

MYCOLOGY

CRITIQUÉ

Mycology Proficiency Testing Program
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Wadsworth Center

New York State Department of Health



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Test Specimens and Grading Policy

Test Specimens

A minimum of two strains of each of the proposed yeast specimens were examined for inclusion in the proficiency test event of June 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 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 – Yeast Only

	Specimen Key	Validated Specimen	Other Acceptable Answers
Y-1	<i>Cryptococcus uniguttulatus</i>	<i>Cryptococcus uniguttulatus</i>	
Y-2	<i>Cryptococcus humicolus</i>		
Y-3	<i>Cryptococcus albidus</i>	<i>Cryptococcus albidus</i>	
Y-4	<i>Candida zeylanoides</i>	<i>Candida zeylanoides</i>	
Y-5	<i>Candida dubliniensis</i>		
Ed. Sp.	<i>Pichia obmeri</i>		<i>Candida guilliermondii</i>

Mycology – Antifungal Susceptibility Testing for Yeasts

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

Mycology – Yeast Only

	Correct Responses/ Total # Labs (%)	Referees (%)
Y - 1 <i>Cryptococcus uniguttulatus</i>	143/144 (99)	10/10 (100)
Y - 2 <i>Cryptococcus humicolus</i> (Not validated)	103/144 (72)	8/10 (80)
Y - 3 <i>Cryptococcus albidus</i>	137/144 (95)	10/10 (100)
Y - 4 <i>Candida zeylanoides</i>	143/144 (99)	10/10 (100)
Y - 5 <i>Candida dubliniensis</i> (Not validated)	84/144 (58)	6/10 (60)

Mycology – Antifungal Susceptibility Testing for Yeasts

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

Mycology – General and Yeast Only

Number of participating laboratories	144
Number of referee laboratories	10
Number of laboratories responding by deadline	144
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	142
Number of laboratories unsuccessfully completing this test	2

Mycology – Antifungal Susceptibility Testing for Yeasts

Number of participating laboratories	21
Number of referee laboratories	3
Number of laboratories responding by deadline	21
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	0

Commercial Identification Systems Used*

AMS Vitek system	58
API 20C AUX	80
Microscan	3
Remel Uni-Yeast-Tek	4
Other	3

(*Includes multiple systems used by some labs)

Source: CSF

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	143
Labs with incorrect ID:	1
(<i>Cryptococcus albidus</i>)	(1)

Clinical Significance: *Cryptococcus uniguttulatus* has not been reported as a causative agent of infections in humans till recently. A case of ventriculitis due to *C. uniguttulatus* was documented in 2001.

Ecology: *C. uniguttulatus* is found on leaves and in soil.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar, after 7 days at 25°C, *C. uniguttulatus* colony was smooth, dull, soft, cream colored (Figure 1).
2. **Microscopic morphology** – On corn meal agar with Tween 80, round blastoconidia are seen (Figure 2). No pseudo- or true hyphae are formed
3. **Differentiation from other yeasts** – *C. uniguttulatus* does not ferment any carbohydrate, does not grow at 37°C or on the media containing cycloheximide. It produces urease enzyme. It does not form brown colonies on caffeic seed agar, thus differentiating from *C. neoformans*. It does not assimilate nitrate, differentiating from *C. albidus*. *C. laurentii* assimilates lactose and dulcitol but *C. uniguttulatus* does not assimilate these carbohydrates.
4. **In vitro susceptibility testing** – A single clinical isolate was susceptible to amphotericin B and itraconazole.
5. **Molecular tests** – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA was reported to differentiate several *Cryptococcus* species including *C. uniguttulatus*.

Further Reading:

1. Kwon-Chung, K.J., Hill, W.B., and Bennett, J.E. 1981. New, special stain for histopathological diagnosis of cryptococcosis. *J. Clin. Microbiol.* 13: 383-387.
2. McCurdy, L.H. and Morrow J.D. 2001. Ventriculitis due to *Cryptococcus uniguttulatus*. *South Med. J.* 94: 65-66.
3. Vilgalys, R. and Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* 172: 4238-4246.

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 AY382328 and AY382334, respectively.

```

CBS 1727 (AF444303)      1                               50
ATCC 66033 (AY382328)  TCCGTAGGTG AACCTGCGGA AGGATCATTA TTGAATTTAG TTGTCTGGCT
                               TCCGTAGGTG AACCTGCGGA AGGATCATTA TTGAATTTAG TTGTCTGGCT

51                               100
TTCGCCGACG ACGATATCAT TATCCATAAC ACCTGTGCAC TGTTGGATGT
TTCGCCGACG ACGATATCAT TATCCATAAC ACCTGTGCAC TGTTGGATGT

101                              150
TTAATACATC CGTTTTACAC TAAACAATAT TGTTACAAAT GTAGTCTTAT
TTAATACATC CGTTTTACAC TAAACAATAT TGTTACAAAT GTAGTCTTAT

151                              200
TATAACATAA TAAACTTTC AACACGGAT CTCTGGGCTC TCGCATCGAT
TATAACATAA TAAACTTTC AACACGGAT CTCTGGGCTC TCGCATCGAT

201                              250
GAAGAACGCA GCGAAATGCG ATAAGTAATG TGAATTGCAG AATTCAGTGA
GAAGAACGCA GC                               AATTCAGTGA

```

Figure 3. Alignment of primary sequences of the ITS1 regions of *C. uniguttulatus* CBS 1727 and PT specimen *C. uniguttulatu* ATCC 66033. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

CBS 1727 (AF444303)      1                               50
ATCC 66033 (AY382334)  TATAACATAA TAAACTTTC AACACGGAT CTCTGGGCTC TCGCATCGAT
                               TATAACATAA TAAACTTTC AACACGGAT CTCTGGGCTC TCGCATCGAT

51                               100
GAAGAACGCA GCGAAATGCG ATAAGTAATG TGAATTGCAG AATTCAGTGA
GAAGAACGCA GCGAAATGCG ATAAGTAATG TGAATTGCAG AATTCAGTGA

101                              150
ATCATCGAAT CTTTGAACGC ACCTTGCGCT CCTTGGTATT CCGAGGAGCA
ATCATCGAAT CTTTGAACGC ACCTTGCGCT CCTTGGTATT CCGAGGAGCA

151                              200
TGCCTGTTTG AGTGTCATGA AACTCTCAA A CTCTTGT TTTT GGATGCAAAT
TGCCTGTTTG AGTGTCATGA AACTCTCAA A CTCTTGT TTTT GGATGCAAAT

201                              250
CCTTGCTTGA GTTTGGACTT GGGTGT T TGC CGGTGATGAA CCGACTCGCC
CCTTGCTTGA GTTTGGACTT GGGTGT T TGC CGGTGATGAA CCGACTCGCC

251                              300
TTAAACATAT TAGCTGGACT TGTCTATATG ACTGGTTTGA CTTGGCATAA
TTAAACATAT TAGCTGGACT TGTCTATATG ACTGGTTTGA CTTGGCATAA

301                              400
TAAGTATTTT GCTAAGGACA TCTTCGGATG GCCAGTACCT AGGCTCTGTG
TAAGTATTTT GCTAAGGACA TCTTCGGATG GCCAGTACCT AGGCTCTGTG

401                              500
TCTGCTAACT AAACCATCAC TTGGAGTGCA TCTTTATGGT GTTGCTTCCT
TCTGCTAACT AAACCATCAC TTGGAGTGCA TCTTTATGGT GTTGCTTCCT

501                              550
GTGTATACTT TGACATCTGA CCTCAAATCA GGTAGGACTA CCCGCTGAAC
GTGTATACTT TGACATCTGA CCTCAAATCA GGTAGGACTA CCCGCTGAAC

551
TTAAGCATAT CAATAAGCGG AGGA
TTAAGCATAT CAATAAGCGG AGGA

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Figure 4. Alignment of primary sequences of the ITS2 regions of *C. uniguttulatus* CBS 1727 and PT specimen *C. uniguttulatu* ATCC 66033. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Comments: Only one participating lab reported this specimen as *C. albidus*. *C. uniguttulatus* can be differentiated from *C. albidus* by its negative nitrate assimilation.

Source: Skin

Scoring:	No. Labs
Referee Labs with correct ID:	8
Labs with correct ID:	103
Labs with incorrect ID:	41
(<i>Trichosporon mucoides</i>)	(31)
(<i>Trichosporon beigeli</i>)	(6)
(<i>Cryptococcus laurentii</i>)	(3)
(<i>Cryptococcus terreus</i>)	(1)

Clinical Significance: There are few reports of *C. humicolus* causing infection in humans. It has been isolated from cancer patients. It can cause infections of nail, eye, and central nervous system in the immunocompromised host.

Ecology: It has been isolated in nature from mushrooms, decaying toadstools, water, and from fruits like strawberries.

Laboratory Diagnosis:

1. Culture – On Sabouraud’s dextrose agar after 7 days at 25°C, colonies were yellow to buff in color, glistening to dull in appearance, smooth in texture (Figure 5).
2. Microscopic morphology – On corn meal agar with Tween 80, *C. humicolus* forms abundantly true hyphae and pseudohyphae. Ovoid blastoconidia are borne on terminal hyphae (Figure 6).
3. Differentiation from other yeasts – *C. humicolus* produces true and pseudo-hyphae on corn meal agar while other *Cryptococcus* species produce only blastoconidia. The assimilation patterns resemble that of *Trichosporon mucoides* but all strains of *Trichosporon* produce arthroconidia while *C. humicolus* does not produce arthroconidia.
4. In vitro susceptibility testing – No information available.
5. Molecular tests – The sequences of the internal transcribed spacer regions (ITS) and 18S rDNA were used to taxonomic heterogeneity of *Cryptococcus humicolus* and its closely related species *C. curvatus*, and the genus *Trichosporon*.

Further Reading:

1. Alvarez Gasca, M.A., Arguero Licea, B., Pliego Castaneda, A., and Garcia Tena, S., 1998. Fungal agents isolated from cancer patients. *Revista Latinoamericana de Microbiologia*. 40: 15-24.
2. Liotet, S., Rouchy, J.P., and Drouchet, E. 1971. *Candida humicola* superinfection in chronic conjunctivitis. *Bulletin des Societes de Ophthalmologie de France*. 71: 737-741.
3. Rogowska-Szadkowska, D., Wiercinska-Drapalo, A., Borzuchowska, A., and Prokopowicz, D. 1997. *Candida humicola* infection of the central nervous system in an HIV-infected patient: a case report. *Przegl. Epidemiol.* 51: 465-469.
4. Sugita, T., Takashima, M., Ikeda, R., Nakase, T., and Shinoda, T. 2000. Phylogenetic and taxonomic heterogeneity of *Cryptococcus humicolus* by analysis of the sequences of the internal transcribed spacer regions and 18S rDNA, and the phylogenetic relationships of *C. humicolus*, *C. curvatus*, and the genus *Trichosporon*. *Microbiol. Immunol.* 44: 455-461.

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 AY382329 and AY382335, respectively.

```

1                               50
UWFP-362 (AF335934) TCCGTAGGTG AACCTGCGGA AGGATCATTG GTGATTGCC TTAGTGGCTA
ATCC 9949 (AY382329) TCCGTAGGTG AACCTGCGGA AGGATCATTG GTGATTGCC TTAGTGGCTA

51                               100
AAAACCTATAT CCCAAACACC TGTGAACTGT TGAATCGCGT CTTCCGGATGT
AAAACCTATAT CCCAAACACC TGTGAACTGT TGAATCGCGT CTTCCGGATGT

101                              150
GATTCTTTTA CAAACATTGT GTAATGAACG TCATAACATT ATAAACAATA
GATTCTTTTA CAAACATTGT GTAATGAACG TCATAACATT ATAAACAATA

151                              200
CAACTTTCAA CAACGGATCT CTTGGCTCTC GCATCGATGA AGAACGCAGC
CAACTTTCAA CAACGGATCT CTTGGCTCTC GCATCGATGA AGAACGCAGC

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Figure 7. Alignment of primary sequences of the ITS1 regions of *C. humicolus* UWFP-362 and PT specimen *C. humicolus* ATCC 9949. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1                               50
UWFP-362 (AF218999) GCATCGATGA AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
ATCC 9949 (AY382335) GCATCGATGA AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA

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

101                              150
GGAGAGCATG CCTGTTTGAG TGTCATGATC TCTCAACCAA TAGGGTTTCT
GGAGAGCATG CCTGTTTGAG TGTCATGATC TCTCAACCAA TAGGGTTTCT

151                              200
TATTGGCTTG GATCTGGGTG TTGCCAGCTT TGTCTGGCTC GCCTTAAAGG
TATTGGCTTG GATCTGGGTG TTGCCAGCTT TGTCTGGCTC GCCTTAAAGG

201                              250
AGTTAGCGAG TAAAGCTCTG TCGTCTGGCG TAATAAGTTT CGCTGGTGTA
AGTTAGCGAG TAAAGCTCTG TCGTCTGGCG TAATAAGTTT CGCTGGTGTA

251                              300
GACAGTGGTG GCGCACGCTT ATAATCGCCT TCGGGCAATT TTTGACTCTG
GACAGTGGTG GCGCACGCTT ATAATCGCCT TCGGGCAATT TTTGACTCTG

301                              350
GCCTCAAATC AGGTAGGACT ACCCGCTGAA CTTAAGCATA TCAATAAGCG
GCCTCAAATC AGGTAGGACT ACCCGCTGAA CTTAAGCATA TCAATAAGCG

351
GAGGA
GAGGA

```

Figure 8. Alignment of primary sequences of the ITS2 regions of *C. humicolus* UWFP-362 and PT specimen *C. humicolus* ATCC 9949. 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. One-fourth of the participating labs reported it as *Trichosporon*. *C. humicolus* resembles *Trichosporon mucoides* morphologically and on assimilation patterns. However, all strains of *Trichosporon* produce arthroconidia while *C. humicolus* does not.

Source: eye

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	137
Labs with incorrect ID:	7
(<i>Cryptococcus laurentii</i>)	(4)
(<i>Cryptococcus neoformans</i>)	(2)
(<i>Cryptococcus glutinis</i>)	(1)

Clinical Significance: *Cryptococcus albidus* is a rare causal agent of sepsis, wound infection, and pneumonia in immunocompromised patients.

Ecology: *C. albidus* is cosmopolitan, found on plants and in water. It is also found on skin of animals and humans.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was soft, mucoid, cream to pink (Figure 9).
2. **Microscopic morphology** – On corn meal agar with Tween 80, large, round budding yeast cells, no true hyphae or pseudohyphae are seen (Figure 10).
3. **Differentiation from other yeasts** – *C. albidus* does not grow on media containing cycloheximide, grows poorly at 37°C, produces urease enzyme, and assimilates nitrate. It is differentiated from *C. neoformans* by its inability to form brown colonies on niger seed agar. Although *C. terreus* is also nitrate-positive, it differs from *C. albidus* in assimilation of sorbitol and N-acetylglucosamine.
4. **In vitro susceptibility testing** – Almost all isolates are susceptible to amphotericin B, flucytosine, and azoles.
5. **Molecular tests** – Ribosomal DNA sequence analysis revealed diversity in *C. albidus*.

Further Reading:

1. Fonseca, A., Scorzetti, G., and Fell, J.W. 2000. Diversity in the yeast *Cryptococcus albidus* and related species as revealed by ribosomal DNA sequence analysis. *Can. J. Microbiol.* 46: 7-27.
2. Gluck, J.L., Myers, J.P., and Pass, L.M. 1987. Cryptococemia due to *Cryptococcus albidus*. *South Med. J.* 80: 511-513.
3. Kordossis, t., Avlami, A., Velegraki, A., Stefanou, I., Georgakopoulos, G., Papalambrou, C., and Legakis, N.J. 1998. First report of *Cryptococcus laurentii* meningitis and a fatal case of *Cryptococcus albidus* cryptococcaemia in AIDS patients. *Med. Mycol.* 36: 335-339.
4. Loison, J., Bouchara, J.P., Gueho, E., de Gentile, L., Cimon, B., Chennebault, J.M., and Chabasse, D. 1996. First report of *Cryptococcus albidus* septicaemia in an HIV patient. *J. Infect.* 33: 139-140.
5. Narayan, S., Batta, K., Colloby, P., and Tan, C.Y. 2000. Cutaneous *Cryptococcus* infection due to *C. albidus* associated with Sezary syndrome. *Br. J. Dermatol.* 143: 632-634.
6. Wells, G.M., Gajjar, A., Pearson, T.A., Hale, K.L., and Shenep, J.L. 1998. Pulmonary cryptosporidiosis and *Cryptococcus albidus* fungemia in a child with acute lymphocytic leukemia. *Med Pediatr. Oncol.* 31: 544-546.

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 AY382330 and AY382336, respectively.

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1                               50
CBS 5592 (AF444370)  TCCGTAGGTG AACCTGCGGA AGGATCATTG ATGATTGACC GTCTGTGCGAG
ATCC 10666 (AY382330) TCCGTAGGTG AACCTGCGGA AGGATCATTG ATGATTGACC GTCTGTGCGAG

51                               100
CTTGCTCACA GGCACATCAT ATCCATAACA CCTGTGCACT TGTCGGATGG
CTTGCTCACA GGCACATCAT ATCCATAACA CCTGTGCACT TGTCGGATGG

101                              150
CTTAGTGAAG ACCGCAAGGT TGAATCTATC CATCTACTTT ACATAACAAT
CTTAGTGAAG ACCGCAAGGT TGAATCTATC CATCTACTTT ACATAACAAT

151                              200
TCTGTAACAA ATGTAGTCTT ATTATAACAT AATAAACTT TCAACAACGG
TCTGTAACAA ATGTAGTCTT ATTATAACAT AATAAACTT TCAACAACGG

201                              250
ATCTCTTGGC TCTCGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA
ATCTCTTGGC TCTCGCATCG ATGAAGAACG CAGC

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Figure 11. Alignment of primary sequences of the ITS1 regions of *C. albidus* CBS 5592 and PT specimen *C. albidus* ATCC 10666. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

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1                               50
CBS 142 (AF145321)  ATCTCTTGGC TCTCGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA
ATCC 10666 (AY382336) ATCTCTTGGC TCTCGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA

51                               100
TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACCTTGGC
TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACCTTGGC

101                              150
CTCCTTGGTA TTCCGAGGAG CATGCCTGTT TGAGTGTCAT GAAAACCCTC
CTCCTTGGTA TTCCGAGGAG CATGCCTGTT TGAGTGTCAT GAAAACCCTC

151                              200
AACCCTAGAT TGGTTAAAAC CTCTCTTTGG TTTGGATTG GACGTTTGGC
AACCCTAGAT TGGTTAAAAC CTCTCTTTGG TTTGGATTG GACGTTTGGC

201                              250
GATGATAAGT CGGCTCGTCT TAAAAGTAAT AGCTGGATCT GTCTCGCGAC
GATGATAAGT CGGCTCGTCT TAAAAGTAAT AGCTGGATCT GTCTCGCGAC

251                              300
ATGGTTTGAC TTGGCGTAAT AAGTATTTTCG CTAAGGACAT CTTCCGATGG
ATGGTTTGAC TTGGCGTAAT AAGTATTTTCG CTAAGGACAT CTTCCGATGG

301                              350
CCGCGTTGCA GGAATAAAGA CCGCTTTCTA ATCCATTGAT CTTCCGATTA
CCGCGTTGCA GGAATAAAGA CCGCTTTCTA ATCCATTGAT CTTCCGATTA

351                              400
ATACTCTTGA CATCTGGCCT CAAATCAGGT AGGACTACCC GCTGAACCTA
ATACTCTTGA CATCTGGCCT CAAATCAGGT AGGACTACCC GCTGAACCTA

401
AGCATATCAA TAAGCGGAGGA
AGCATATCAA TAAGCGGAGGA

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Figure 12. Alignment of primary sequences of the ITS2 regions of *C. albidus* CBS 142 and PT specimen *C. albidus* ATCC 10666. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Comments: *C. albidus* can be distinguished from *C. laurentii* by absence of growth on the media containing cycloheximide and nitrate positive test. It is different from *C. neoformans* by nitrate positive test.

Source: Urine

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	143
Labs with incorrect ID:	1
(<i>Candida krusei/inconspicua</i>)	(1)

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

Ecology: *C. zeylanoides* is cosmopolitan, found in water, meat, and on human body.

Laboratory Diagnosis:

1. Culture – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was smooth, cream-colored, butyrous raised (Figure 13).
2. Microscopic morphology – On corn meal agar with Tween 80, *C. zeylanoides* forms long pseudohyphae, with verticillate, ovoid blastoconidia (Figure 14). Blastoconidia are produced in whorls around the pseudohyphae.
3. Differentiation from other yeasts – *C. zeylanoides* does not ferment any carbohydrates, grows at 37°C, grows on media containing cycloheximide, and assimilates limited carbohydrates.
4. In vitro susceptibility testing – *C. zeylanoides* is susceptible to amphotericin B and to the commonly used azoles.
5. Molecular tests – Multiplex PCR using ITS1 and ITS2 was reported for rapid detection and identification of yeast strains.

Further Reading:

1. Bisbe, J., Vilardell, J., Valls, M., Moreno, A., Brancos, M., and Andreu, J. 1987. Transient fungemia and *Candida* arthritis due to *Candida zeylanoides*. *European J. Clin. Microbiol.* 6: 668-669.
2. Crozier, W.J. 1993. Two cases of onychomycosis due to *Candida zeylanoides*. *Australasian J. Dermatology.* 34: 23-25.
3. Fujita, S.I., Senda, Y., Nakaguchi, S., and Hashimoto, T. 2001. Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. *J. Clin. Microbiol.* 39: 3617-22.
4. Levenson, D., Pfaller, M.A., Smith, M.A., Hollis, R. Gerarden, T., Tucci, C.B., and Isenberg, H.D. 1991. *Candida zeylanoides*: another opportunistic yeast. *J. Clin. Microbiol.* 29: 1689-1692.
5. Liao, W.-Q., Li, Z.-G., Guo, M., and Zhang, J.-Z. 1993. *Candida zeylanoides* causing candidiasis as tinea cruris. 1993. *Chinese Medical J.* 106: 542-545.
6. Whitby, S., Madu, E.C., and Bronze, M.S. 1996. *Candida zeylanoides* infective endocarditis complicating infection with the human immunodeficiency virus. *Am. J. Medical Sciences.* 312: 138-139.

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 AY382331 and AY382337, respectively.

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1                                     50
ATCC 7351 (AF335930) TCCGTAGGTG AACCTGCGGA AGGATCATTA CAGTATTCTT TTGCCAGCGC
NRRL 1774 (AY382331) TCCGTAGGTG AACCTGCGGA AGGATCATTA CAGTATTCTT TTGCCAGCGC

51                                     100
TTAATTGCGC GCGGAAAAAC CTTACACACT ATGTTTTTTT GATTTGAAAC
TTAATTGCGC GCGGAAAAAC CTTACACACT ATGTTTTTTT GATTTGAAAC

101                                    150
TTTGTGCTTG GTCTGACTTA GAAATGAGTT GGGCCAAAGG TTTTATACTA
TTTGTGCTTG GTCTGACTTA GAAATGAGTT GGGCCAAAGG TTTTATACTA

151                                    200
AAACTTCAAT TTTATTATG AATTGTAAAT TAATTATATT GTCAATTTGT
AAACTTCAAT TTTATTATG AATTGTAAAT TAATTATATT GTCAATTTGT

201                                    250
TGATTAAATT CAAAAATCTT CAAAACCTTC AACACGGAT CTCTTGGTTC
TGATTAAATT CAAAAATCTT CAAAACCTTC AACACGGAT CTCTTGGTTC

251
TCGCATCGAT GAAGAACGCA GC
TCGCATCGAT GAAGAACGCA GC

```

Figure 15. Alignment of primary sequences of the ITS1 regions of *C. zeylanoides* ATCC 7351 and PT specimen *C. zeylanoides* NRRL 1774. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1                                     50
ATCC 7351T (AF218976) GCATCGATGA AGAACGCAGC GAAATGCGAT AAGTAATATG AATTGCAGAT
NRRL 1774 (AY382337) GCATCGATGA AGAACGCAGC GAAATGCGAT AAGTAATATG AATTGCAGAT

51                                     100
TTTCGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCT ATGGTATTCC
TTTCGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCT ATGGTATTCC

101                                    150
ATAGGGCATG CCTGTTTGTG CGTCATTTCT CTCTCAAATC TTCGGATTG
ATAGGGCATG CCTGTTTGTG CGTCATTTCT CTCTCAAATC TTCGGATTG

151                                    200
GTTTTGAGTG ATACTCTTAG TCAGACTAAG CGTTTGCTTG AAATGTATTG
GTTTTGAGTG ATACTCTTAG TCAGACTAAG CGTTTGCTTG AAATGTATTG

201                                    250
GCATGAGTGG TACTAGATAG TGCTGAACTG TTTCAATGTA TTAGGTTTAT
GCATGAGTGG TACTAGATAG TGCTGAACTG TTTCAATGTA TTAGGTTTAT

251                                    300
CCAACCTCGTT GACCAGTATA GTATTTGTTT ATTACACAGG CTCGGCCTTA
CCAACCTCGTT GACCAGTATA GTATTTGTTT ATTACACAGG CTCGGCCTTA

301                                    350
CAACAACAAA CAAAGTTTGA CCTCAAATCA GGTAGGACTA CCCGCTGAAC
CAACAACAAA CAAAGTTTGA CCTCAAATCA GGTAGGACTA CCCGCTGAAC

351
TTAAGCATAT CAATAAGCGG AGGA
TTAAGCATAT CAATAAGCGG AGGA

```

Figure 16. Alignment of primary sequences of the ITS2 regions of *C. zeylanoides* ATCC 7351T and PT specimen *C. zeylanoides* NRRL 1774. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Comments: One lab reported this specimen as *C. krusei/inconspicua*, which ferments glucose but does not assimilate trehalose, while *C. zeylanoides* does not ferment any carbohydrates but assimilate trehalose.

Source: Oropharynx

Scoring:	No. Labs
Referee Labs with correct ID:	6
Labs with correct ID:	84
Labs with incorrect ID:	60
(<i>Candida albicans</i>)	(60)

Clinical Significance: Isolates of *Candida dubliniensis* were initially recovered from the oral cavities of HIV-infected individuals and AIDS patients causing erythematous and/or pseudomembranous oral candidiasis or angular cheilitis. Most of the *C. dubliniensis* isolates have been recovered from the oral cavities of HIV infected patients, but the organism has also been isolated from other body sites including lungs, vagina, blood, and feces. More recently, this species has been reported as a cause of blood stream infection in the United States.

Ecology: Even though *C. dubliniensis* was first isolated and described in Ireland, it is reported from many parts of world including Israel, Netherlands, USA, etc.

Laboratory Diagnosis:

- Culture** – On Sabouraud’s dextrose agar after 7 days at 25°C, colony is white to cream, smooth, and soft (Figure 17). Phenotypically, *C. dubliniensis* is practically indistinguishable from *C. albicans*. On initial isolation, *C. dubliniensis* produces dark green colonies on CHROMagar Candida while *C. albicans* colonies appear light blue-green but after repeated transfer or storage, the ability to produce the dark green colonies on this specialized medium may be lost.
- Microscopic morphology** – It has been reported that *C. dubliniensis* produces abundant chlamydospores, often in contiguous pairs or triplets but at least one study has not found this to be consistent, and therefore, the relative abundance of chlamydospores may not be a definite criterion. As illustrated in the Figure 18, the test isolate produced chlamydospore in clusters.
- Differentiation from other yeasts** – One physiologic feature that does appear to be fairly stable based on the literature is that *C. dubliniensis* grows poorly or not at all at 42°C while *C. albicans* grows well at this temperature. Tests at 45°C might be even more promising to distinguish these two species than growth at 42°C. In addition, *C. dubliniensis* is able to assimilate glycerol, but not xylose nor trehalose. However, *C. albicans* is the opposite.
- In vitro susceptibility testing** – Several isolates of *C. dubliniensis* have been found to have higher resistance to fluconazole than other pathogenic species of *Candida*, and the resistance to fluconazole may be induced in some originally sensitive strains. This fact may have serious implications for immunocompromised individuals on prolonged regimen of fluconazole.
- Molecular tests** – Genetically, *C. dubliniensis* has been found to be distinct from *C. albicans* in DNA fingerprinting studies even though the two species are closely related phylogenetically. Several *C. dubliniensis* molecular probes are available in reference laboratories.

Further Reading:

- Gales, A.C., Pfaller, M.A., Houston, A.K., Joly, S., Sullivan, D.J., Coleman, D.C., and Soll, D.R. 1999. Identification of *Candida dubliniensis* based on temperature and utilization of xylose and α-methyl-D-glucoside as determined with the API 20C AUX and Vitek YBC systems. *J. Clin. Microbiol.* 37: 3804-3808.
- Gugnani, H.C., Becker, K., Fegeler, W., Basu, S., Chattopadhyay, D., Baveja, U., Satyanarayana, S., Kalghatgi, T., Murlidhar, A. 2003. Oropharyngeal carriage of *Candida* species in HIV-infected patients in India. *Mycoses* 46: 281-288.
- Kim, J.O., Garofalo, L., Blecker-Shelly, D., McGowan, K.L. 2003. *Candida dubliniensis* infections in a pediatric population: retrospective identification from clinical laboratory isolates of *Candida albicans*. *J. Clin. Microbiol.* 41: 3354-3357.
- Lees, E. and Barton, R.C. 2003. The use of Niger seed agar to screen for *Candida dubliniensis* in the clinical microbiology laboratory. *Diagn. Microbiol. Infect. Dis.* 46:13-7.
- Park, S. Wong, M., Marras, S.A., Cross, E.W., Kiehn, T.E., Chaturvedi, V., Tyagi, S., and Perlin, D.S. 2000. Rapid identification of *Candida dubliniensis* using a species-specific molecular beacon. *J. Clin. Microbiol.* 38: 2829-2836.
- Pincus, D.H., Coleman, D., Pruitt, W.R., Padhye, A.A., Salkin, I.F., Geimer, M., Bassel, A., Sullivan, D.J., and Hearn, V. 1999. Rapid identification of *Candida dubliniensis* with commercial yeast identification systems. *J. Clin. Microbiol.* 37: 3533-3539.
- Sullivan, D. and Coleman, D. 1998. Minireview. *Candida dubliniensis*: characteristics and identification. *J. Clin. Microbiol.* 36: 329-334.

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 AY382332 and AY382338, respectively.

```

1                               50
UWFP-92 (AF336833)  TCCGTAGGTG AACCTGCGGA AGGATCATTA CTGATTTGCT TAATTGCACC
NRRL 17841 (AY382332) TCCGTAGGTG AACCTGCGGA AGGATCATTA CTGATTTGCT TAATTGCACC

51                               100
ACATGTGTTT TGTTCTGGAC AAACCTGCTT TGGCGGTGGG CCCCTGCCTG
ACATGTGTTT TGTTCTGGAC AAACCTGCTT TGGCGGTGGG CCCCTGCCTG

101                              150
CCGCCAGAGG ACATAAACTT ACAACCAAAT TTTTATAAAA CTTGTCACGA
CCGCCAGAGG ACATAAACTT ACAACCAAAT TTTTATAAAA CTTGTCACGA

151                              200
GATTATTTTT AATAGTCAAA ACTTTCAACA ACGGATCTCT TGGTTCTCGC
GATTATTTTT AATAGTCAAA ACTTTCAACA ACGGATCTCT TGGTTCTCGC

210
ATCGATGAAG AACGCAGC
ATCGATGAAG AACGCAGC

```

Figure 19. Alignment of primary sequences of the ITS1 regions of *C. dubliniensis* UWFP-92 and PT specimen *C. dubliniensis* NRRL 17841. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1                               50
UWFP-92 (AF218993)  GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATATG AATTGCAGAT
NRRL 17841 (AY382338) GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATATG AATTGCAGAT

51                               100
ATTTCGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCT CTGGTATTCC
ATTTCGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCT CTGGTATTCC

101                              150
GGAGGGCATG CCTGTTTGTG CGTCGTTTCT CCCTCAAACC CCTAGGGTTT
GGAGGGCATG CCTGTTTGTG CGTCGTTTCT CCCTCAAACC CCTAGGGTTT

151                              200
GGTGTGAGC AATACGACTT GGGTTTGCTT GAAAGATGAT AGTGGTATAA
GGTGTGAGC AATACGACTT GGGTTTGCTT GAAAGATGAT AGTGGTATAA

201                              250
GGCGGAGATG CTTGACAATG GCTTAGGTGT AACCAAAAAC ATTGCTAAGG
GGCGGAGATG CTTGACAATG GCTTAGGTGT AACCAAAAAC ATTGCTAAGG

251                              300
CGGTCTCTGG CGTCGCCCAT TTTATTCTTC AAACCTTTGAC CTCAAATCAG
CGGTCTCTGG CGTCGCCCAT TTTATTCTTC AAACCTTTGAC CTCAAATCAG

301
GTAGGACTAC CCGCTGAACT TAAGCATATC AATAAGCGGA GGA
GTAGGACTAC CCGCTGAACT TAAGCATATC AATAAGCGGA GGA

```

Figure 20. Alignment of primary sequences of the ITS2 regions of *C. dubliniensis* UWFP-92 and PT specimen *C. dubliniensis* NRRL 17841. 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. All labs with incorrect identification reported this specimen as *C. albicans*. This specimen was sent out earlier as an educational specimen in the Mycology PTP October 1997 event, and as a real test specimen in October 2000 PT event. Although many more labs reported correct ID this time, there were still about 42% labs that failed to identify *C. dubliniensis*. Interestingly, the ability to correctly identify this specimen was independent of the commercial identification system used. It was not clear why labs that used API AUX20C and VITEK II systems, which have *C. dubliniensis* in their database, still reported this isolate as *C. albicans*. As summarized earlier in this section, a number of physiological differences could be used to distinguish these two closely related *Candida* species.

Source: Blood

Scoring:	No. Labs
Labs with correct ID:	136
Labs with incorrect ID:	8
(Unidentified)	(3)
(<i>Candida parapsilosis</i>)	(2)
(<i>Blastoschizomyces capitatus</i>)	(1)
(<i>Candida famata</i>)	(1)
(<i>Pichia anomala</i>)	(1)

Clinical Significance: *Pichia obmeri* is a frequent causal agent of nosocomial fungemia in immunosuppressed patients. Also, it is an infrequent casual agent of urinary tract infections, brain abscess, and ocular infections.

Ecology: *P. obmeri* is cosmopolitan in distribution.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar after 7 days at 25°C, colony was flat, smooth, cream-yellow (Figure 21).
2. **Microscopic morphology** – On corn meal agar with Tween 80, few short pseudohyphae with blastoconidia and some ascospores are seen (Figure 22).
3. **Differentiation from other yeasts** – *P. obmeri* ferments glucose, sucrose, and trehalose, grows at 37°C, and grows on media containing cycloheximide. It does not form pink pigment thereby differentiating from *Rhodotorula* species. It does not produce true hyphae, which differentiates it from *Candida ciferrii* and *Trichosporon beigeli*. Unlike *C. lusitanae*, it is unable to grow at 45°C.
4. **In vitro susceptibility testing** – Most clinical isolates are susceptible to amphotericin B, 5FC, and azoles such as fluconazole, itraconazole, and ketoconazole; a few isolates are reported to have high MIC to azoles.
5. **Molecular tests** – Primers for large ribosomal subunit DNA sequences were used in PCR to differentiate *P. obmeri* from *C. famata/Debaryomyces hansenii* complex. Isolates of *P. obmeri* were identified using PCR to amplify ribosomal DNA, followed by restriction digestion of the PCR product.

Further Reading:

1. Hitomi, S., Kumao, T., Onizawa, K., Miyajima, Y., and Wakatsuki, T. 2002. A case of central-venous-catheter-associated infection caused by *Pichia obmeri*. *J. Hosp. Infect.* 51: 75-77.
2. Joao, I., Duarte, J., Cotrim, C., Rodrigues, A., Martins, C., Fazendas, P., Oliveira, L.M., Diogo, J., and Carrageta, M. 2002. Native valve endocarditis due to *Pichia obmeri*. *Heart Vessels.* 16: 260-263.
3. Maute, A.J., Visser, M.R., Lipovsk, M., Schuitemaker, F.J., and Hoepelman, A.I.M. 2000. A case of disseminated infection with *Pichia obmeri*. *Eur. J. Clin. Microbiol. Infect. Dis.* 19: 971-973.
4. Puerto, J.L., Garcia-Martos, P., Saldarreaga, A., Ruiz-Aragon, J., Garcia-Agudo, R., and Aoufi, S. 2002. First report of urinary tract infection due to *Pichia obmeri*. *Eur. J. Clin. Microbiol. Infect. Dis.* 21: 630-631.
5. Reina, J.P., Larone, D.H., Sabetta, J.R., Krieger, K.K., Hartman, B.J. 2002. *Pichia obmeri* prosthetic valve endocarditis and review of the literature. *Scand. J. Infect. Dis.* 34: 140-141.
6. Shin, D.H., Park, J.H., Shin, J.H., Suh, S.P., Ryang, D.W., and Kim, S.J. 2003. *Pichia obmeri* fungemia associated with phlebitis: successful treatment with amphotericin B. *J. Infect. Chemother.* 9: 88-89

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 AY382333 and AY382339, respectively.

```

1
ATCC 46053 (AF335946) TCCGTAGGTG AACCTGCGGA AGGATCATT ACATAATATT CTTACACACT
NRRL 1932 TCCGTAGGTG AACCTGCGGA AGGATCATT ACATAATATT CTTACACACT

51
GTTTTTTTAC AACAAAAAAA ATCTATCTAA AAACAATTCT TTACAAGAAA
GTTTTTTTAC AACAAAAAAA ATCTATCTAA AAACAATTCT TTACAAGAAA

101
TTCTTAAAC TTTCAACAAC GGATCTCTTG GTTCTCGCAT CGATGAAGAA
TTCTTAAAC TTTCAACAAC GGATCTCTTG GTTCTCGCAT CGATGAAGAA

151
CGCAGC
CGCAGC

```

Figure 23. Alignment of primary sequences of the ITS1 regions of *P. ohmeri* ATCC 46053 and PT specimen *P. ohmeri* NRRL 1932. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1
ATCC 46053T (AF218977) GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATACG AATCGCAGCT
NRRL 1932 GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATACG AATCGCAGCT

51
CTCGGAATCA TCGAATCTTT GAACGCACAT TGCACCATTG GGTATTCCCA
CTCGGAATCA TCGAATCTTT GAACGCACAT TGCACCATTG GGTATTCCCA

101
ATGGTATGCT TGTTTGAGCG AATACTTCCC TAATCCTCAC GGATTGTATT
ATGGTATGCT TGTTTGAGCG AATACTTCCC TAATCCTCAC GGATTGTATT

151
GTGTTTGCAC GAAAATAATG ACGACAGTAC TCTACAAAAC GGTACCGTCA
GTGTTTGCAC GAAAATAATG ACGACAGTAC TCTACAAAAC GGTACCGTCA

201
GTACACTCAT TTTTTTTCCT CAAATCAAGT AGGACTACCC GCTGAACTTA
GTACACTCAT TTTTTTTCCT CAAATCAAGT AGGACTACCC GCTGAACTTA

251
AGCATATCAA TAAGCGGAGGA
AGCATATCAA TAAGCGGAGGA

```

Figure 24. Alignment of primary sequences of the ITS2 regions of *P. ohmeri* ATCC 46053T and PT specimen *P. ohmeri* NRRL 1932. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Comments: *P. ohmeri* is the teleomorph of *Candida guilliermondii*. Most participating labs were able to correctly identify this specimen. *P. ohmeri* is able to grow on the media with cycloheximide, which distinguishes from *C. parapsilosis*. *P. ohmeri* is different from *P. anomala* by its negative reaction on nitrate. *P. ohmeri* ferments glucose, sucrose, and trehalose but *B. capitatus* does not. *C. famata* infrequently assimilates melezitose and raffinose (60%), while *P. ohmeri* assimilates these two carbohydrates more frequently (90%).

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. In it the results are read after 24hr incubation rather than after 48hr (1).

Materials & Methods:

Nineteen microbiology laboratories within the United States and one reference lab each from Canada and United Kingdom participated in this event. Two NCCLS quality control strains, *Candida parapsilosis* ATCC 22019 (S-4) and *Candida krusei* ATCC 6258 (S-3), and three NCCLS reference strains, *Candida parapsilosis* ATCC 90018 (S-1), *Candida albicans* ATCC 90028 (S-2), and *Candida albicans* ATCC 24433 (S-5) (3, 4), were included in the June 4, 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 ± 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 21 labs participated in this antifungal susceptibility testing event, and the performances of all of the labs were satisfactory. Of the 21 participating laboratories, 7 labs used the broth microdilution method, 2 labs used Etest, and 12 labs used YeastOne Colorimetric microdilution method. The supplementary information on antifungal susceptibility testing procedures is summarized in Table 1. The MIC results submitted by the 21 participants are illustrated in Figure 25. For amphotericin B, good performance was noted for *C. parapsilosis* ATCC 90018 and 22019, *C. krusei* ATCC 6258, and *C. albicans* ATCC 24433, irrespective of the methodology used by the laboratories. For fluconazole, good performance was seen for *C. parapsilosis* ATCC 90018, *C. albicans* ATCC 90028, and *C. tropicalis* ATCC 750. Overall agreement with the NCCLS reference ranges was 99% against amphotericin B and 98% against fluconazole for all five isolates, after with the expansion of the reference range by ± 2 dilutions. 83% of answers for amphotericin B and 87% 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.
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.

Individual Isolates:

S-1 *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

Thirteen labs reported values within the NCCLS reference range, 6 labs reported values within the expanded range for amphotericin B. Twenty labs reported values within the NCCLS reference range and 1 lab reported values within the expanded range for fluconazole.

S-2 *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

Twelve labs reported values within the NCCLS reference range, 6 labs reported values within the expanded range, and 1 lab reported a MIC value lower than the expanded range for amphotericin B. Sixteen labs reported values within the NCCLS reference range and 5 labs reported values within the expanded range for fluconazole.

S-3 *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 labs reported values within the NCCLS reference range for amphotericin B. Nineteen labs reported values within the NCCLS reference range and 2 labs reported values within the expanded values for fluconazole.

S-4 *Candida parapsilosis* ATCC 22019

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

Seventeen labs reported values within the NCCLS reference range and 2 labs reported a value within the expanded range for amphotericin B. Eighteen labs reported values within the NCCLS reference range, 2 labs reported a value within the expanded range, and 1 lab reported a MIC value lower than the expanded range for fluconazole.

S-5 *Candida albicans* ATCC 24433

Summary	NCCLS Reference range	Expanded Range
Amphotericin B	0.25-1.0 µg/ml	0.125-2.0 µg/ml
Fluconazole	0.25-1.0 µg/ml	0.125-2.0 µg/ml

Eighteen labs reported values within the NCCLS reference range, 1 lab reported values within the expanded range for amphotericin B. Eighteen labs reported values within the NCCLS reference range, 2 labs reported values within the expanded range, and 1 lab reported a MIC value higher than the expanded range for fluconazole.

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.

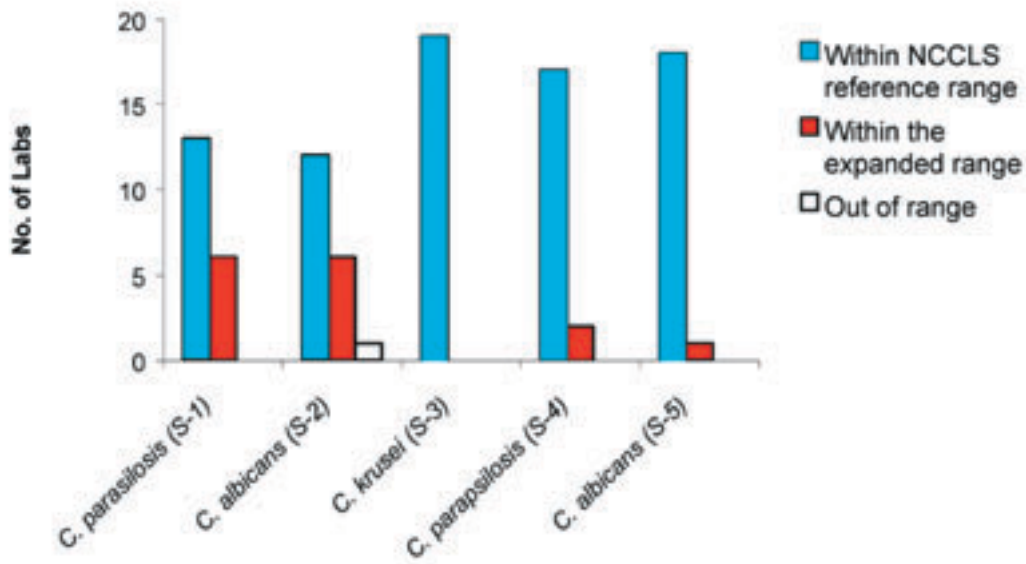
Table 1. Summary of supplementary information on antifungal susceptibility testing by participating laboratories

	No. Participant Labs
Test Method	
NCCLS broth microdilution	7
NCCLS broth macrodilution	0
Sensititre YeastOne Colorimetric	12
Etest	2
Medium employed	
RPMI 1640	10 *
RPMI 1640 w / alamar blue	2
Antibiotic medium 3	1
Sabouraud dextrose	4
YeastOne broth	6
Inoculum preparation	
Spectrophotometric	8*
MacFarland	14
Inoculum size	
0.5-2.5 × 10 ³	8
1.5-8 × 10 ³	10
0.5-1.0 × 10 ⁴	3
Incubation temperature	
35°C	19
37°C	2
Incubation duration	
24 hr	14 *
48 hr	10
Endpoint reading	
Visual	12
Spectrophotometric	0
Colorimetric	9
Scoring endpoint¹	
100% inhibition	10 *
95% inhibition	1
80% inhibition	4
50% inhibition	4
Other (color change)	7
QC organism	
NCCLS recommended strains	21
Unknown	0

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

* More than one value reported by individual laboratories

A



B

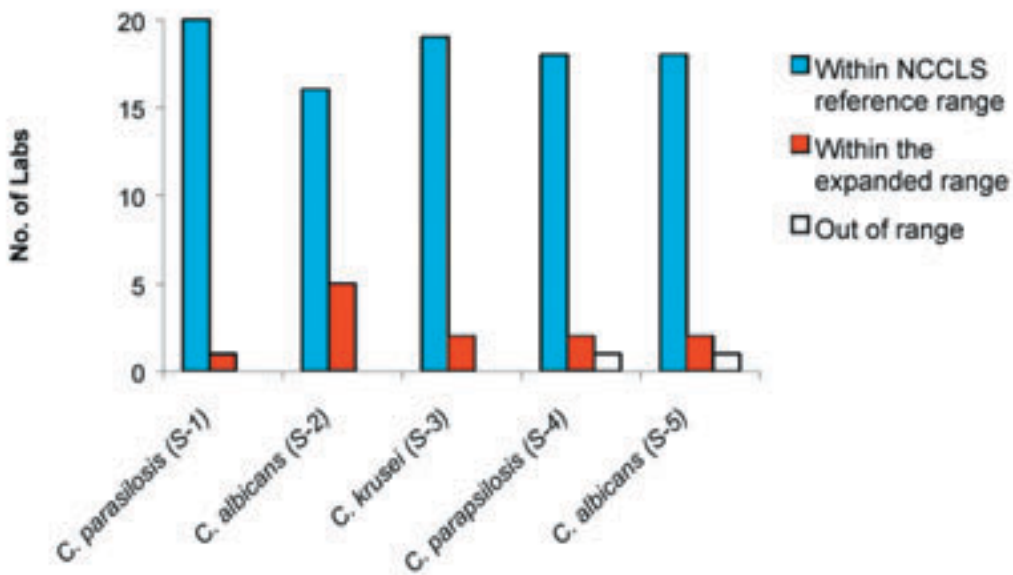


Figure 25. Summary of the results submitted by the participating labs for 5 isolates, for amphotericin B (A) and fluconazole (B).



Figure 1. Seven-day-old, soft smooth colony of *Cryptococcus uniguttulatus* on Sabouraud's dextrose agar.

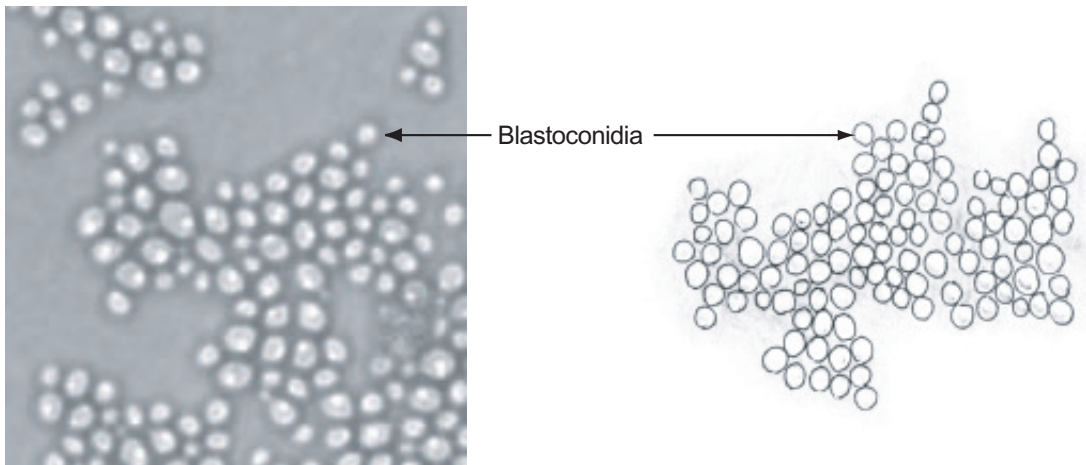


Figure 2. Microscopic morphology of *Cryptococcus uniguttulatus*. On corn meal agar culture, round blastoconidia are seen (left; 200× magnification, right; line drawing not to scale).



Figure 5. Seven-day-old, pale yellow to buff, smooth colony of *Cryptococcus humicolus* on Sabouraud's dextrose agar.

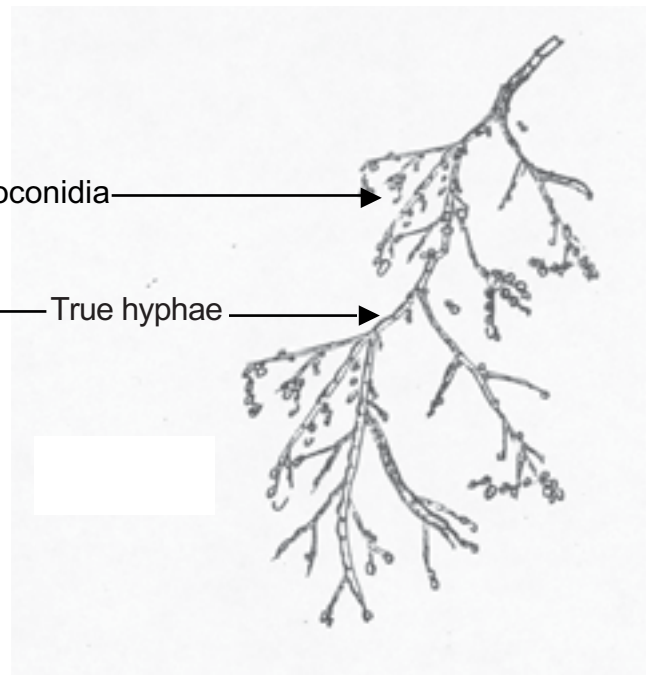
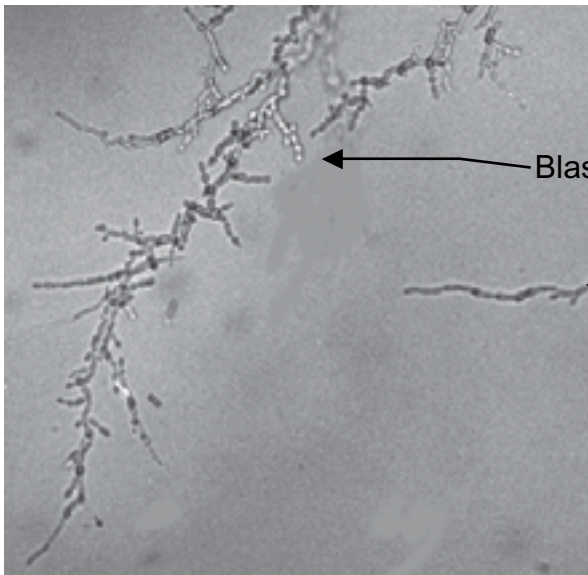


Figure 6. Microscopic morphology of *Cryptococcus humicolus* on corn meal agar showing true hyphae and blastoconidia (left; 100× magnification, right; line drawing not to scale).



Figure 9. Seven-day-old, mucoid, soft colony of *Cryptococcus albidus* on Sabouraud's dextrose agar.

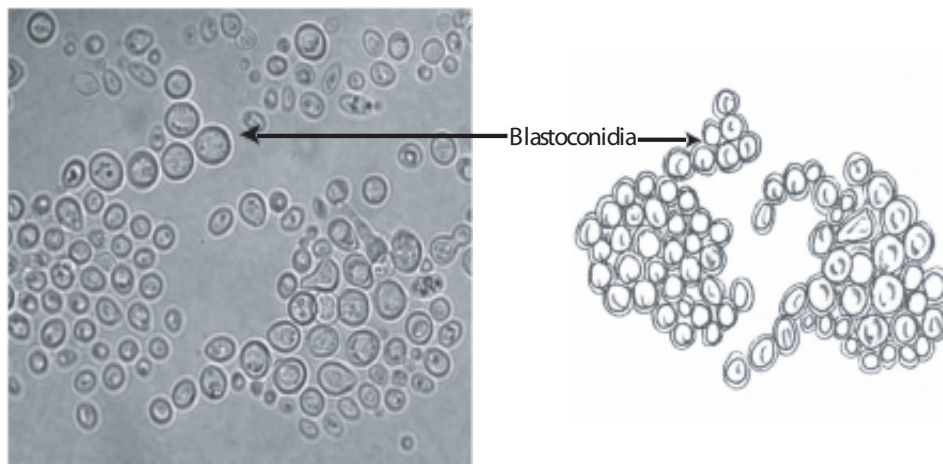


Figure 10. Microscopic morphology of *Cryptococcus albidus*. On corn meal agar culture, large, round blastoconidia are seen (left; 400× magnification, right; line drawing not to scale).



Figure 13. Seven-day-old, creamish white, butyrous, raised colony of *Candida zeylanoides* on Sabouraud's dextrose agar.

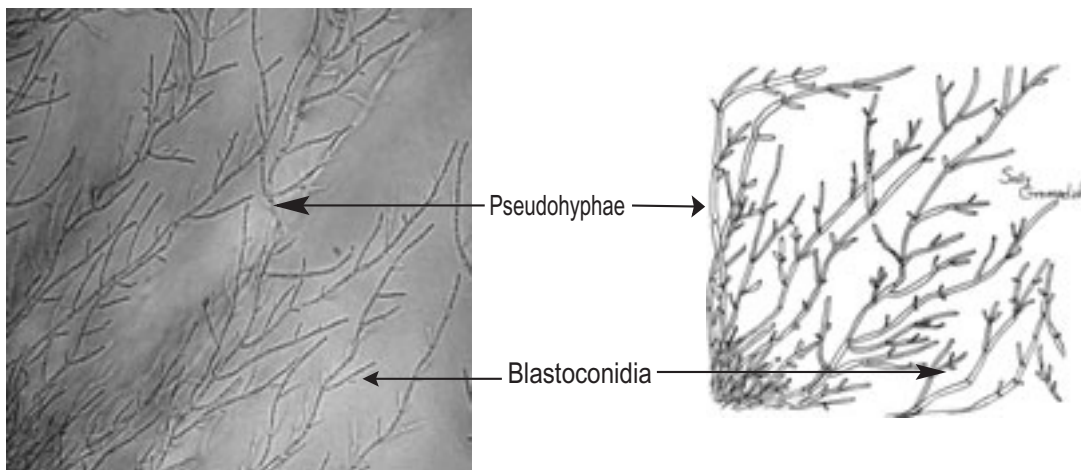


Figure 14. Microscopic morphology of *Candida zeylanoides* on corn meal agar showing long pseudohyphae with verticillate, ovoid blastoconidia (left; 100× magnification, right; line drawing not to scale).



Figure 17. Seven-day-old, white to cream, smooth, and soft colony of *Candida dubliniensis* on Sabouraud's dextrose agar.

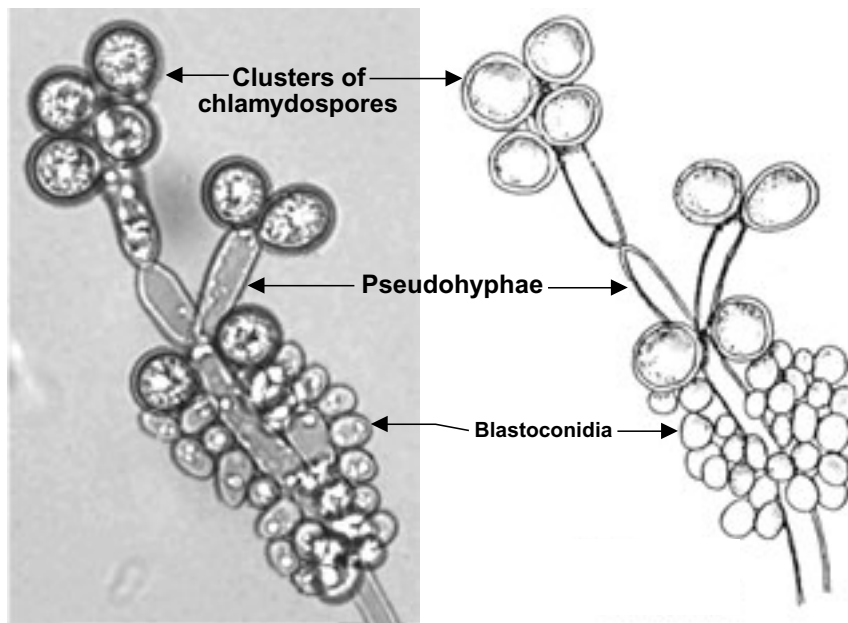


Figure 18. Microscopic morphology of *Candida dubliniensis* on corn meal agar showing clusters of chlamyospores and blastoconidia (left; 400× magnification, right; line drawing not to scale).



Figure 21. Seven-day-old, flat, smooth, cream-yellow colony of *Pichia obmeri* on Sabouraud's dextrose agar

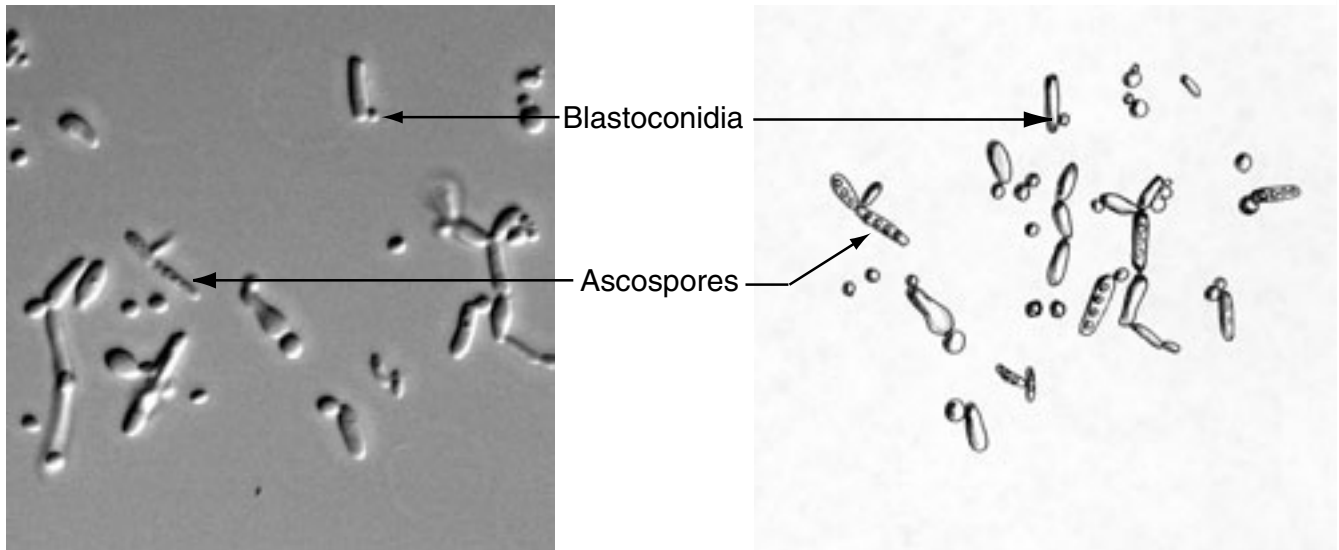


Figure 22. Microscopic morphology of *Pichia obmeri*. On corn meal agar with Tween 80 culture, short pseudohyphae with blastoconidia and some ascospores are seen (left; 200× magnification, right; line drawing not to scale).

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