphology** – On corn meal agar with Tween 80, true hyphae are produced. Anneloconidia emerge from the annellides (A percurrent, indeterminate conidiogenous cell in which the first conidium is holoblastic and each successive conidium is enteroblastic. Anellations occur with the release of annelloconidia. Anellides become longer and narrower with the production of each new conidium.) (Figure 34), and may undergo schizolytic division. The resulting conidia simulate the appearance of arthroconidia, as seen in the genus *Trichosporon* and *Geotrichum*.
3. **Differentiation from other yeasts** – *B. capitatus* can be differentiated from *G. candidum* by the lack of growth on a medium containing D-xylose as a carbon source. It can be differentiated from *T. beigelii* by lack of urease and its growth at 45°C. *B. capitatus* is included in the database of commercial yeast identification systems.
4. **In vitro susceptibility testing** – *B. capitatus* is susceptible to amphotericin B; fluconazole resistant strains have been reported from cancer patients.
5. **Molecular tests** – Primers for large ribosomal subunit DNA sequences were used in PCR to differentiate between *C. famata* and *C. guilliermondii*. The amplification of 340 bp of the large rDNA led to rapid and specific identification of *C. famata*. RAPD-PCR analysis was applied to identify *C. famata* in dairy product.

**Comments:** *B. capitatus* and its synonym *Geotrichum capitatum* were both accepted as correct answer in this testing event. However, *G. candidum* reported by three laboratories is different form *B. capitatus* based on xylose assimilation test. *G. candidum* can not grow on the medium containing xylose as a carbon source, but *B. capitatus* can. *B. capitatus* can be differentiated from *Candida norvegensis* by assimilation of galactose but not cellobiose.

### Further Reading:

1. Buchta, V., Zak, P., Kohout, A., and Otcenasek, M. 2001. Case report. Disseminated infection of *Blastoschizomyces capitatus* in a patient with acute myelocytic leukaemia. *Mycoses*. 44: 505-512.
2. Cheung, M.Y., Chiu, N.C., Chen, S.H., Liu, H.C., Ou, C.T., Liang, D.C. 1999. Mandibular osteomyelitis caused by *Blastoschizomyces capitatus* in a child with acute myelogenous leukemia. *J. Formos. Med. Assoc.* 98: 787-789.
3. D’Antonio, D., Romano, F., Iacone, A., Violante, B., Fazii, P., Pontieri, E., Staniscia, T., Caracciolo, C., Bianchini, S., Sferra, R., Vetuschi, A., Gaudio, E., Carruba, G. 1999. Onychomycosis caused by *Blastoschizomyces capitatus*. *J. Clin. Microbiol.* 37: 2927-2930.
4. Nieto Martin, D., Vallejo Maroto, I., Aparicio Santos, R., and Muniz Grijalvo, O. 2003. Persistent lung colonization by *Blastoschizomyces capitatus* in patient with AIDS. *Med. Clin. (Barc)*. 120: 435-436.
5. Perez-Sanchez, I., Anguita, J., Martin-Rabadan, P., Munoz, P., Serrano, D., Escudero, A., and Pintado, T. 2000. *Blastoschizomyces capitatus* infection in acute leukemia patients. *Leuk. Lymphoma*. 39: 209-212.

## **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 (3). 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 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 (1).

## **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 parapsilosis* ATCC 22019 (S-1) and *Candida krusei* ATCC 6258 (S-5), and three NCCLS reference strains, *Candida tropicalis* ATCC 750 (S-2), *Candida parapsilosis* ATCC 90018 (S-3), and *Candida albicans* ATCC 90028 (S-4) (3, 4), were included in the October 1, 2003 antifungal proficiency testing event. These isolates have been well characterized, and their MIC ranges against amphotericin B and fluconazole have been published (4, 6). MICs within  $\pm 2$  dilutions of the reference result (range of MICs for a particular yeast described in NCCLS, M27-A2) are the acceptable results in this event (3).

## **Results:**

A total of 23 laboratories participated in this antifungal susceptibility testing event. The performances of 21 laboratories were satisfactory and two laboratories had unsatisfactory performances. Of the 23 participating laboratories, 8 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 35. For amphotericin B, good performance was noted for *C. tropicalis* ATCC 750, *C. parapsilosis* ATCC 90018, *C. albicans* ATCC 90028, and *C. krusei* ATCC 6258, irrespective of the methodology used by the laboratories. For fluconazole, good performance was seen for *C. krusei* ATCC 6258. Overall agreement with the NCCLS reference ranges was 99% against amphotericin B and 94% against fluconazole for all five isolates, after with the expansion of the reference range by  $\pm 2$  dilutions. 93% of answers for amphotericin B and 86% answers for fluconazole were within the NCCLS reference range.

## **Further Reading:**

1. Barry, A. and Brown, S. 1996. Fluconazole disk diffusion procedure for determining susceptibility of *Candida* species. *J. Clin. Microbiol.* 34: 2154-2157.
2. National Committee for Clinical Laboratory Standards. 1996. Minutes US-NCCLS antifungal susceptibility subcommittee meeting on interpretive breakpoints. NCCLS, Villanova, PA.
3. 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.

## Individual Isolates:

### S-1 *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

Seventeen laboratories reported values within the NCCLS reference range, 1 laboratory reported value within the expanded range, and 1 laboratory reported a MIC value higher than the expanded range for amphotericin B. Twenty-one laboratories reported values within the NCCLS reference range, 1 laboratory reported value within the expanded range, and 1 laboratory reported a MIC value lower than the expanded range for fluconazole.

### S-2 *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

Eighteen 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, 1 laboratory reported a MIC value higher than the expanded range, and 1 laboratory reported a MIC value lower than the expanded range for fluconazole.

### S-3 *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

Eighteen laboratories reported values within the NCCLS reference range and 1 laboratory reported value within the expanded range for amphotericin B. Nineteen laboratories reported values within the NCCLS reference range, 1 laboratory reported value within the expanded range, 1 laboratory reported a MIC value lower than the expanded range, and 2 laboratories reported MIC values higher than the expanded range for fluconazole.

### S-4 *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

Sixteen laboratories reported values within the NCCLS reference range and 3 laboratories reported value within the expanded range for amphotericin B. Eighteen laboratories reported values within the NCCLS reference range, 4 laboratories reported values within the expanded values, and 1 laboratory reported a MIC value higher than the expanded range for fluconazole.

### S-5 *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 laboratories reported values within the NCCLS reference range, 3

## Further Reading: (cont'd)

4. Pfaller, M.A., Bale, M., Buschelman, B., Lancaster, M., Espinel-Ingroff, A., Rex, J.H., Rinaldi, M.G., Cooper, C.R., and McGinnis, M.R. 1995. Quality control guidelines for National Committee for Clinical Laboratory Standards recommended broth macrodilution testing of amphotericin B, fluconazole, and flucytosine. *J. Clin. Microbiol.* 33: 1104-7.

5. Rex, J.H., Pfaller, M.A., Galgiani, J., Bartlett, M., Espinel-Ingroff, A., Ghannoum, M., Lancaster, M., Odds, F., Rinaldi, M., Walsh, T., 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. Diseases.* 24: 235-247.

6. 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

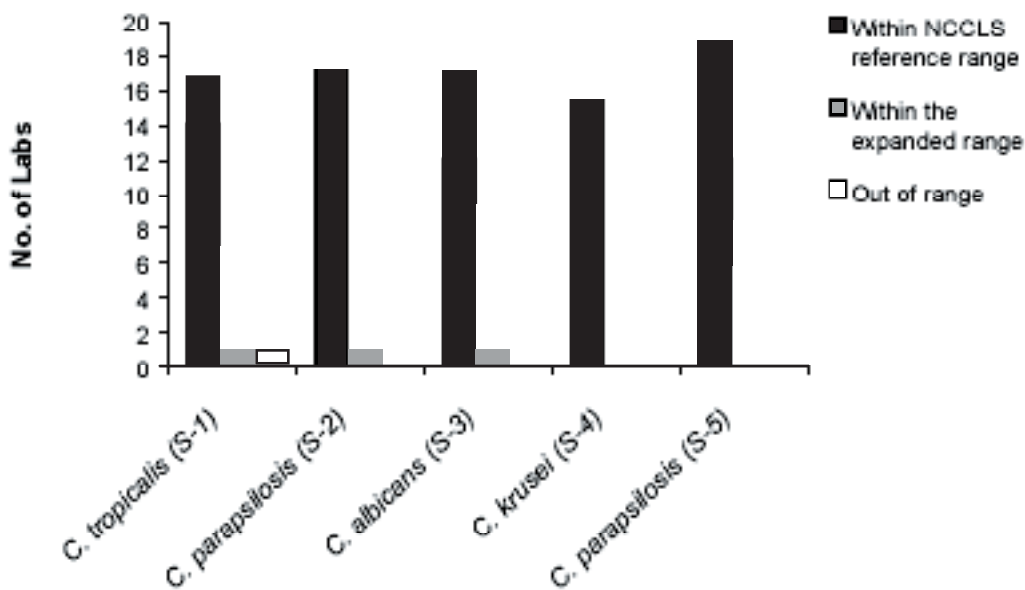
Table 1. Summary of supplementary information on antifungal susceptibility testing by participating laboratories

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

<sup>1</sup>Most labs used 100% inhibition for amphotericin B and either 80 or 50% for azoles.

\* More than one value reported by individual laboratories

**A**



**B**

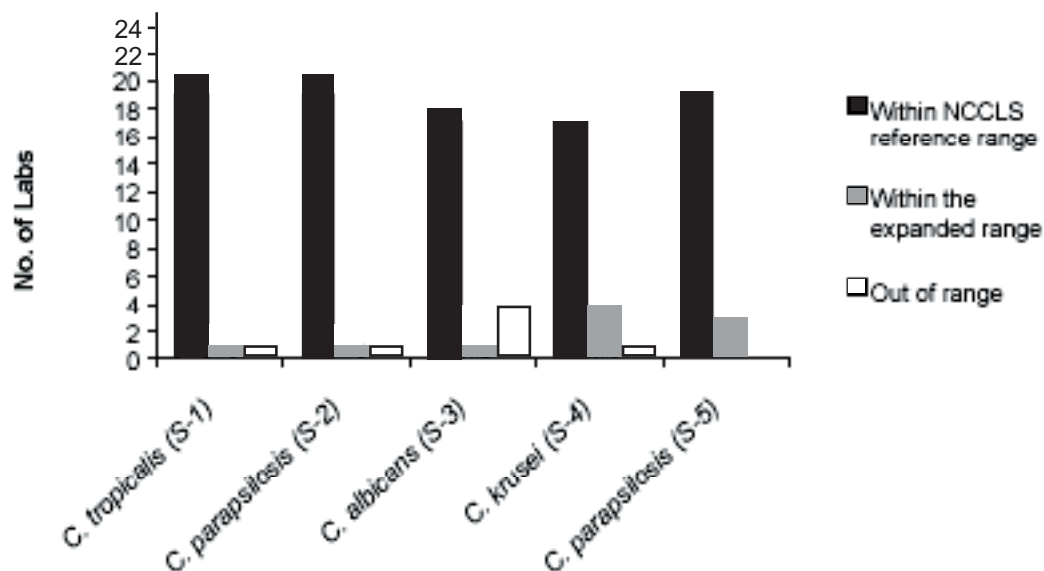
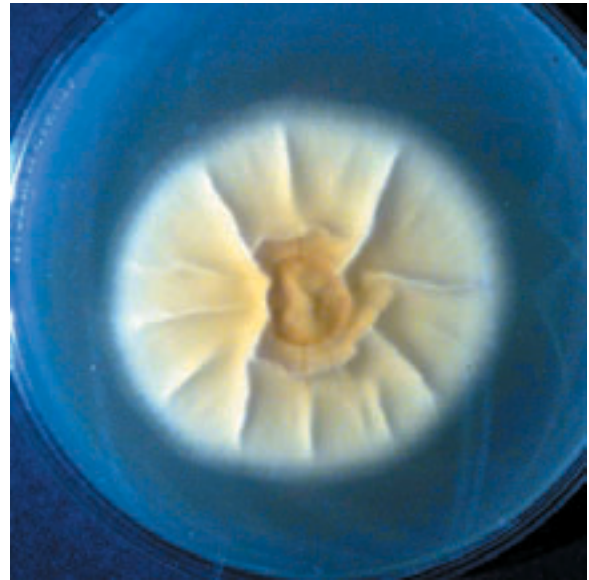


Figure 33. Summary of the results submitted by the participating labs for 5 isolates, for amphotericin B (A) and fluconazole (B).

Figure 1



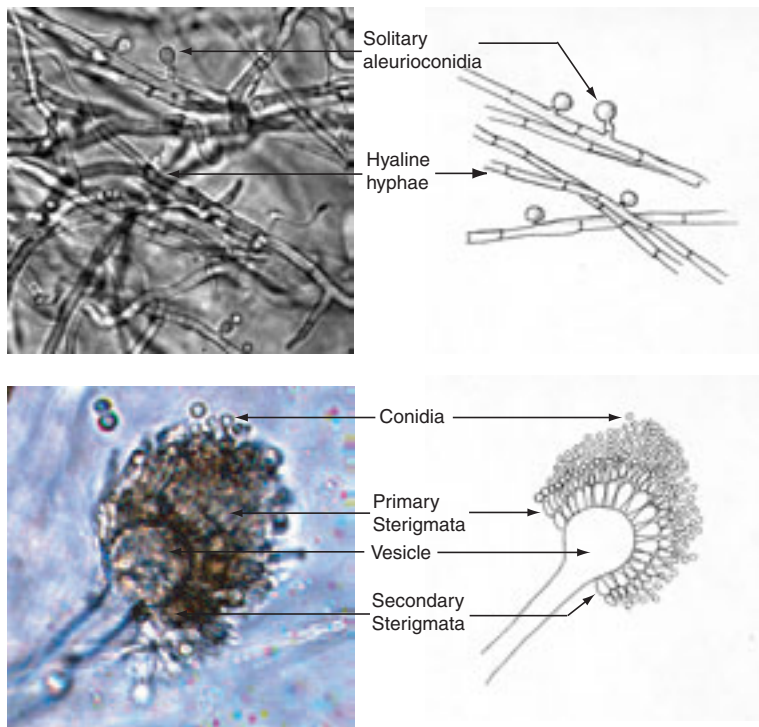
Seven-day-old, pale yellow color colony of *Aspergillus terreus* on Sabouraud's dextrose agar.



The reverse shows pale yellow to brown.

---

Figure 2

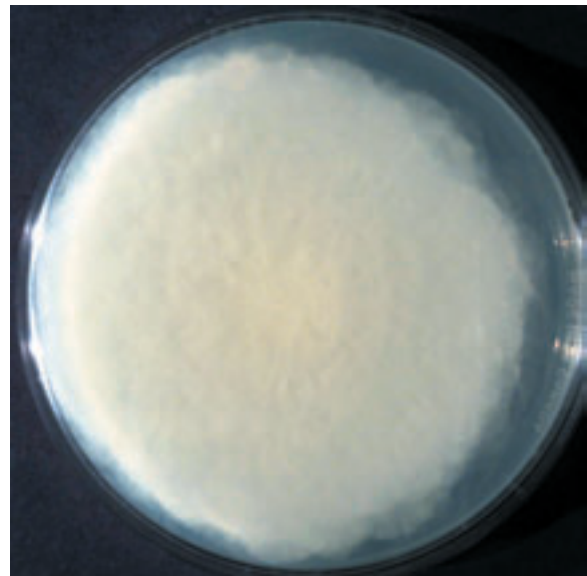


Microscopic morphology of *Aspergillus terreus* showing hyaline hyphae with solitary aleurioconidia attached (Top panel) and typical compact column formed conidial head with biseriate phialides and round, smooth conidia (Bottom panel) (left; 400× magnification, right; line drawing not to scale).

Figure 3



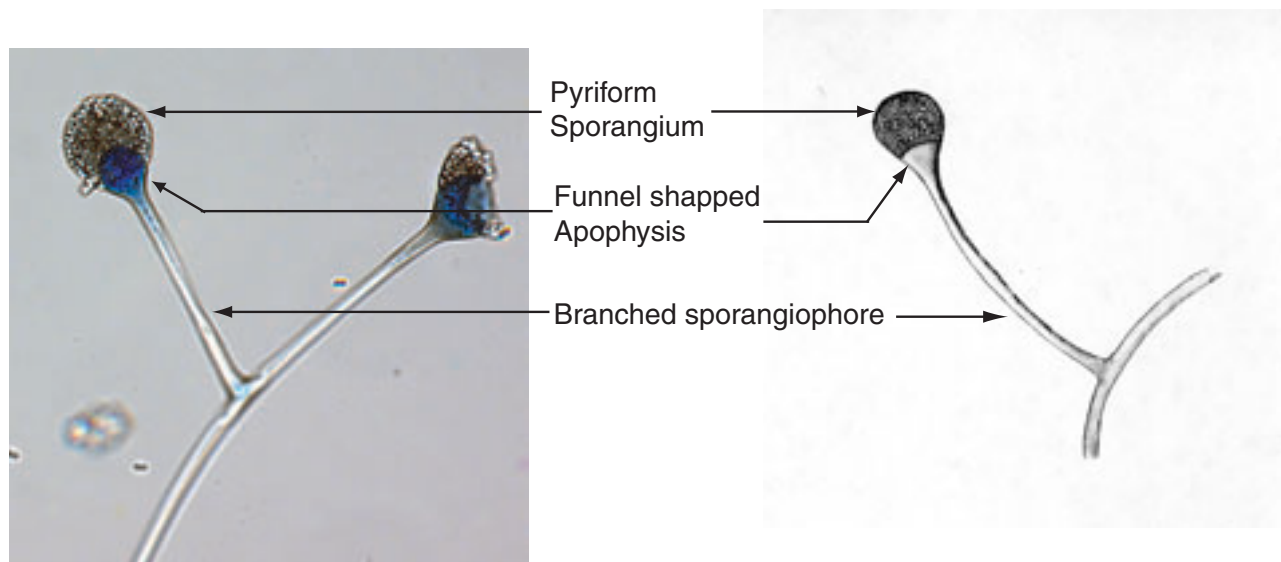
Seven-day-old, gray with wooly texture colony of *Absidia corymbifera* on Sabouraud's dextrose agar.



The reverse of the colony shows pale to yellowish.

---

Figure 4



Microscopic morphology of *Absidia corymbifera* showing branched sporangiophores with a funnel-shaped swelling (apophysis) under the pyriform sporangium (left; 400× magnification, right; line drawing not to scale).

Figure 6



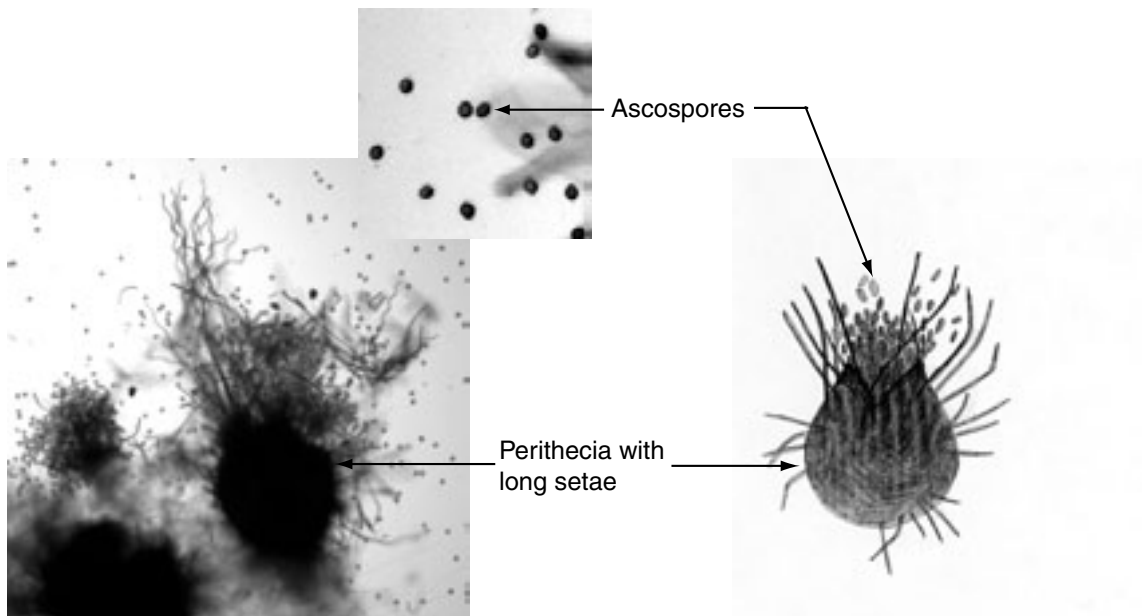
Seven-day-old, white to gray, yellowish colony of *Chaetomium* sp. on Sabouraud's dextrose agar.



The reverse side of the colony is pale yellow.

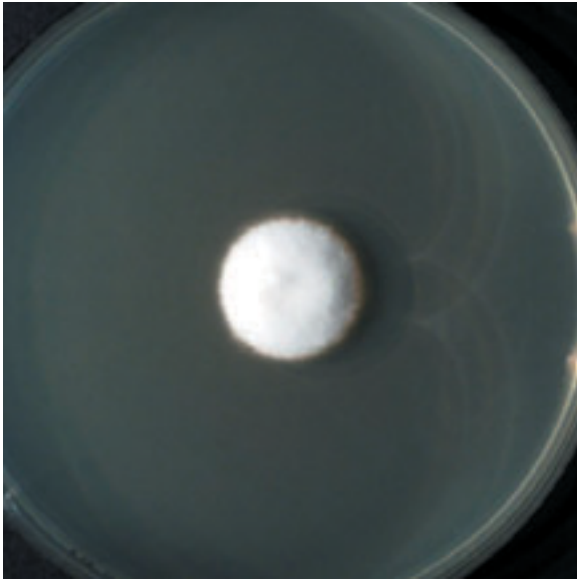
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Figure 7

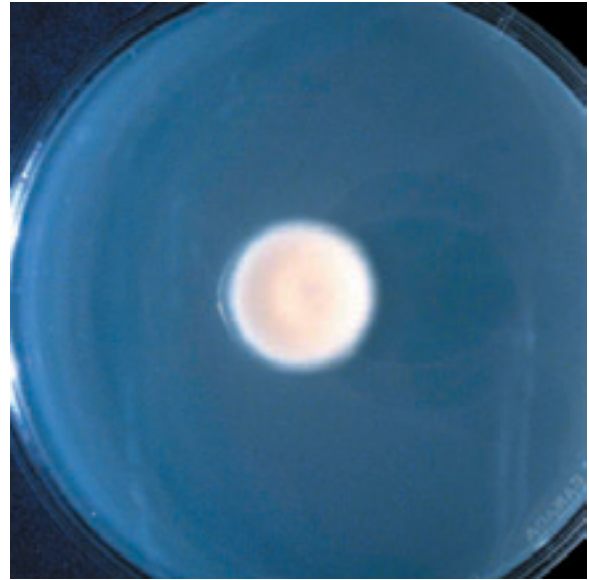


Microscopic morphology of *Chaetomium* sp. showing round, oval, or flask-shaped perithecia with long setae. Ascospores, oval to lemon shaped, release from the ostiole of the perithecium (left; 400× magnification, right; line drawing not to scale).

Figure 10



Seven-day-old, white colored velvety colony of *Emmonsia parva* on Sabouraud's dextrose agar.



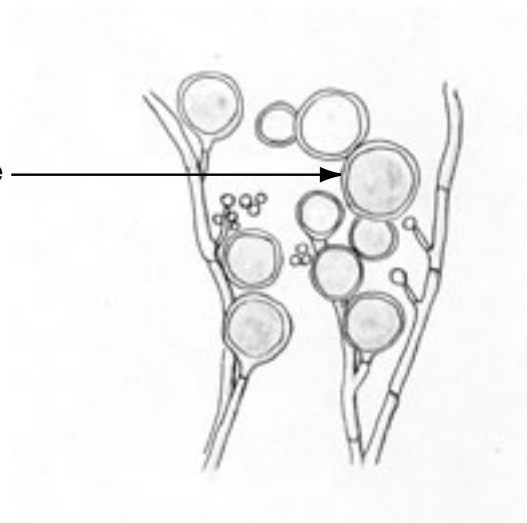
The reverse of the colony appears yellowish to pale.

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Figure 11



Adiaspore

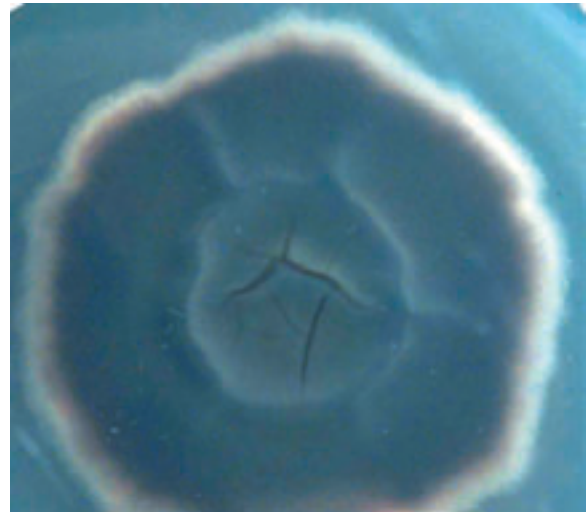


Microscopic morphology of *Emmonsia parva* showing hyaline septate hyphae with simple, or sometimes branched conidiophores and adiaspores (left; 400× magnification, right; line drawing not to scale).

Figure 14



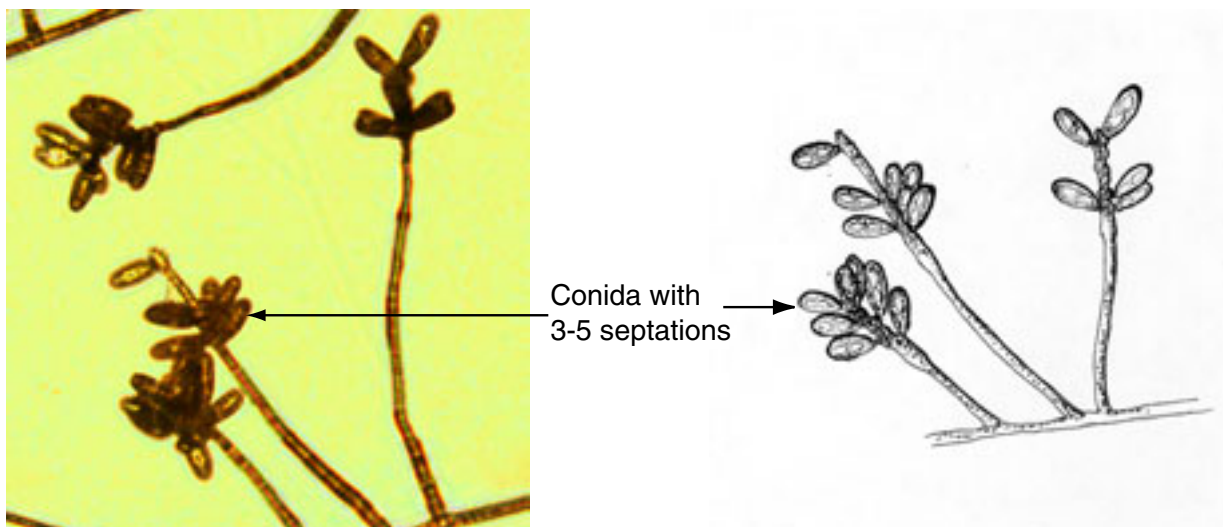
Four-day-old, white to gray, black colored downy texture colony of *Bipolaris* sp. on Sabouraud's dextrose agar.



The reverse of the colony is black with brown at the edge.

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Figure 15



Microscopic morphology of *Bipolaris* sp. showing brown, thick walled conidia with 3-5 septations (left; 200× magnification, right; line diagram not to scale).

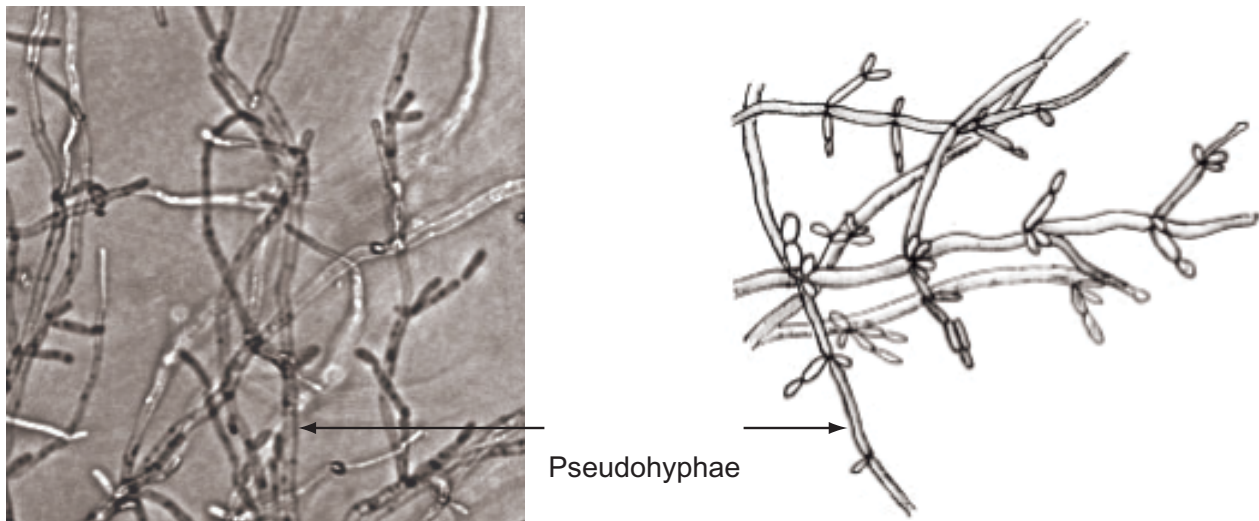
Figure 18



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

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Figure 19



Microscopic morphology of *Candida krusei* on corn meal agar showing long, branched pseudohyphae with oval blastoconidia (left; 200× magnification, right; line drawing not to scale).

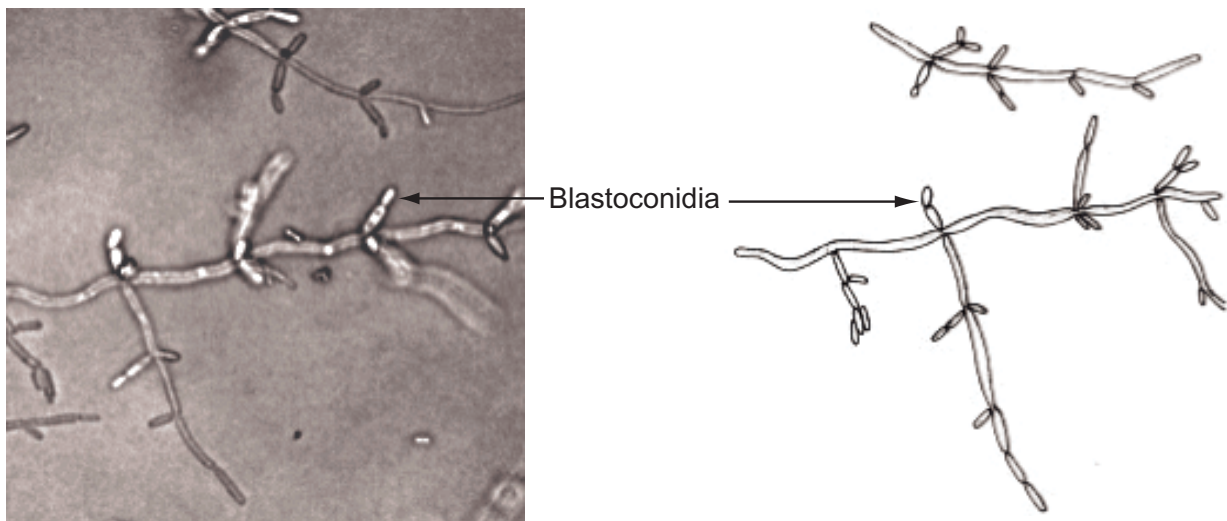
Figure 21



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

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Figure 22



Microscopic morphology of *Candida lambica*. On corn meal agar with Tween 80 culture, long, branched pseudohyphae with oval blastoconidia are seen (left; 200× magnification, right; line drawing not to scale).

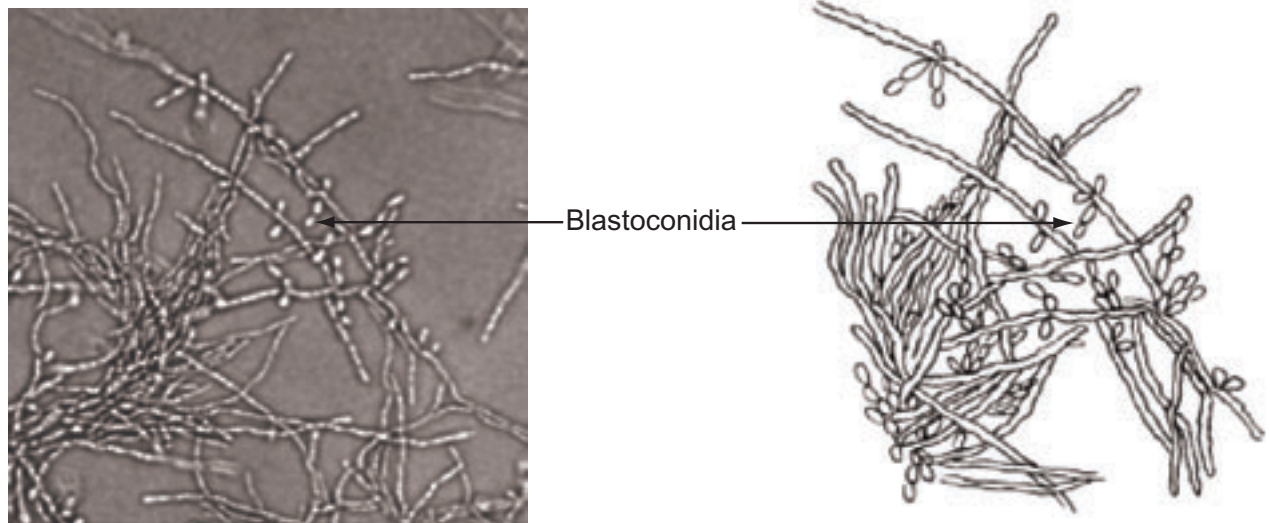
Figure 25



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

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Figure 26



Microscopic morphology of *Candida rugosa*. On corn meal agar with Tween 80 culture, branched pseudohyphae with elongated blastoconidia are seen (left; 200× magnification, right; line drawing not to scale).

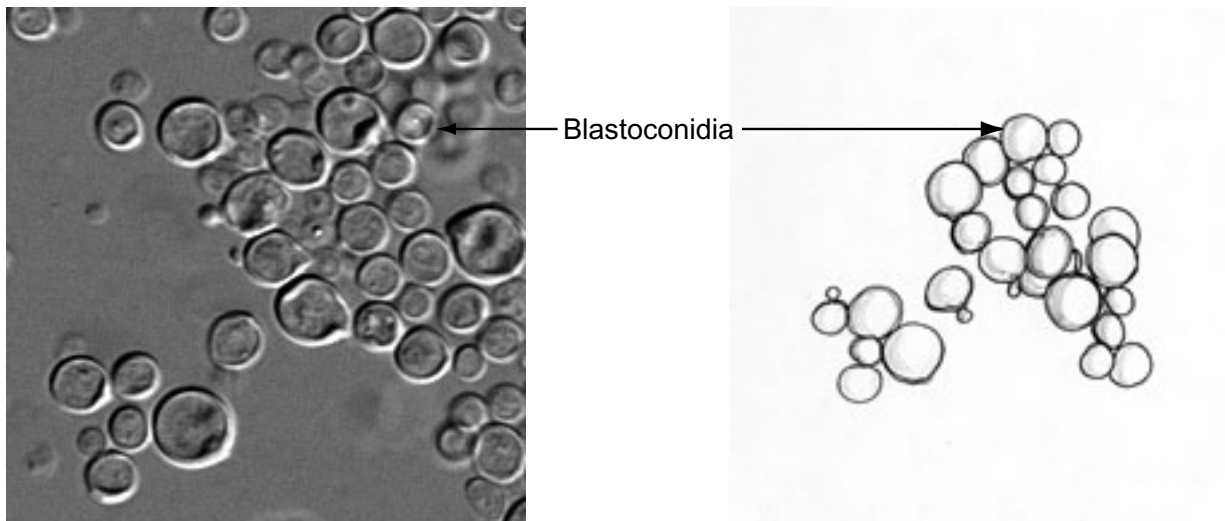
Figure 29



Seven-day-old, smooth-to-wrinkled, pink to red colored colony of *Rhodotorula glutinis* on Sabouraud's dextrose agar.

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Figure 30



Microscopic morphology of *Rhodotorula glutinis*, on corn meal agar with Tween 80 shows single or small clusters of round blastoconidia (left; 400× magnification, right; line drawing not to scale).

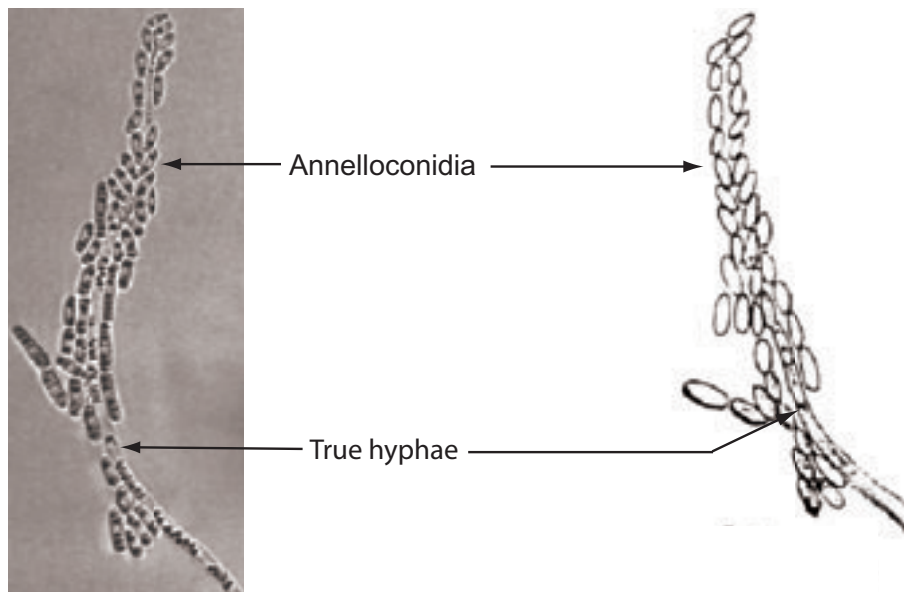
Figure 33



Seven-day-old, white, smooth to slightly wrinkled, raised colony of *Blastoschizomyces capitatus* on Sabouraud's dextrose agar.

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Figure 34



Microscopic morphology of *Blastoschizomyces capitatus* on corn meal agar with Tween 80, shows annelloconidia formed from true hyphae (left; 200× magnification, right; line drawing not to scale).



# **Wadsworth Center**

New York State Department of Health

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