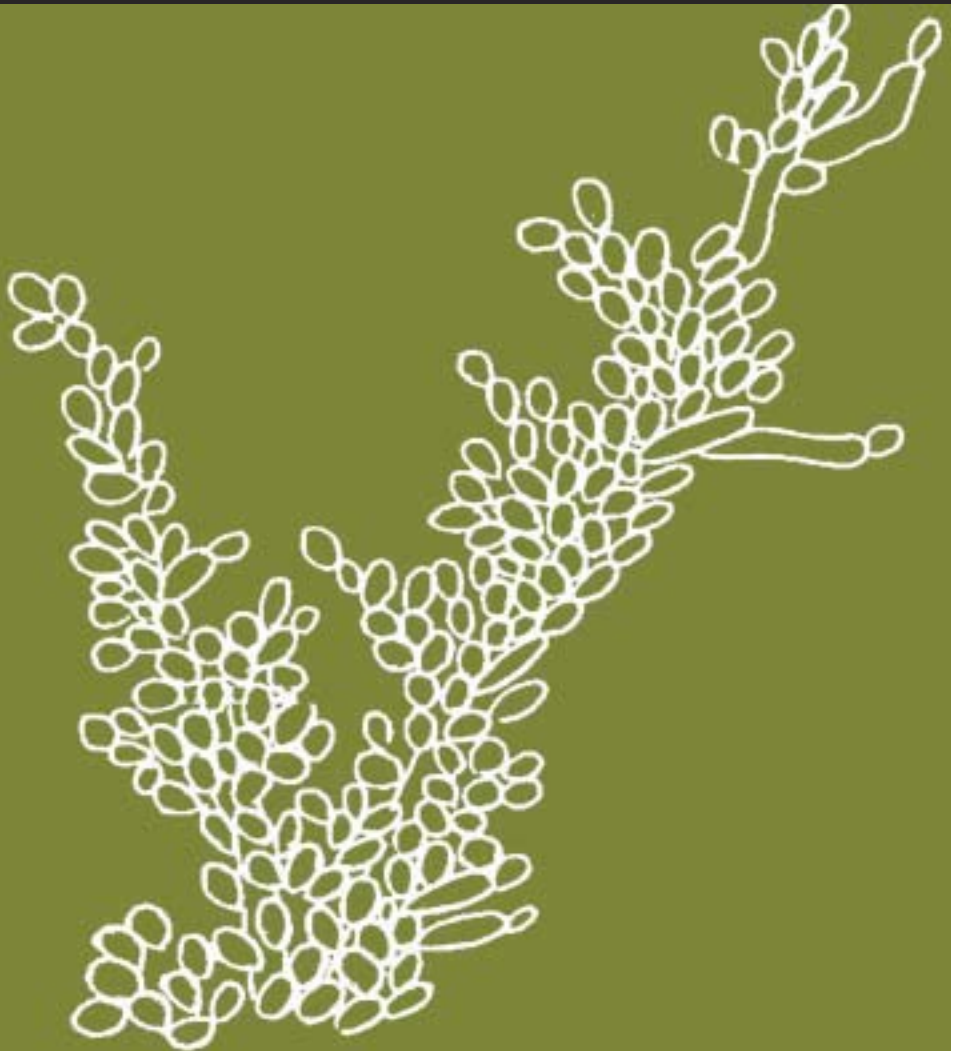


MYCOLOGY CRITIQUÉ

**Mycology Proficiency Testing Program
June 2002**

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 2002. 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 pathogen was used in the test.

Grading Policy

A laboratory's response for each sample is compared with the response that reflects 90 percent agreement of 10 referee laboratories or 90 percent of all participating laboratories. The referee laboratories are selected at random from among hospital laboratories participating in the program. They represent all geographical areas of New York State and must have a record of excellent performance during the preceding three years. The grading formula used for each specimen is:

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

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

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

ANSWER KEY

Mycology - General and Yeast Only

	Specimen Key	Validated Specimen	Acceptable Answers
Y-1	<i>Candida lusitaniae</i>	<i>Candida lusitaniae</i>	
Y-2	<i>Candida kefyr</i>	<i>Candida kefyr</i>	<i>Candida pseudotropicalis</i>
Y-3	<i>Candida norvegensis</i>		
Y-4	<i>Candida albicans</i>	<i>Candida albicans</i>	
Y-5	<i>Candida glabrata</i>	<i>Candida glabrata</i>	<i>Torulopsis glabrata</i>
Ed.sp.	<i>Candida viswanathii</i>		

Mycology - Antifungal Susceptibility Testing for Yeasts

Specimen Key

S-1	<i>Candida tropicalis</i> ATCC 750
S-2	<i>Candida parapsilosis</i> ATCC 90018
S-3	<i>Candida parapsilosis</i> ATCC 22019
S-4	<i>Candida albicans</i> ATCC 24433
S-5	<i>Candida krusei</i> ATCC 6258
Ed.sp.	<i>Cryptococcus neoformans</i>

LABORATORY RESULTS

Mycology - General and Yeast Only

	Correct Responses / Total # Labs (%)	Referees (%)
Y - 1 <i>Candida lusitanae</i>	143/148 (97)	10/10 (100)
Y - 2 <i>Candida kefyr</i>	147/148 (99)	10/10 (100)
Y - 3 <i>Candida norvegensis</i> (Not validated)	98/148 (66)	9/10 (90)
Y - 4 <i>Candida albicans</i>	144/148 (97)	10/10 (100)
Y - 5 <i>Candida glabrata</i>	148/148 (100)	10/10 (100)

Mycology - Antifungal Susceptibility Testing for Yeasts

	Correct Responses / Total # Labs (%)	Correct Responses / Total # Labs (%)
	Amphotericin B	Fluconazole
S- 1 <i>Candida tropicalis</i> ATCC 750	18/18 (100)	18/18 (100)
S- 2 <i>Candida parapsilosis</i> ATCC 90018	18/18 (100)	18/18 (100)
S- 3 <i>Candida parapsilosis</i> ATCC 22019	18/18 (100)	18/18 (100)
S- 4 <i>Candida albicans</i> ATCC 24433	18/18 (100)	18/18 (100)
S- 5 <i>Candida krusei</i> ATCC 6258	18/18 (100)	18/18 (100)

TEST STATISTICS

Mycology - General and Yeast Only

Number of participating laboratories	148
Number of referee laboratories	10
Number of laboratories responding by deadline	148
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	148
Number of laboratories unsuccessfully completing this test	0

Mycology - Antifungal Susceptibility Testing for Yeasts

Number of participating laboratories	18
Number of referee laboratories	3
Number of laboratories responding by deadline	18
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	18
Number of laboratories unsuccessfully completing this test	0

Commercial Identification Systems Used*

AMS Vitek system.....	57
API 20C AUX.....	83
Microscan.....	4
Remel Uni-Yeast-Tek.....	7
Other	3

(* Includes multiple systems used by some labs)

Y-1 CANDIDA LUSITANIAE

Source: Sputum

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	144
Labs with incorrect ID:	4
(<i>Candida guilliermondii</i>)	(2)
(<i>Candida famata</i>)	(2)
(<i>Candida tropicalis</i>)	(1)

Clinical Significance: *Candida lusitaniae* is rarely isolated from clinical specimens. Mainly, it was recovered from blood, urine, and respiratory tract of immunocompromised and debilitated patients such as with cancer, diabetes, asthma, etc. or intensive-cared neonates.

Epidemiology: Infection of *C. lusitaniae* in humans has occurred all over the world.

Ecology: *C. lusitaniae* has been found from water, citrus juice, animal manure, etc.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar, at 25°C for 3 to 5 days, *C. lusitaniae* colony shows white to cream color; smooth and glistening; appears soft (Figure 1).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *C. lusitaniae* produces many short, branched (“bushy”) pseudohyphae. Along the length of the pseudohyphae, elongated blastoconidia form in short chains (Figure 2). The latter could be confused with *C. parapsilosis*.
3. **Differentiation from other yeasts** – *C. lusitaniae* and *C. parapsilosis* are close to each other microscopically and physiologically. Both species are able to grow at 37°C, but not on the media containing cycloheximide. Both are able to assimilate glucose, maltose, sucrose, galactose, and xylose. They do not hydrolyze urea and assimilate nitrate. However, *C. lusitaniae* is able to ferment and assimilate cellobiose, which differentiates it from *C. parapsilosis*.
4. **In vitro susceptibility testing** – *C. lusitaniae* is susceptible to amphotericin B, but the resistant variants may develop during the course of treatment. *C. lusitaniae* also has been reported more susceptible to voriconazole than fluconazole.
5. **Molecular tests** – Specific nucleic acid probes targeting the large subunit rRNA genes have been developed for identification of *C. lusitaniae*. Three pulsed-field electrophoretic methods and a random amplified polymorphic DNA (RAPD) method were also reported to delineate strains of *C. lusitaniae*.

Further Reading:

1. Behar, S.M. and Chertow, G.H. 1998. Olecranon bursitis caused by infection with *Candida lusitaniae*. *J. Rheumatol.* 25: 598-600.
2. Fowler, S.L., Rhoton, B., Springer S.C., Messer S.A., Hollis, R.J., and Pfaller, M.A. 1998. Evidence for person-to-person transmission of *Candida lusitaniae* in a neonatal intensive-care unit. *Infect. Contr. & Hospit. Epidemiol.* 19: 343-345.
3. King, D., Rhine-Chalberg, J., Pfaller, M.A., Moser, S.A., and Merz, W.G. 1995. Comparison of four DNA-based methods for strain delineation of *Candida lusitaniae*. *J. Clin. Microbiol.* 33: 1467-1470.
4. McClenny, N.B., Fei, H., Baron, E.J., Gales, A.C., Houston, A., Hollis, R.J., and Pfaller, M.A. 2002. Change in colony morphology of *Candida lusitaniae* in association with development of amphotericin B resistance. *Antimicrob. Agents Chemother.* 46: 1325-1328.
5. Minari, A., Hachem, R., and Raad, I. 2001. *Candida lusitaniae*: a cause of breakthrough fungemia in cancer patients. *Clin. Infect. Dis.* 32: 186-190.
6. Pelletier, R., Loranger, L., Marcotte, H., and De Carolis, E. 2002. Voriconazole and fluconazole susceptibility of *Candida* isolates. *J. Med. Microbiol.* 51: 479-483.
7. Sandhu, G.S., Kline, B.C., Stockman, L., and Roberts, G.D. 1995. Molecular probes for diagnosis of fungal infections. *J. Clin. Microbiol.* 33: 2913-2919.

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 and ITS2 rDNA. The sequences are deposited in GenBank with the accession numbers AY139787 and AY139788, respectively.

```

1
ATCC 34449 (AF336844)   TCCGTAGGTG AACCTGCGGA AGGATCATTG AAAAATACAT TACACATTGT TTTTGCGAAC AAAAAATAA ATTTTTTTAT
ATCC 200950 (AY139787) TCCGTAGGTG AACCTGCGGA AGGATCATTG AAAAATACAT TACACATTGT TTTTGCGAAC AAAAAATAA ATTTTTTTAT

81
TCGAATTCT TAATATCAA ACTTTCAACA ACGGATCTCT TGGTTCTCGC ATCGATGAAG AACGCAGC
TCGAATTCT TAATATCAA ACTTTCAACA ACGGATCTCT TGGTTCTCGC ATCGATGAAG AACGCAGC

```

Figure 3. Alignment of primary sequences of the ITS1 regions of *C. lusitaniae* ATCC34449 and PT specimen *C. lusitaniae* ATCC 200950. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in brackets.

```

1
ATCC 34449 (AF218970)   GCATCGATGA AGAACGCAGC GAATTGCGAT ACGTAGTATG ACTTGCAGAC GTGAATCATC GAATCTTTGA ACGCACATTG
ATCC 200950 (AY139788) GCATCGATGA AGAACGCAGC GAATTGCGAT ACGTAGTATG ACTTGCAGAC GTGAATCATC GAATCTTTGA ACGCACATTG

81
CGCCTCGAGG CATTCTCGA GGCATGCCTG TTTGAGCGTC GCATCCCCTC TAACCCCGG TTAGGCGTTG CTCGGAATA
CGCCTCGAGG CATTCTCGA GGCATGCCTG TTTGAGCGTC GCATCCCCTC TAACCCCGG TTAGGCGTTG CTCGGAATA

161
TCAACCGCGC TGTCAAACAC GTTTACAGCA CGACATTTG CCCTCAAATC AGGTAGG-AC TACCCGCTGA ACTTAAGCAT
TCAACCGCGC TGTCAAACAC GTTTACAGCA CGACATTTG CCCTCAAATC AGGTAGGGAC TACCCGCTGA ACTTAAGCAT

241
ATCAATAAGC GGAGGA
ATCAATAAGC GGAGGA

```

Figure 4. Alignment of primary sequences of the ITS2 regions of *C. lusitaniae* ATCC34449 and PT specimen *C. lusitaniae* ATCC 200950. Gaps are indicated by dashes. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in brackets.

Comments: Two labs each reported this specimen as *Candida gulliermondii* and *Candida famata*, and one lab identified it as *Candida tropicalis*. *C. lusitaniae* is not able to grow on the media containing cycloheximide, but *C. gulliermondii* does. *C. lusitaniae* grows at 45°C, but *C. famata* does not. *C. lusitaniae* is able to both assimilate and ferment cellobiose, which differentiate it from *C. tropicalis*.

Y-2 CANDIDA KEFYR

Source: Blood

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	147
Labs with incorrect ID:	1
(<i>Saccharomyces cerevisiae</i>)	(1)

Clinical Significance: *Candida kefyri* is a rarely isolated *Candida* species in the clinical laboratory. The infection is reported from the reproductive and digestive tract and the mucous linings.

Ecology: *C. kefyri* is isolated from milk, other dairy products, grains, and some mammals carry this yeast.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar at 25°C for 3 to 5 days, colonies appeared smooth, creamy, and soft (Figure 5).
2. **Microscopic morphology** – On corn meal agar with Tween 80, microscopic morphology of *C. kefyri* shows plenty of long pseudohyphae, and oval to elongated blastoconidia (Figure 6). Ascospores in asci are observed when *C. kefyri* is cultured on V-8 or malt extract agar.
3. **Differentiation from other yeasts** – *C. kefyri* grows at 45°C and on the cycloheximide containing media. *C. kefyri* ferments glucose, sucrose, lactose, galactose, but not maltose, trehalose, and cellobiose, which differentiate it from other medically important *Candida* species .
4. **In vitro susceptibility testing** – *C. kefyri* shows susceptibility to amphotericin B, different azoles, and 5-fluorocytosine.
5. **Molecular tests** – Randomly amplified polymorphic DNA-polymerase chain reaction (RADP-PCR) was applied for the identification of *C. kefyri*.

Further Reading:

1. Andrighetto, C., Psomas, E., Tzanetakis, N., Suzzi, G., and Lombardi, A. 2000. Randomly amplified polymorphic DNA (RAPD) PCR for the identification of yeasts isolated from dairy products. *Lett. Appl. Microbiol.* 30: 5-9.
2. Farina, C., Vailati, F., Manisco, A., and Goglio, A. 1999. Fungaemia survey: a 10-year experience in Bergamo, Italy. *Mycoses* 42: 543-548.
3. Garcia-Martos, P., Dominguez, I., Marin, P., Garcia-Agudo, R., Aoufi, S., and Mira, J. 2001. Antifungal susceptibility of emerging yeast pathogens. *Enferm. Infecc. Microbiol. Clin.* 19: 249-256.
4. Listemann, H., Schulz, K.D., Wasmuth, R., Begemann, F., and Meigel, W. 1998. Oesophagitis caused by *Candida kefyri*. *Mycoses* 41: 343-344.
5. Loeffler, J., Hebart, H., Magga, S., Schmidt, D., Klingspor, L., Tollemar, J., Schumacher, U., and Einsele, H. 2000. Identification of rare *Candida* species and other yeasts by polymerase chain reaction and slot blot hybridization. *Diagnos. Microbiol. Infect. Dis.* 38: 207-212.
6. Maiwald, M., Kappe, R., and Sonntag, H.G. 1994. Rapid presumptive identification of medically relevant yeasts to the species level by polymerase chain reaction and restriction enzyme analysis. *J. Med. Vet. Mycol.* 32: 115-122.
7. Mitrovic, S., Milosevic, D., Dankuc, D., and Jovic, R. 2000. Mycotic disease of the mucous membranes of the head and neck. *Med. Pregl.* 53: 85-88.
8. Neoff, P., Oswald, U., and Hausteil, U.F. 1999. In vitro susceptibility of yeasts for fluconazole and itraconazole. Evaluation of a microdilution test. *Mycoses* 42: 4290639.
9. Sandhu, G.S., Kline, B.C., Stockman, L., and Roberts, G.D. 1995. Molecular probes for diagnosis of fungal infections. *J. Clin. Microbiol.* 33: 2913-2919.

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 and ITS2 rDNA. The sequences are deposited in GenBank with the accession numbers AY139785 and AY139786, respectively.

```

1
UWFP-208 (AF336841) TCCGTAGGTG AACCTGCCGA AGGATCATT AAGATTATGA ATGAATAGAT TACTGGGGGA ATCGTCTGAA CAAGGCCTGC
NYSDOH PFI240601 (AY139785) TCCGTAGGTG AACCTGCCGA AGGATCATT AAGATTATGA ATGAATAGAT TACTGGGGGA ATCGTCTGAA CAAGGCCTGC

81
GCTTAATTGC GCGGCCAGTT CTTGATTCTC TGCTATCAGT TTTCTATTTT TCATCCTAAA CACAATGGAG TTTTTTCTCT
GCTTAATTGC GCGGCCAGTT CTTGATTCTC TGCTATCAGT TTTCTATTTT TCATCCTAAA CACAATGGAG TTTTTTCTCT

161
ATGAACTACT TCCCTGGAGA GCTCGTCTCT CCAGTGGACA TAAACACAAA CAATATTTTG TATTATGAAA AACTATTATA
ATGAACTACT TCCCTGGAGA GCTCGTCTCT CCAGTGGACA TAAACACAAA CAATATTTTG TATTATGAAA AACTATTATA

241
CTATAAAATT TAATATTCAA AACTTTCAAC AACGGATCTC TTGGTTCTCG CATCGATGAA GAACGCAGC
CTATAAAATT TAATATTCAA AACTTTCAAC AACGGATCTC TTGGTTCTCG CATCGATGAA GAACGCAGC

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Figure 7. Alignment of primary sequences of the ITS1 regions of *C. kefyi* UWFP-208 and PT specimen *C. kefyi* NYSDOH PFI 240601. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in brackets.

```

1
UWFP-208 (AF218997) GCATCGATGA AGAACGCAGC GAATTGCGAT ATGTATTGTG AATTGCAGAT TTTCTGTAAT CATCAAATCT TTG-AACGCA
NYSDOH PFI240601 (AY139786) GCATCGATGA AGAACGCAGC GAATTGCGAT ATGTATTGTG AATTGCAGAT --TCGTGAAT CATCAAATCT TTGGAACGCA

81
CATTGCGCCC TCTGGTATTC CAGGGGGCAT GCCTGTTTGA GCGTCATTTT TCTCTCAAA CTTTGGGTTT GGTAGTGAGT
CATTGCGCCC TCTGGTATTC CAGGGGGCAT GCCTGTTTGA GCGTCATTTT TCTCTCAAA CTTTGGGTTT GGTAGTGAGT

161
GATACTCGTC TCGGGTTAAC TTGAAAGTGG CTAGCCGTTG CCATCTGCGT GAGCAGGGCT GCGTGTCAAG TCTATGGACT
GATACTCGTC TCGGGTTAAC TTGAAAGTGG CTAGCCGTTG CCATCTGCGT GAGCAGGGCT GCGTGTCAAG TCTATGGACT

241
CGACTCTTGC ACATCTACGT CTTAGGTTTG CGCCAATTCG TGTAAGCTT GGGTCATAGA GACTCATAGG TGTATAAAG
CGACTCTTGC ACATCTACGT CTTAGGTTTG CGCCAATTCG TGTAAGCTT GGGTCATAGA GACTCATAGG TGTATAAAG

321
ACTCGCTGGT GTTTGTCTCC TTGAGGCATA CGGCTTTAAC CAAAACCTCT AAAGTTTGAC CTCAAATCAG GTAGGAGTAC
ACTCGCTGGT GTTTGTCTCC TTGAGGCATA CGGCTTTAAC CAAAACCTCT AAAGTTTGAC CTCAAATCAG GTAGGAGTAC

401
CCGCTGAACT TAAGCATATC AATAAGCGGA GGA
CCGCTGAACT TAAGCATATC AATAAGCGGA GGA

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Figure 8. Alignment of primary sequences of the ITS2 regions of *C. kefyi* UWFP-208 and PT specimen *C. kefyi* NYSDOH PFI 240601. Gaps are indicated by dashes. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in brackets.

Comments: One participating lab identified this isolate as *Saccharomyces cerevisiae* probably based on the ascospores. However, many long pseudohyphae seen on the corn meal agar with Tween 80 for *C. kefyi*, but almost no pseudohyphae are seen for *S. cerevisiae*. In addition, *C. kefyi* ferments lactose but not maltose unlike *S. cerevisiae*.

Y-3 CANDIDA NORVEGENSIS

Source: Catheter

Scoring:	No. Labs
Referee Labs with correct ID:	9
Labs with correct ID:	98
Labs with incorrect ID:	50
(<i>Blastoschizomyces capitatus</i>)	(3)
(<i>Candida krusei</i>)	(41)
(<i>Candida lambica</i>)	(3)
(<i>Candida lipolytica</i>)	(1)
(<i>Candida zeylanoides</i>)	(1)
(<i>Saccharomyces cerevisiae</i>)	(1)

Clinical Significance: *Candida norvegensis* is infrequently isolated from clinical specimens, and only a few cases of documented from human infections are known. However, there are indications that *C. norvegensis* may be an emerging new pathogen in severely immunocompromised patients.

Ecology: *C. norvegensis* is found on fruits, dairy products, and humans.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar at 25°C for 3 to 5 days, colonies are white to cream, dull with smooth surface (Figure 9).
2. **Microscopic morphology** – On corn meal agar with Tween 80, simple pseudohyphae with blastoconidia are seen (Figure 10).
3. **Differentiation from other yeasts** – *C. norvegensis* only ferments glucose, but not maltose, sucrose, lactose and trehalose. It does not grow on the media containing cycloheximide, which differentiates it from *Candida lipolytica*, *Geotrichum capitatum* and *G. candidum*. It does not assimilate sucrose, differentiating it from *C. parapsilosis* and *C. lusitaniae*. As API 20C AUX product insert indicates *C. norvegensis* is positive on esculin hydrolysis test, which differentiates it from *C. krusei*.
4. **In vitro susceptibility testing** – Clinical isolates are susceptible to amphotericin B but resistant to fluconazole.
5. **Molecular tests** – *C. norvegensis* was differentiated from *C. krusei*, and *C. inconspicua* by restriction analysis of PCR amplified internally transcribed spacer (ITS) regions and 5.8 S rRNA gene.

Further Reading:

1. Bouakline, A., Lacroix, C., Roux, N., Gangneux, J.P., and Derouin, F. 2000. Fungal contamination of food in hematology units. *J. Clin. Microbiol.* 38: 4272-4273.
2. Hood, S.V., Moore, C.B., and Denning, D.W. 1996. Isolation of *Candida norvegensis* from clinical specimens: four case reports. *Clin. Infect. Dis.* 23: 1185-1187.
3. Krcmery, V.Jr., Oravcova, E., Spanik, S., Mrazova-Studena, M., Trupl, J., Kunova, A., Stoplova-Grey, K., Kukuckova, E., Krupova, I., Demitrovicova, A., and Kralovicova, K. 1998. Nosocomial breakthrough fungaemia during antifungal prophylaxis or empirical antifungal therapy in 41 cancer patients receiving antineoplastic chemotherapy: analysis of aetiology risk factors and outcome. *J. Antimicrob. Chemother.* 41: 373-380.
4. Nho, S., Anderson, M.J., Moore, C.B., and Denning, D.W. 1997. Species differentiation by internally transcribed spacer PCR and HhaI digestion of fluconazole-resistant *Candida krusei*, *Candida inconspicua*, and *Candida norvegensis* strains. *J. Clin. Microbiol.* 35: 1036-1039.
5. Nielsen, H. and Stenderup, J. 1996. Invasive *Candida norvegensis* infection in immunocompromised patients. *Scand. J. Infect. Dis.* 28:311-312.
6. Nielsen, H., Stenderup, J., Bruun, B., and Ladefoged, J. 1990. *Candida norvegensis* peritonitis and invasive disease in a patient on continuous ambulatory peritoneal dialysis. *J. Clin. Microbiol.* 28: 1664-1665.
7. Nolla-Salas, J., Torres-Rodriguez, J.M., Grau, S., Isbert, F., Torrella, T., Riveiro, M., and Sitges-Serra, A. 2000. Successful treatment with liposomal amphotericin B of an intraabdominal abscess due to *Candida norvegensis* associated with a Gore-Tex mesh infection. *Scand. J. Infect. Dis.* 32: 560-562.
8. Sandven, P., Nilsen, K., Digranes, A., Tjade, T., and Lassen, J. 1997. *Candida norvegensis*: a fluconazole-resistant species. *Antimicrob. Agents Chemother.* 41:1375-1376.

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS2 rDNA. The sequence is deposited in GenBank with the accession numbers AY139790.

```

1
ATCC 22977 (FNU70509)
NYSDOH 83MR168 (AY139790)
GCATCGATGA AGAACGCAGC GAAATGCGAT ACCTAGTGTG AATTGCAGCC ATCGTGAATC ATCGAGTTCT TGAACGCACA
81
CCT TCTTGCACAA GCAGAAGTTG GGGTTGCCAC
TTGCGCCCTC CGGCATTCCG GGGGGCATGC CTGTTTGAGC GTCGTTTCCT TCTTGCACAA GCAGA-GTTG GGGTTGCCAC
161
GGCCCGTGCG GCCTGTGTGT GGCTCCCC-G AAACGGAACG GCAGCGGGAC TGAGCGAAGT ACACAACACT CGCGCTTGGC
GGCCCGTGCG GCCTGTGTGT GGCTCCCCG AAACGGAACG GCAGCGGGAC TGAGCGAAGT ACACAACACT CGCGCTTGGC
241
CGCCCGAACT TTTTTT--A ATCTAAG
CGCCCGAACT TTTTTTTAA ATCTAAGCTC GACCTCAAT CAGGTAGGAA TACCCGCTGA ACTTAAGCAT ATCAATAAGC
321
GGAGGA

```

Figure 11. Alignment of primary sequences of the ITS2 regions of *C. norvegensis* and PT specimen *C. norvegensis* NYSDOH 83MR168. Gaps are indicated by dashes. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in brackets.

Comments: This specimen was not validated. Forty-one out of fifty labs with the incorrect responses for this specimen identified it as *C. krusei*. Three of the participating labs reported this isolate as *B. capitatus*, or *C. lambica*. One lab each identified it as *C. lipolytica*, *C. zeylanoides*, or *Saccharomyces cerevisiae*. In the API 20C AUX yeast identification database, this organism has the same biocode as *G. capitatum*, *C. krusei/inconspicua*, *C. lambica*, and *C. lipolytica*. Additional tests are recommended by the manufacturer to differentiate among these organisms, such as esculin hydrolysis test, which differentiates *C. norvegensis* (positive) from *C. krusei* and *C. lambica* (negative). *B. capitatus* produces arthroconidia on corn meal agar, *C. zeylanoides* and *C. lipolytica* grow on the media with cycloheximide, however *C. norvegensis* neither produces arthroconidia nor grows on media with cycloheximide. *S. cerevisiae* does not form any pseudohyphae on the corn meal agar with Tween 80.

Y-4 CANDIDA ALBICANS

Source: Bronchial lavage

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	144
Labs with incorrect ID:	4
(<i>Candida dubliniensis</i>)	(4)

Clinical Significance: *Candida albicans* is the most common cause of candidiasis. It is ubiquitous in humans, who probably encounter initial infection during passage through the birth canal. The infection is generally seen in immunocompromised patients.

Ecology: *C. albicans* is found as a commensal on humans and a number of other mammals. Also found on leaves, flowers, water, and soil.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar at 25°C for 3 to 5 days, colonies are white to cream, glossy, smooth and soft (Figure 12).
2. **Microscopic morphology** – On corn meal agar with Tween 80, round blastoconidia bunched together with pseudohyphae are easily seen. Thick walled, mostly terminal chlamydoconidia are prominent (Figure 13).
3. **Differentiation from other yeasts** – On morphology, *C. albicans* is difficult to distinguish from *C. dubliniensis* and *C. stellatoidea*. However, *C. albicans* grows well at 42°C, but *C. dubliniensis* grows poorly or not at all at 42°C. *C. albicans* is able to assimilate sucrose, but not *C. stellatoidea*. *C. albicans* has the positive germ tube test and chlamydoconidia which are the important characteristics to differentiate it from *C. tropicalis*. *C. albicans* ferments glucose and maltose and grows on the media containing cycloheximide. It gives negative reactions with urease and nitrate.
4. **In vitro susceptibility testing** – Both fluconazole-resistant and -sensitive isolates of *C. albicans* are reported.
5. **Molecular tests** – Many molecular tests are available for identification of *C. albicans* such as PCR fingerprinting, random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLPs), etc. Combination of RFLPs generated by different restriction digestion of the PCR products of the V3 region of the 25S rDNA gene (rDNA) or from ITS were reported to be able to differentiate *Candida albicans* subgroups, *C. dubliniensis* and *C. stellatoidea*.

Further Reading:

1. Bartie, K.L., Williams, D.W., Wilson, M.J., Potts, A.J., and Lewis, M.A. 2001. PCR fingerprinting of *Candida albicans* associated with chronic hyperplastic candidosis and other oral conditions. *J. Clin. Microbiol.* 39: 4066-4075.
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8. Sato, Y., Aoyagi, T., Kobayashi, T., and Inoue, J. 2001. Occurrences of candidiasis in a Fisher's lovebird and a budgerigar. *J. Vet. Med. Sci.* 63: 939-941.
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10. Tamura, M., Watanabe, K., Mikami, Y., Yacawa, K., and Nishimura, K. 2001. Molecular characterization of new clinical isolates of *Candida albicans* and *C. dubliniensis* in Japan: analysis reveals a new genotype of *C. albicans* with group I intron. *J. Clin. Microbiol.* 39: 4309-4315.

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 and ITS2 rDNA. The sequences are deposited in GenBank with the accession numbers AY139781 and AY139782, respectively.

```

UWFP-60 (AF336832)      1
ATCC 24433 (AY139781)  TCCGTAGGTG AACCTGCGGA AGGATCATTG CTGATTGCTT TAATTGCACC ACATGTGTTT TTCTTTGAAA CAAACTTGCT
                        80
                        81
                        TTGGCGGTGG GCCCAGCCTG CCGCCAGAGG TCTAAACTTA CAACCAATTT TTTATCAACT TGTCACACCA GATTATTACT
                        160
                        TTGGCGGTGG GCCCAGCCTG CCGCCAGAGG TCTAAACTTA CAACCAATTT TTTATCAACT TGTCACACCA GATTATTACT

UWFP-60 (AF336832)      161
ATCC 24433 (AY139781)  AATAGTCAAA ACTTTCAACA ACGGATCTCT TGGTTCTCGC ATCGATGAAG AACGCAGC
                        320
                        AATAGTCAAA ACTTTCAACA ACGGATCTCT TGGTTCTCGC ATCGATGAAG AACGCAGC

```

Figure 14. Alignment of primary sequences of the ITS1 regions of *C. albicans* UWFP-60 and PT specimen *C. albicans* ATCC24433. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in brackets.

```

UWFP-60 (AF335964)      1
ATCC 24433 (AY139782)  GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATATG AATTGCAGAT ATTCGTGAAT CATCGAATCT TTGAACGCAC
                        80
                        GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATATG AATTGCAGAT ATTCGTGAAT CATCGAATCT TTGAACGCAC

UWFP-60 (AF335964)      81
ATCC 24433 (AY139782)  ATTGCGCCCT CTGGTATTCC GGAGGGCATG CCTGTTTGGG CGTCGTTTCT CCCTCAAACC GCTGGGTTTG GTGTTGAGCA
                        160
                        ATTGCGCCCT CTGGTATTCC GGAGGGCATG CCTGTTTGGG CGTCGTTTCT CCCTCAAACC GCTGGGTTTG GTGTTGAGCA

UWFP-60 (AF335964)      161
ATCC 24433 (AY139782)  ATACGACTTG GGTTTGCTTG AAAGACGGTA GTGGTAAGGC GGGATCGCTT TGACAAATGGC TTAGGTCTAA CCAAAAACAT
                        240
                        ATACGACTTG GGTTTGCTTG AAAGACGGTA GTGGTAAGGC GGGATCGCTT TGACAAATGGC TTAGGTCTAA CCAAAAACAT

UWFP-60 (AF335964)      241
ATCC 24433 (AY139782)  TGCTTGCGGC GGTAACGTCC ACCACGTATA TCTTCAAAC TTAGCCTCAA ATCAGGTAGG ACTACCCGCT GAACCTAAGC
                        320
                        TGCTTGCGGC GGTAACGTCC ACCACGTATA TCTTCAAAC TTAGCCTCAA ATCAGGTAGG ACTACCCGCT GAACCTAAGC

UWFP-60 (AF335964)      321
ATCC 24433 (AY139782)  ATATCAATAA GCGGAGGA
                        340
                        ATATCAATAA GCGGAGGA

```

Figure 15. Alignment of primary sequences of the ITS2 regions of *C. albicans* UWFP-60 and PT specimen *C. albicans* ATCC24433. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in brackets.

Comments: The prominent difference between *C. albicans* and *C. dubliniensis* is that *C. albicans* grows well at 42°C and up to 45°C, but *C. dubliniensis* grows poorly or not at all at 42°C. Three of the four labs which reported this isolates as *Candida dubliniensis* knew this difference and indeed, performed the growth tests at 42°C. Unfortunately, their test somehow yielded false negative results. Most recent report by Kim *et al.* (2002) found that only *C. albicans* could generate germ tubes in YPD at 39°C but not other *Candida* species. This result might also be another approach one can apply for the identification of *C. albicans* in clinical laboratories as long as this feature is consistently seen in clinical isolates.

Y-5 CANDIDA GLABRATA

Source: Urine

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	148
Labs with incorrect ID:	0

Clinical Significance: Incidence of candidiasis caused by *Candida glabrata* has increased in immunosuppressed patients due to more intensive anticancer chemotherapy, marrow, and organ transplantations, etc. Urinary tract infections and vaginitis are the other most common infections caused by *C. glabrata*.

Ecology: Humans, lower mammals, and birds are the carriers of *C. glabrata*.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar at 25°C for 3 to 5 days, colony is white to cream, smooth and soft, shiny (Figure 16).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *C. glabrata* blastoconidia are tiny, round or elliptical shaped (Figure 17).
3. **Differentiation from other yeasts** – *C. glabrata* grows at 42°C but does not grow on media containing cycloheximide. It ferments glucose and trehalose. *C. glabrata* forms only blastoconidia and no pseudohyphae or true hyphae.
4. **In vitro susceptibility testing** – *C. glabrata* is susceptible to amphotericin B and 5FC but resistant to azoles like fluconazole and itraconazole.
5. **Molecular tests** – PCR amplification of a mitochondrial rRNA gene fragment, which is species specific, was developed to identify *C. glabrata*. Diversity of karyotype by pulse-field gel electrophoresis was used to confirm *C. glabrata* infection. Comparative sequence analysis of cytochrome oxidase gene has been reported for typing of *C. glabrata*.

Further Reading:

1. Becker, K., Badehorn, D., Keller, B., Schulte, M., Bohm, K.H., Peters, G., and Fegeler, W. 2001. Isolation and characterization of a species-specific DNA fragment for identification of *Candida (Torulopsis) glabrata* by PCR. *J. Clin. Microbiol.* 39: 3356-3359.
2. Fairchild, K.D., Tomkoria, S., Sharp, E.C., and Mena, F.V. 2002. Neonatal *Candida glabrata* sepsis: clinical and laboratory features compared with other *Candida* species. *Pediatr. Infect. Dis.* 21: 39-43.
3. Fodor, E., Dosa, E., Nagy, A., Nagy, E., and Ferenczy, L. 2002. Karyotyping of *Candida albicans* and *Candida glabrata* isolates from recurrent vaginal infections by pulsed-field gel electrophoresis. *Acta. Microbiol. Immunol. Hung.* 49: 59-68.
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6. Sanson, G.F., and Briones, M.R. 2000. Typing of *Candida glabrata* in clinical isolates by comparative sequence analysis of the cytochrome oxidase subunit 2 gene distinguishes two clusters of strains associated with geographical sequence polymorphisms. *J. Clin. Microbiol.* 38: 227-235.

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 and ITS2 rDNA. The sequences are deposited in GenBank with the accession numbers AY139783 and AY139784, respectively.

```

ATCC 2001 (AF336836)          1
NYSDOH 181-89 (AY139783)    TCCGTAGGTG AACCTGCGGA AGGATCATT AAGAAATTTA ATTGATTTGT CTGAGCTCGG AGAGAGACAT CTCTGGGGAG
                                TCCGTAGGTG AACCTGCGGA AGGATCATT AAGAAATTTA ATTGATTTGT CTGAGCTCGG AGAGAGACAT CTCTGGGGAG

81
GACCAAGTGA GACACTCAGG AGGCTCCTAA AATATTTTCT CTGCTGTGAA TGCTATTTCT CCTGCCTCGG CTTAAGTGCG
GACCAAGTGA GACACTCAGG AGGCTCCTAA AATATTTTCT CTGCTGTGAA TGCTATTTCT CCTGCCTCGG CTTAAGTGCG

161
CGGTTGGTGG GTGTTCTGCA GTGGGGGGAG GGAGCCGACA AAGACCTGGG AGTGTGCGTG GATCTCTCTA TTCCAAGGA
CGGTTGGTGG GTGTTCTGCA GTGGGGGGAG GGAGCCGACA AAGACCTGGG AGTGTGCGTG GATCTCTCTA TTCCAAGGA

241
GGTGTTTTAT CACACGACTC GACACTTTCT AATTACTACA CACAGTGGAG TTTACTTTAC TACTATTCTT TTGTTCGGTTG
GGTGTTTTAT CACACGACTC GACACTTTCT AATTACTACA CACAGTGGAG TTTACTTTAC TACTATTCTT TTGTTCGGTTG

321
GGGGAACGCT CTCTTTCGGG GGGGAGTTCT CCCAGTGGAT GCAAACACAA ACAATATTT TTTTAAACTA ATTCAGTCAA
GGGGAACGCT CTCTTTCGGG GGGGAGTTCT CCCAGTGGAT GCAAACACAA ACAATATTT TTTTAAACTA ATTCAGTCAA

401
CACAAAGATT CTTTTAGTAG AAAACAACCT CAAAACCTTC AACAATGGAT CTCTTGGTTC TCGCATCGAT GAAGAACGCA
CACAAAGATT CTTTTAGTAG AAAACAACCT CAAAACCTTC AACAATGGAT CTCTTGGTTC TCGCATCGAT GAAGAACGCA

481
GC
GC
GC

```

Figure 18. Alignment of primary sequences of the ITS1 regions of *C. glabrata* ATCC2001 and PT specimen *C. glabrata* NYSDOH 181-89. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in brackets.

```

UWFP-116 (AF219008)          1
NYSDOH 181-89 (AY139784)    GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATGTG AATTGCAGAA TTCCGTGAAT CATCGAATCT TTGAACGCAC
                                GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATGTG AATTGCAGAA TTCCGTGAAT CATCGAATCT TTGAACGCAC

81
ATTGCGCCCT CTGGTATTCC GGGGGGCATG CCTGTTGAG CGTCATTCC TTCTCAAACA CGTTGTGTTT GGTAGTGAGT
ATTGCGCCCT CTGGTATTCC GGGGGGCATG CCTGTTGAG CGTCATTCC TTCTCAAACA CGTTGTGTTT GGTAGTGAGT

161
GATACTCTCG TTTTGTAGTT AACTTGAAAT TGTAGGCCAT ATCAGTATGT GGGACACGAG CGCAAGCTTC TCTATTAATC
GATACTCTCG TTTTGTAGTT AACTTGAAAT TGTAGGCCAT ATCAGTATGT GGGACACGAG CGCAAGCTTC TCTATTAATC

241
TGCTGCTCGT TTGCGCGAGC GGCGGGGGTT AATACTGTAT TAGGTTTAC CAACTCGGTG TTGATCTAGG GAGGGATAAG
TGCTGCTCGT TTGCGCGAGC GGCGGGGGTT AATACTGTAT TAGGTTTAC CAACTCGGTG TTGATCTAGG GAGGGATAAG

321
TGAGTGTCTT GTGCGTGCTG GGCAGACAGA CGTCTTAAAG TTTGACCTCA AATCAGGTAG GGTACCCGC TGAACCTAAG
TGAGTGTCTT GTGCGTGCTG GGCAGACAGA CGTCTTAAAG TTTGACCTCA AATCAGGTAG GGTACCCGC TGAACCTAAG

401
CATATCAATA AGCGGAGGA
CATATCAATA AGCGGAGGA

```

Figure 19. Alignment of primary sequences of the ITS2 regions of *C. glabrata* UWFP-116 and PT specimen *C. glabrata* NYSDOH 181-89. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in brackets.

Comments: The test isolate was sent earlier as an educational specimen in May 2001 PT Event. At that time, 11 out of 150 labs could not identify it. All the participating labs had the correct answers for this specimen in the resent test event.

ED. SP. *CANDIDA VISWANATHII*

Source: CSF

Referee Labs with correct ID:	1
Labs with correct ID:	15
Labs with no ID:	2
Labs with incorrect ID:	131
(<i>Candida tropicalis</i>)	(98)
(<i>Candida parapsilosis</i>)	(12)
(<i>Candida</i> species)	(6)
(<i>Candida lusitanae</i>)	(5)
(<i>Candida famata</i>)	(3)
(<i>Candida albicans</i>)	(2)
(<i>Candida krusei</i>)	(1)
(<i>Candida lipolytica</i>)	(1)
(<i>Candida stellatoidea</i>)	(1)
(<i>Candida zeylanoides</i>)	(1)
(<i>Trichosporon beigelii</i>)	(1)

Clinical Significance: *C. viswanathii* is rarely isolated from clinical specimens. Only a few case reports exist on its recovery from the cerebrospinal fluid or sputum of patients with meningitis.

Ecology: *C. viswanathii* was found in the sputum and CSF from meningitis patient in India, and shrimp from Gulf of Mexico.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar at 25°C for 3 to 5 days, colonies are white or cream, a little wrinkled, moist (Figure 20).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *C. viswanathii* forms long pseudohyphae and elongated, oval shaped blastoconidia. The truncated scar can be observed at the attached site (Figure 21).
3. **Differentiation from other yeasts** – *C. viswanathii* grows on the media containing cycloheximide and grows well at 37°C. It ferments glucose and maltose, and many other physiological characteristics are very similar to *C. albicans* and *C. tropicalis*. However, on microscopic morphology, *C. viswanathii* has the long pseudohyphae with elongated oval shaped conidia with truncate scars, which differentiates *C. viswanathii* from *C. tropicalis*, and also no chlamydospore and germ tube differentiates it from *C. albicans*.
4. **In vitro susceptibility testing** – No information available.
5. **Molecular tests** – DNA hybridization and electrokaryotype or restriction enzyme analysis of PCR products obtained from the gene coding for the small ribosomal subunit 18S-rRNA were applied for differentiation of *C. viswanathii* from other medically relevant yeasts.

Further Reading:

1. Lee, F.L., Fu, H.M., and Hsu, W.H. 1998. DNA hybridization and electrokaryotype study of some *Candida* species. *Int. J. Syst. Bacteriol.* 48: 1463-1466.
2. Maiwald, M., Kappe, R., and Sonntag, H.G. 1994. Rapid presumptive identification of medically relevant yeasts to the species level by polymerase chain reaction and restriction enzyme analysis. *J. Med. Vet. Mycol.* 32: 115-122.
3. Quindos, G., Lipperheide, V., and Ponton, J. 1993. Evaluation of two commercialized systems for the rapid identification of medically important yeasts. *Mycoses* 36: 299-303.
4. Sandhu, D.K., Sandhu, R.S., and Misra, V.C. 1976. Isolation of *Candida viswanathii* from cerebrospinal fluid. *Sabouraudia* 14: 251-254.

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS2 rDNA. The sequence is deposited in GenBank with the accession numbers AY139792.

```

1
ATCC 22981 (U70510)
ATCC 22981 (AY139792)
CTCCCTCAA CCCGCGGGTT
GAATGAACGC ACATTGCGCC CTTTGGTATT CAAAAGGGCA TGCCTGTTTG AGCGTCATT CTCCCTCAA CCCGCGGGTT

81
TGGTGGTTGAG CAATACGCCA GGGTTGGTTG AAAGACGTAC GTGGAGACCA TATTAGCGAC TTAGGTTCTA CAAAACGCT
TGGTGGTTGAG CAATACGCCA GGGTTGGTTG AAAGACGTAC GTGGAGACCA TATTAGCGAC TTAGGTTCTA CAAAACGCT

161
TGTGCAGTCG GCCCACACA -CAGTGTAA CTAACA
TGTGCAGTCG GCCCACACA GCTTTGTAA CTTTGACCT CAAATCAGGT AGGACTACCC GCTGAACCTA AGCATATCAA

241
TAAGCGGAGG A

```

Figure 22. Alignment of primary sequences of the ITS2 regions of *C. viswanathii* ATCC 22981 and PT specimen *C. viswanathii* ATCC 22981. Gaps are indicated by dashes. Unmatched nucleotide bases are shaded. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in brackets.

Comments: There is no bicode for *C. viswanathii* in the API 20C AUX database. *C. viswanathii* grows on the media containing cycloheximide, differentiating it from *C. parapsilosis*, *C. lusitaniae*, *C. krusei*, *C. stellatoidea*, and *C. zeylanoides*. *C. viswanathii* ferments glucose, but *C. lipolytica* does not. *Trichosporon beigelii* produces arthroconidia and urease positive, but *C. viswanathii* does not. *C. viswanathii* is different from *C. albicans* by not having chlamydospores and germ tube test negative. *C. viswanathii* has prominent truncated scars on the blastoconidia differentiate it from other physiologically closed yeasts include *C. tropicalis*.

Y-1 *CANDIDA LUSITANIAE*



Figure 1. Four-day-old, white, smooth colony of *Candida lusitaniae* on Sabouraud's dextrose agar.

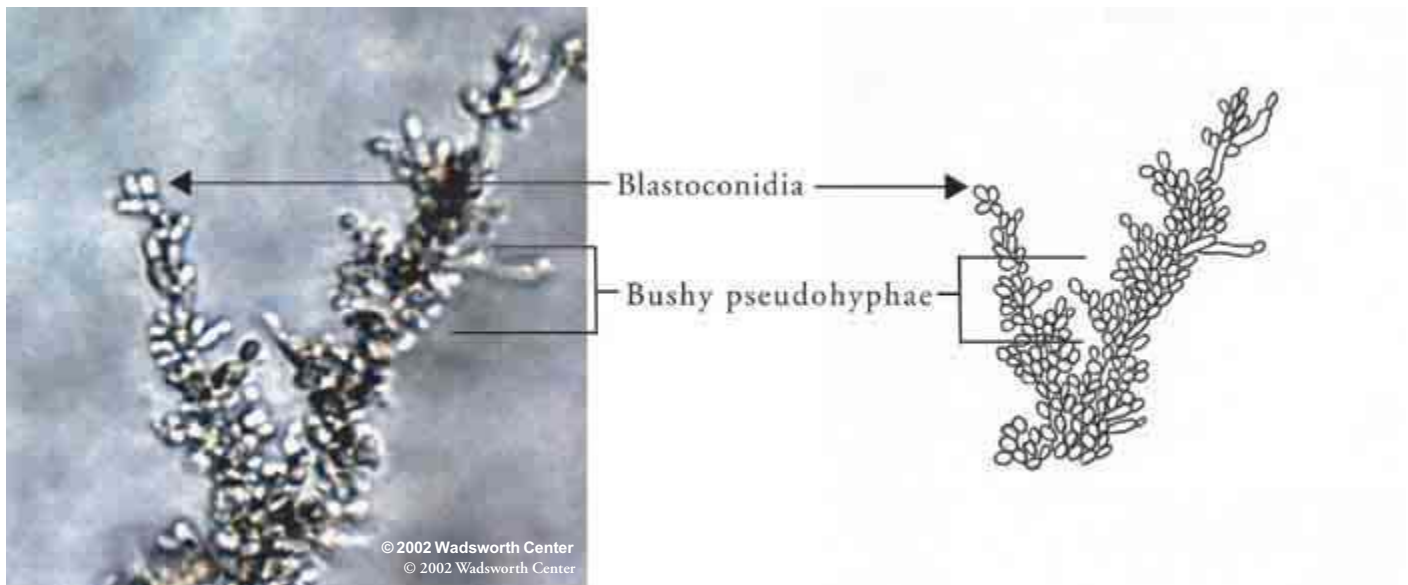


Figure 2. Microscopic morphology of *Candida lusitaniae* on corn meal agar with Tween 80 shows bushy pseudohyphae and blastoconidia (left; 400 X magnification, right; line diagram not to scale).

Y-2 CANDIDA KEFYR



Figure 5. Four-day-old, cream, smooth, and soft colony of *Candida kefyra* on Sabouraud's dextrose agar.



Pseudohyphae

Blastoconidia

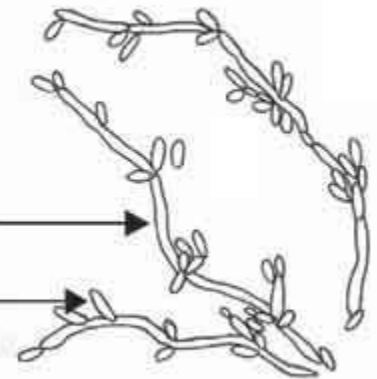


Figure 6. Microscopic morphology of *Candida kefyra* on corn meal agar with Tween 80 shows long pseudohyphae with oval to elongated blastoconidia (left; 400 X magnification, right; line diagram not to scale).

Y-3 CANDIDA NORVEGENSIS



Figure 9. Four-day-old, cream, smooth colony of *Candida norvegenis* on Sabouraud's dextrose agar.

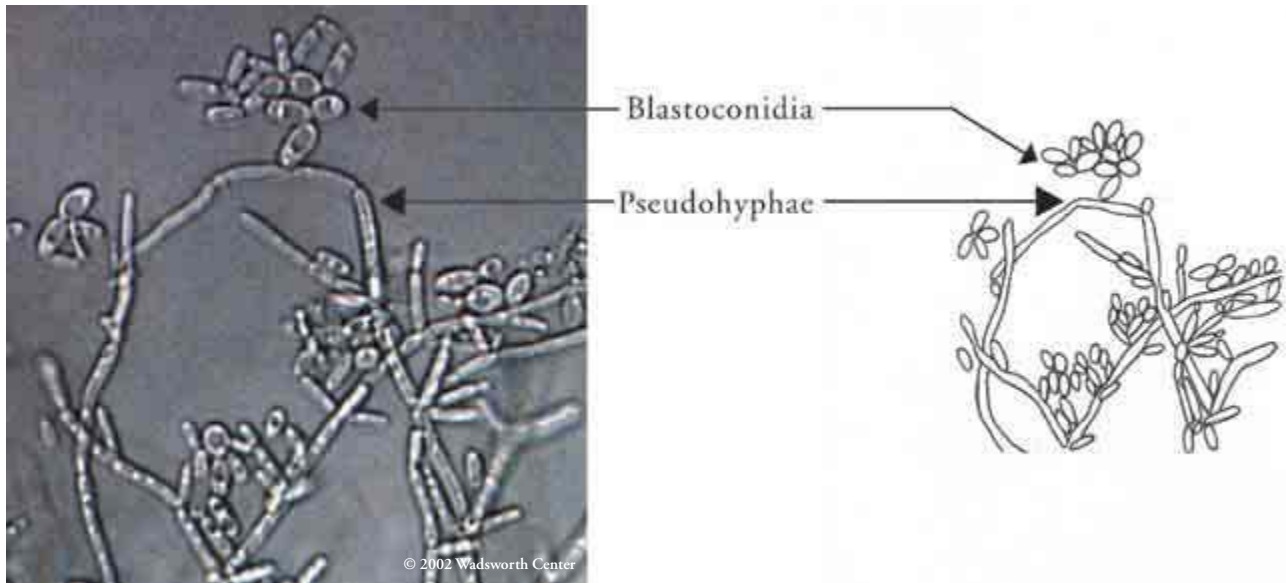


Figure 10. Microscopic morphology of *Candida norvegenis* on corn meal agar with Tween 80 shows simple pseudohyphae with blastoconidia (left; 400 X magnification, right; line diagram not to scale).

Y-4 *CANDIDA ALBICANS*



Figure 12. Four-day-old, white, glossy, and smooth colony of *Candida albicans* on Sabouraud's dextrose agar.

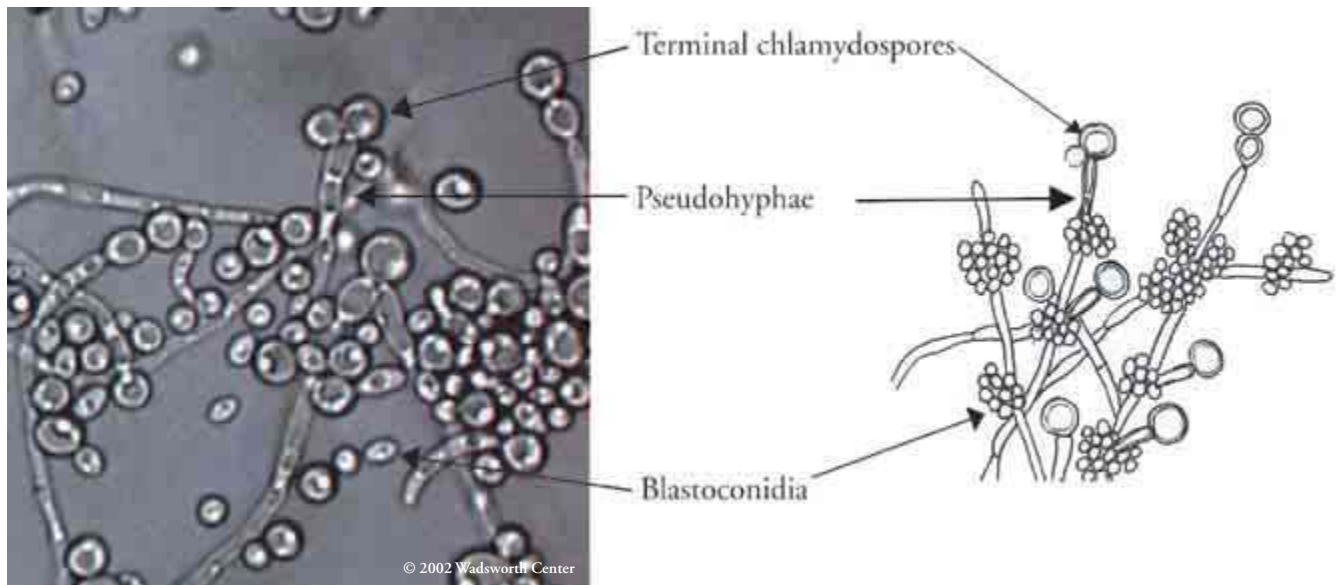


Figure 13. Microscopic morphology of *Candida albicans* on corn meal agar with Tween 80 shows terminal chlamydoconidia on pseudohyphae with blastoconidia (left; 400 X magnification, right; line diagram not to scale).

Y-5 CANDIDA GLABRATA



Figure 16. Four-day-old, white and shiny colony of *Candida glabrata* on Sabouraud's dextrose agar.

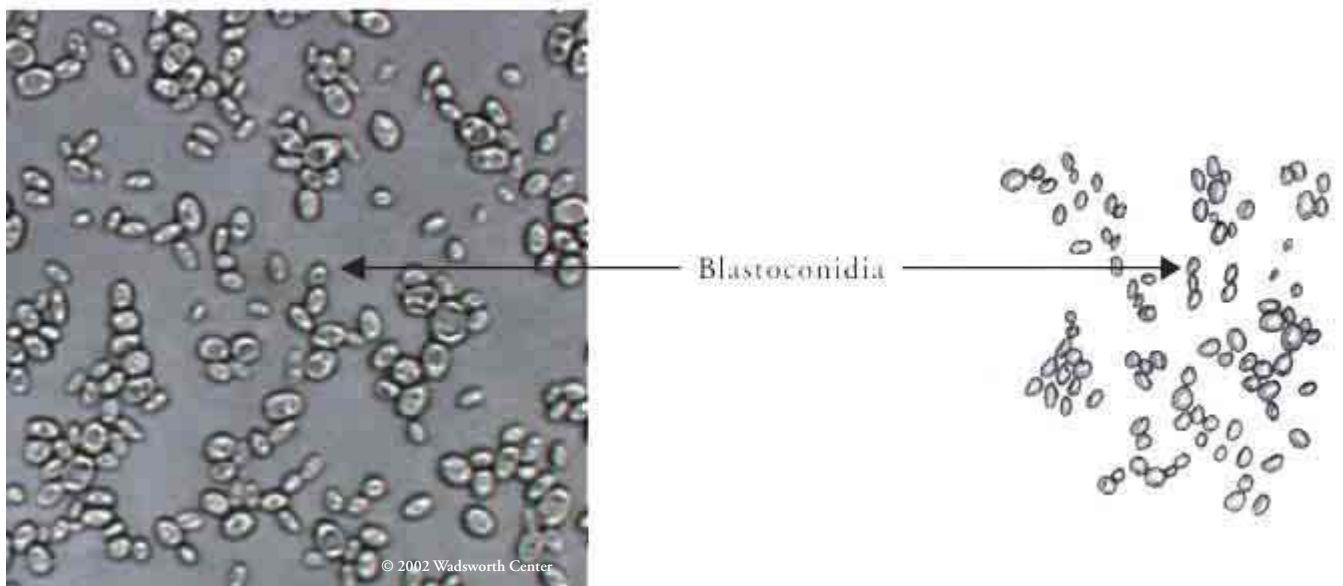


Figure 17. Microscopic morphology of *Candida glabrata* on corn meal agar with Tween 80 shows elliptical shaped blastoconidia (left; 400 X magnification, right; line diagram not to scale).

ED. SP. *CANDIDA VISWANATHII*



Figure 20. Four-day-old, white, a little wrinkled, and moist colony of *Candida viswanathii* on Sabouraud's dextrose agar.

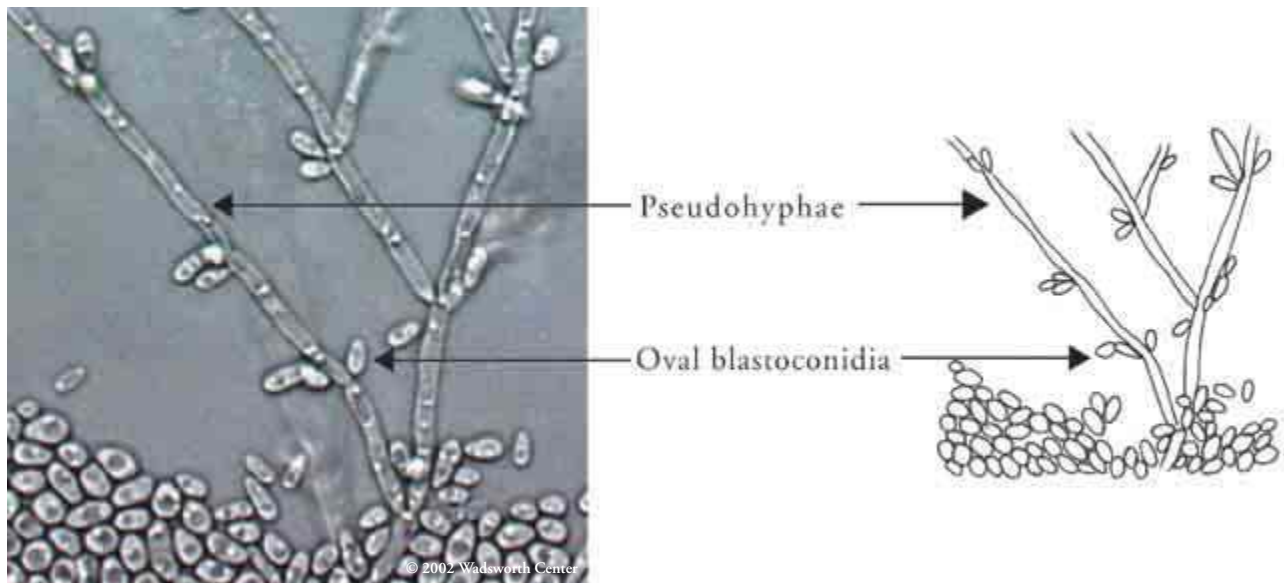


Figure 21. Microscopic morphology of *Candida viswanathii* on corn meal agar with Tween 80 shows long pseudohyphae and oval shaped blastoconidia (left; 400 X magnification, right; line diagram not to scale).

ANTIFUNGAL SUSCEPTIBILITY TESTING

Introduction: M27-A document published by the National Committee for Clinical Laboratory Standards (NCCLS) Subcommittee on Antifungal Susceptibility Testing is the current standard reference method described for determining the antifungal susceptibility testing of pathogenic yeasts (2). It includes broth microdilution and broth macrodilution two methods. Various commercial systems are also being developed for antifungal susceptibility testing of yeasts such as Sensititre YeastOne Colorimetric Panel and Etest. NCCLS M27-A document also described in detail on how to testing *C. neoformans*. The aim to give *C. neoformans* as an educational specimen is to provide an opportunity for the participating laboratories to gain expertise with *C. neoformans* testing.

Materials & Methods: Fourteen microbiology laboratories within 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-3) and *Candida krusei* ATCC 6258 (S-5), three NCCLS reference strains *Candida albicans* ATCC 24433 (S-4), *Candida parapsilosis* ATCC 90018, and *Candida tropicalis* ATCC 750 (S-1) (3, 4), and an educational specimen *Cryptococcus neoformans* (S-edu.) were included in June 5, 2002 antifungal proficiency testing event. These isolates except *C. neoformans* have been well characterized, and their MIC ranges against amphotericin B and fluconazole have been published (3, 4). MICs within ± 2 dilution from the reference result (range of MICs for a particular yeast described in NCCLS, M27-A) is the acceptable results in this event (2).

Results: A total of 18 labs participated in this antifungal susceptibility testing event, and all the labs reported the correct answers. Of the 18 participating laboratories, 6 labs used broth microdilution method, 3 labs used Etest, and 1 lab used broth macrodilution. Of the 14 labs, 8 labs used commercially prepared YeastOne Colorimetric microdilution method, while the other 6 labs performed testing according to NCCLS M27-A guidelines. The supplementary information on antifungal susceptibility testing procedures is summarized in Table 1. No matter which method was used for testing, all labs results were within the reference range of all the isolates including educational specimen *Cryptococcus neoformans*. 91% of answers for Amphotericin B and 92% answers for fluconazole were within the NCCLS reference range.

Further Reading:

1. College of American Pathologists. 2001. Antifungal Susceptibility Testing. F-B Survey: 8-11.
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Individual Isolates:

S-1 *Candida tropicalis* ATCC 750

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

Fifteen labs reported values within NCCLS reference range and 3 labs reported values within the expanded range for amphotericin B. All the participating labs reported values within NCCLS reference range for fluconazole.

S-2 *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

Sixteen labs reported values within NCCLS reference range and 2 labs reported values within the expanded range for amphotericin B. Fourteen labs reported values within NCCLS reference range and 4 labs reported values within the expanded values for fluconazole.

S-3 *Candida parapsilosis* ATCC 22019

Summary	NCCLS Reference range	Expanded Range
Amphotericin B	0.25-1.0 µg/ml	0.12-2.0 µg/ml
Fluconazole	2.0-8.0 µg/ml	1.0-16 µg/ml

Seventeen labs reported values within NCCLS reference range and 1 lab reported values within the expanded range for both drugs.

S-4 *Candida albicans* ATCC 24433

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

Seventeen labs reported values within NCCLS reference range and 1 lab reported values within the expanded range for amphotericin B. Sixteen labs reported values within NCCLS reference range and 2 labs reported values within the expanded values for fluconazole.

S-5 *Candida krusei* ATCC 6258

Summary	NCCLS Reference range	Expanded range
Amphotericin B	0.5-2.0 µg/ml	0.25-4.0 µg/ml
Fluconazole	16-64 µg/ml	8.0->64 µg/ml

Seventeen labs reported values within NCCLS reference range and 1 lab reported values within the expanded range for amphotericin B. All the participating labs reported values within NCCLS reference range for fluconazole.

S-Education specimen *Cryptococcus neoformans*

Summary	Reference labs range
Amphotericin B	0.06-1.0 µg/ml
Fluconazole	8.0->64 µg/ml

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

Test Method	No. Participants Labs
NCCLS broth microdilution	6
NCCLS broth macrodilution	1
Sensititre YeastOne Colorimetric	8
Etest	3
Media employed	
RPMI 1640	12
RPMI 1640 w / alamar blue	1
Antibiotic medium 3	1
Casitone agar	1
Sabouraud dextrose	1
YeastOne broth	4
Inoculum preparation	
Spectrophotometric	11
MacFarland	9
Inoculum size	
0.5-2.5 X 10 ³	9
1.5-8 X 10 ³	8
0.5-1.0 X 10 ⁴	1
Incubation temperature	
35°C	15
37°C	3
Incubation duration	
24 hour	9
48 hour	10
72 hour ¹	8
Endpoint reading	
Visual	13
Spectrophotometric	1
Colorimetric	4
Scoring endpoint²	
100% inhibition	9
95% inhibition	1
80% inhibition	6
50% inhibition	4
other (color change)	4
QC organism	
NCCLS recommended strains	17
Unknown	1

¹For *Cryptococcus neoformans* incubation time.

²Most labs used 100% inhibition for amphotericin B and either 80 or 50% for azoles.

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SUMMARY OF SURVEY ON MOLECULAR TESTS/ANTIGEN DETECTION

Total 148 copies of survey sheet were sent out and 140 labs responded. Following are the summary of results on the numbers of laboratories performing molecular test/antigen detection:

❖ Use PCR for fungal identifications/characterization	2
❖ Nucleotide sequences for fungal identifications/characterization	1
❖ DNA/RNA probe-based identification	3
❖ Gen-Probes	15
❖ Others	1
➤ NOCARDIA PCR	1
❖ Molecular typing	2
➤ Restriction Fragment Length Polymorphism (RFLP)	1
➤ Random Amplified Polymorphic DNA (RAPD)	1
➤ Pulse Field Gel Electrophoresis (PFGE)	2
➤ Repeat probes	1
❖ Antigen detection	15
➤ <i>Histoplasma capsulatum</i>	5
➤ <i>Candida albicans</i>	3
➤ <i>Cryptococcus neoformans</i>	10
➤ <i>Coccidioides immitis</i>	3
➤ <i>Blastomyces dermatitidis</i>	3
➤ <i>Aspergillus flavus</i>	1
➤ <i>Aspergillus fumigatus</i>	1
➤ <i>Aspergillus niger</i>	1

SUMMARY OF SURVEY ON ISOLATION OF DIMORPHIC FUNGI

Total 148 copies of survey sheet were sent out and 140 labs responded. The numbers of dimorphic fungi reported are summarized in Figure 23. Following are some related result statistics.

❖ Use Gen-Probe or similar kits for identification		
➤ Yes		12
➤ No		125
➤ No Answer		3
❖ Send cultures to reference laboratories for identification		
➤ Yes		105
▪ NYSDOH Mycology Lab	59	
▪ Other Mycology Lab	46	
➤ No		22
➤ No Answer		13
❖ Keep the cultures for reference or destroy them		
➤ Keep		37
➤ Keep for less than 1 year		13
➤ Destroy		53
➤ No Answer		37
❖ Receive requests for susceptibility testing on dimorphic fungi		
➤ Yes		13
➤ No		98
➤ No Answer		29

SUMMARY OF SURVEY ON ISOLATION OF DIMORPHIC FUNGI

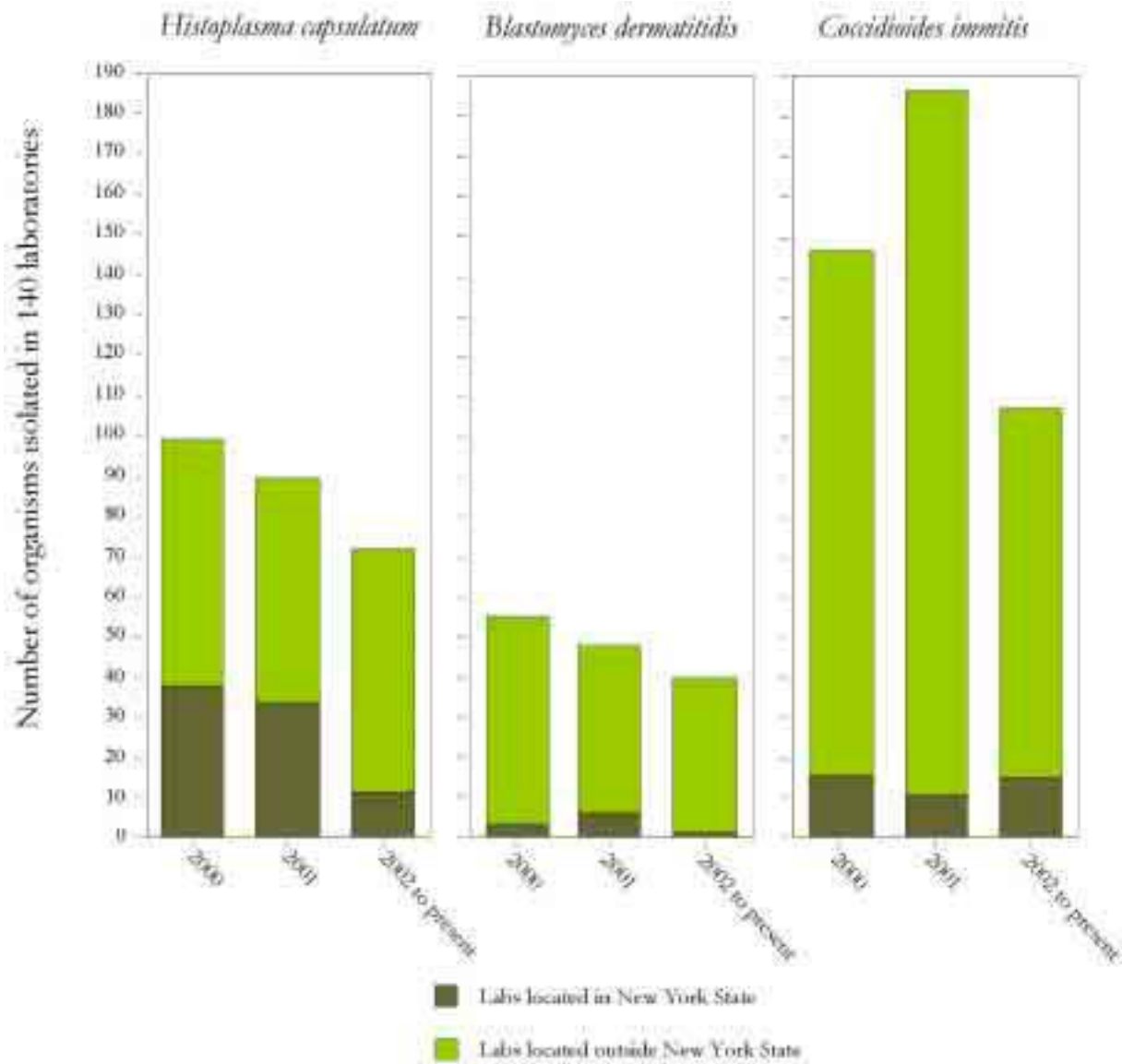



Figure 23. Summary of the numbers of three dimorphic fungi isolated from New York State (110 laboratories) and out of New York State (30 laboratories) by year.



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

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

P h o t o g r a p h y & I l l u s t r a t i o n D e p a r t m e n t

P T C o o r d i n a t o r : D r . P i n g R e n

G r a p h i c D e s i g n e r : C h r i s t i n e L e e

L i n e D r a w i n g s : S a l l y G r o m a d z k i

P h o t o g r a p h e r : E a r l E i c h e l l e

S e q u e n c i n g : M o l e c u l a r G e n e t i c s C o r e

C o p y r i g h t © 2 0 0 2 W a d s w o r t h C e n t e r