



# MYCOLOGY

# CRITIQUÉ

**Mycology Proficiency Testing Program  
June 2004**

**Wadsworth Center**

**New York State Department of Health**



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# *Test Specimens and Grading Policy*

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## Test Specimens

Minimal of two strains of each of the proposed yeast specimens was examined for inclusion in the proficiency test event of June 2004. The colony morphology of all yeast strains was studied on corn meal agar with Tween 80 plates inoculated by Dalmau or streak-cut method. Carbohydrate assimilation was studied with the API 20C AUX identification kit. The fermentations of carbohydrates, i.e., glucose, maltose, sucrose, lactose, trehalose, and cellobiose, were also investigated. Additionally, characteristics, such as nitrate assimilation, urease activity, and cycloheximide sensitivity, were investigated with the appropriate test media. The single strain that best demonstrated the morphologic and physiologic characteristics of each of the proposed yeast pathogens was used in the test.

## Grading Policy

A laboratory's response for each sample is compared with the response that reflects 80 percent agreement of 10 referee laboratories or 80 percent of all participating laboratories. The referee laboratories are selected at random from among hospital laboratories participating in the program. They represent all geographical areas of New York State and must have a record of excellent performance during the preceding three years. The maximum score for each specimen is 20 based on the formula:

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

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

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

\*The use of brand and/or trade names in this report does not constitute an endorsement of the products on the part of the Wadsworth Center or the New York State Department of Health.

**Mycology – Yeast Only**

	<b>Specimen Key</b>	<b>Validated Specimen</b>	<b>Other Acceptable Answers</b>
<b>Y-1</b>	<i>Candida boidinii</i>	<i>Candida boidinii</i>	
<b>Y-2</b>	<i>Prototheca wickerhamii</i>	<i>Prototheca wickerhamii</i>	
<b>Y-3</b>	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>	
<b>Y-4</b>	<i>Candida ciferrii</i>	<i>Candida ciferrii</i>	
<b>Y-5</b>	<i>Candida magnolia</i>	(Not validated)	

**Mycology – Antifungal Susceptibility Testing for Yeasts**

	<b>Specimen Key</b>	<b>Validated Specimen</b>
<b>S-1</b>	<i>Candida albicans</i> ATCC 24433	(Not validated)
<b>S-2</b>	<i>Candida albicans</i> ATCC 90028	<i>Candida albicans</i> ATCC 90028
<b>S-3</b>	<i>Candida parapsilosis</i> ATCC 22019	<i>Candida parapsilosis</i> ATCC 22019
<b>S-4</b>	<i>Candida parapsilosis</i> ATCC 90018	<i>Candida parapsilosis</i> ATCC 90018
<b>S-5</b>	<i>Candida krusei</i> ATCC 6258	<i>Candida krusei</i> ATCC 6258

**Mycology – Yeast Only**

	<b>Correct Responses/ Total # Laboratories (%)</b>	<b>Referees (%)</b>
<b>Y - 1</b> <i>Candida boidinii</i>	124/143 (87)	9/10 (90)
<b>Y - 2</b> <i>Prototheca wickerhamii</i>	139/143 (97)	10/10 (100)
<b>Y - 3</b> <i>Saccharomyces cerevisiae</i>	142/143 (99)	10/10 (100)
<b>Y - 4</b> <i>Candida ciferrii</i>	136/143 (95)	10/10 (100)
<b>Y - 5</b> <i>Candida magnolia</i> (Not validated)	121/143 (85)	8/10 (80)

**Mycology – Antifungal Susceptibility Testing for Yeasts**

	<b>Correct Responses/ Total # Laboratories (%)</b>	
	<b>Amphotericin B</b>	<b>Fluconazole</b>
<b>S - 1</b> <i>Candida albicans</i> ATCC 24433 (Not validated)	22/22 (100)	21/26 (81)
<b>S - 2</b> <i>Candida albicans</i> ATCC 90028	21/22 (95)	26/26 (100)
<b>S - 3</b> <i>Candida parapsilosis</i> ATCC 22019	22/22 (100)	26/26 (100)
<b>S - 4</b> <i>Candida parapsilosis</i> ATCC 90018	22/22 (100)	26/26 (100)
<b>S - 5</b> <i>Candida krusei</i> ATCC 6258	22/22 (100)	25/26 (96)

**Mycology – General and Yeast Only**

Number of participating laboratories	143
Number of referee laboratories	10
Number of laboratories responding by deadline	143
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	135
Number of laboratories unsuccessfully completing this test	8

**Mycology – Antifungal Susceptibility Testing for Yeasts**

Number of participating laboratories	27
Number of referee laboratories	3
Number of laboratories responding by deadline	26
Number of laboratories responding after deadline	1
Number of laboratories not responding	0
Number of laboratories successfully completing this test	26
Number of laboratories unsuccessfully completing this test	1

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

AMS Vitek system	49
API 20C AUX	115
IDS Rapid System	3
Microscan	3
Remel Uni-Yeast-Tek	11
Other	6

(\*Includes multiple systems used by some labs)

**Further Reading:**

1. Lin, Y.H., Lee, F.-L., and Hsu, W.-H. 1996. Molecular and chemical taxonomic differentiation of *Candida boidinii* Ramirez strains. *Int. J. Syst. Bacteriol.* 46: 352-355.

Source: Skin

Scoring:	No. Labs
Referee Labs with correct ID:	9
Labs with correct ID:	124
Labs with incorrect ID:	19
( <i>Candida lipolytica</i> )	(5)
( <i>Candida lambica</i> )	(3)
( <i>Blastoschizomyces capitatus</i> )	(2)
( <i>Candida kefir</i> )	(1)
( <i>Candida lusitanae</i> )	(1)
( <i>Candida parapsilosis</i> )	(1)
( <i>Candida</i> sp.)	(1)
( <i>Candida rugosa</i> )	(1)
( <i>Candida zeylanoides</i> )	(1)
( <i>Hansenula polymorpha</i> )	(1)
( <i>Pichia obmeri</i> )	(1)
(Unidentified)	(1)

**Clinical Significance:** *Candida boidinii* is not a known pathogen; it is occasionally isolated as a contaminant on the floor of hospital wards, in soft drinks, and water.

**Ecology:** *C. boidinii* is found in soil.

**Laboratory Diagnosis:**

1. Culture – On Sabouraud’s dextrose agar, after 7 days at 25°C, *C. boidinii* colony was wrinkled, white turning tan with age (Figure 1).
2. Microscopic morphology – On corn meal agar with Tween 80, *C. boidinii* showed pseudohyphae consisting of long branched chains of cells with blastoconidia (Figure 2).
3. Differentiation from other yeasts – *C. boidinii* fermented glucose only. It grew on media containing cycloheximide, which was differentiated from *C. lambica*. *C. boidinii* was distinguished from *C. lipolytica* by its ability to assimilate nitrite.
4. In vitro susceptibility testing – No information available.
5. Molecular tests – There was limited molecular study on differentiation of *C. boidinii*.

**Comments:** Although *C. boidinii* is not a known pathogen, it has been isolated from hospital environment. It can be distinguished from *C. lipolytica* by its ability to assimilate nitrite, from *C. lambica*, *C. lusitanae*, *C. parapsilosis*, and *C. rugosa* by its ability to grow on media containing cycloheximide, from *C. zeylanoides* by its ability to ferment glucose.

Source: Skin

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	139
Labs with incorrect ID:	4
( <i>Candida famata</i> )	(2)
( <i>Candida glabrata</i> )	(1)
( <i>Hansenula polymorpha</i> )	(1)

**Clinical Significance:** *Prototheca wickerhamii* causes protothecosis in humans. Most commonly, these yeast-like algae cause cutaneous lesions and subcutaneous lesions like bursitis. Rarely, *P. wickerhamii* causes systemic infections. The infection is acquired through traumatic implantation of the yeast in subcutaneous tissue.

**Ecology:** *P. wickerhamii* has been isolated from various environmental sources like sewage, slime, and stream sediment.

**Laboratory Diagnosis:**

1. **Culture** – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was moist, cream-colored, yeast-like (Figure 3).
2. **Microscopic morphology** – On corn meal agar with Tween 80, sporangia of various sizes, some filled with sporangiospores (endospores), were seen (Figure 4). There was no budding, true or pseudohyphae formation.
3. **Differentiation from other yeasts** – *P. wickerhamii* required thiamine for growth, did not grow on media containing cycloheximide, grew well at 25°C and 37°C. The cells of *P. wickerhamii* were smaller than those of *P. zopfii*. On the API 20C AUX, a specific assimilation biocode differentiates it from other *Prototheca* species. The isolates of *P. zopfii* are resistant to 50-µg clotrimazole disk at 37°C while *P. wickerhamii* isolates produced a zone of inhibition.
4. **In vitro susceptibility testing** – Almost all isolates are susceptible to amphotericin B but resistant to fluconazole and 5 FC, variably susceptible to itraconazole and ketoconazole.
5. **Molecular tests** – Sequence analysis of the mitochondrial small subunit rRNA from *P. wickerhamii* showed higher homology with mitochondrial sequence from plants.

**Comments:** Two participating laboratories reported this specimen as *Candida famata*, which is able to assimilate sucrose, maltose, cellobiose, and trehalose. One lab each reported it as *Candida glabrata* and *Hansenula polymorpha*. *C. glabrata* is able to ferment dextrose; *Hansenula polymorpha* is nitrate positive.

**Further Reading:**

1. Casal, M.J. and Gutierrez aroca, J. 1995. Simple new test for rapid differentiation of *Prototheca stagnora* from *P. wickerhamii* and *P. zopfii*. *Mycopathologia*. 130: 93-94.
2. Chao, S.C., Hsu, M.M., and Lee, J.Y. 2002. Cutaneous protothecosis: report of five cases. *Br. J. Dermatol.* 146: 688-693.
3. Leimann, B.C., Monteiro, P.C., Lazera, M., Candanoza, E.R., and Wanke, B. 2004. Protothecosis. *Med Mycol.* 42: 95-106.
4. Nedelcu, A.M. 1997. Fragmented and scrambled mitochondrial ribosomal RNA coding regions among green algae: a model for their origin and evolution. *Mol. Biol. Evol.* 14: 506-517.
5. Okuyama, Y., Hamaguchi, T., Teramoto, T., and Takiuchi, I. 2001. A human case of protothecosis successfully treated with itraconazole. *Nippon Ishinkin Gakkai Zasshi.* 42: 143-147.
6. Torres, H.A., Bodey, G.P., Tarrand, J.J., and Kontoyiannis, D.P. 2003. Protothecosis in patients with cancer: case series and literature review. *Clin. Microbiol. Infect.* 9: 786-792.

Source: Lung Biopsy

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	42
Labs with incorrect ID:	1
( <i>Pichia ohmeri</i> )	(1)

**Clinical Significance:** *Saccharomyces cerevisiae* can cause disseminated infection in the immunocompromised hosts.

**Ecology:** *S. cerevisiae* is cosmopolitan in distribution.

**Laboratory Diagnosis:**

1. Culture – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was cream, smooth, dull, butyrous (Figure 5).
2. Microscopic morphology – On corn meal agar with Tween 80, round to oval yeast cells with no pseudohyphae or rudimentary pseudohyphae were seen (Figure 6). On special media like V8 juice agar or malt agar, characteristic ascospores encased in asci were seen.
3. Differentiation from other yeasts – *S. cerevisiae* fermented glucose, maltose, and sucrose, did not grow on the media containing cycloheximide, and grew at 37°C. On the API 20C AUX, a specific assimilation biocode identifies this organism.
4. In vitro susceptibility testing – Most isolates are susceptible to amphotericin B, 5 FC, and to azoles like fluconazole, miconazole, etc.
5. Molecular tests – *S. cerevisiae* is the most intensely studied model organism also being the first eukaryote to have its entire genome sequenced and mapped.

**Further Reading:**

1. Barchiesi, F., Arzeni, D., Compagnucci, P., Di Francesco, L.F., Giacometti, A., and Scalise, G. 1998. *In vitro* activity of five antifungal agents against clinical isolates of *Saccharomyces cerevisiae*. *Med. Mycol.* 36: 437-440.
2. Fiore, N.F., Conway, J.H., West, K.W., and Kleiman, M.B. 1998. *Saccharomyces cerevisiae* infections in children. *Pediatr. Infect. Dis. J.* 17: 1177-1179.
3. Konecny, P., Drummond, F.M., Tish, K.N., and Tapsall, J.W. 1999. *Saccharomyces cerevisiae* oesophagitis in an HIV – infected patient. *Int. J. STD. AIDS.* 10: 821-822.
4. McCullough, M.J., Clemons, K.V., Farina, C., McCusker, J.H., Stevens, D.A. 1998. Epidemiological investigation of vaginal *Saccharomyces cerevisiae* isolates by a genotypic method. *J. Clin. Microbiol.* 36: 557-562.
5. Ogawa, H., Fujimura, M., and Tofuku, Y. 2004 Allergic bronchopulmonary fungal disease caused by *Saccharomyces cerevisiae*. *J. Asthma.* 41: 223-228.
6. Posteraro, B., Sanguinetti, M., Masucci, L., Romano, L., Morace, G., and Fadda, G. 2000. Reverse cross blot hybridization assay for rapid detection of PCR – amplified DNA from *Candida* species, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae* in colonial samples. *J. Clin. Microbiol.* 38: 1609-1614.
7. Xu, J., Boyd, C.M., Livingston, E., Meyer, W., Madden, J.F., and Mitchell, T.G. 1999. Species and genotypic diversities and similarities of pathogenic yeasts colonizing women. *J. Clin. Microbiol.* 37: 3835-3843.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA. The sequences are deposited in GenBank under the accession numbers AY722406 and AY722407, respectively.

```

1                                                    50
UWFP-382 (AF335952) TCCGTAGGTG AACCTGCGGA AGGATCATT AAGAAATTTA ATAATTTTGA
UAMH196 (AY722406) TCCGTAGGTG AACCTGCGGA AGGATCATT AAGAAATTTA ATAATTTTGA

51                                                    100
AAATGGATTT TTTTGTTTTG GCAAGAGCAT GAGAGCTTTT ACTGGGCAAG
AAATGGATTT TTTTGTTTTG GCAAGAGCAT GAGAGCTTTT ACTGGGCAAG

101                                                   150
AAGACAAGAG ATGGAGAGTC CAGCCGGGCC TGCCTTAAG TGC GCGGTCT
AAGACAAGAG ATGGAGAGTC CAGCCGGGCC TGCCTTAAG TGC GCGGTCT

151                                                   200
TGCTAGGCTT GTAAGTTTCT TTCTTGCTAT TCCAAACGGT GAGAGATTTT
TGCTAGGCTT GTAAGTTTCT TTCTTGCTAT TCCAAACGGT GAGAGATTTT

201                                                   250
TGTGCTTTTG TTATAGGACA ATTAAAACCG TTTCAATACA ACACACTGTG
TGTGCTTTTG TTATAGGACA ATTAAAACCG TTTCAATACA ACACACTGTG

251                                                   300
GAGTTTTTCAT ATCTTTGCAA CTTTTTCTTT GGGCATTCTGA GCAATCGGGG
GAGTTTTTCAT ATCTTTGCAA CTTTTTCTTT GGGCATTCTGA GCAATCGGGG

301                                                   350
CCCAGAGGTA ACAAACACAA ACAATTTTAT CTATTCATTA AATTTTTGTC
CCCAGAGGTA ACAAACACAA ACAATTTTAT CTATTCATTA AATTTTTGTC

351                                                   400
AAAAACAAGA ATTTTCGTAA CTGGAAATTT TAAAATATTA AAAACTTTCA
AAAAACAAGA ATTTTCGTAA CTGGAAATTT TAAAATATTA AAAACTTTCA

401
ACAACGGATC TCTTGGTTCT CGCATCGATG AAGAACGCAGC
ACAACGGATC TCTTGGTTCT CGCATCGATG AAGAACGCAGC

```

Figure 7. Alignment of primary sequences of the ITS1 region of *Saccharomyces cerevisiae* UWFP-382 and PT specimen *S. cerevisiae* UAMH196. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1
UWFP-387 (AF219005) TCCTCCGCTT ATTGATATGC TTAAGTTCAG CGGGTACTCC TACCTGATTT
UAMH196 (AY722407) TCCTCCGCTT ATTGATATGC TTAAGTTCAG CGGGTACTCC TACCTGATTT

51
GAGGTCAAAC TTTAAGAACA TTGTTGCCT AGACGCTCTC TTCTTATCGA
GAGGTCAAAC TTTAAGAACA TTGTTGCCT AGACGCTCTC TTCTTATCGA

101
TAACGTTCCA ATACGCTCAG TATAAAAAAG ATTAGCCGCA GTTGGTAAAA
TAACGTTCCA ATACGCTCAG TATAAAAAAG ATTAGCCGCA GTTGGTAAAA

151
CCTAAAACGA CCGTACTTGC ATTATACCTC AAGCACGCAG AGAAACCTCT
CCTAAAACGA CCGTACTTGC ATTATACCTC AAGCACGCAG AGAAACCTCT

201
CTTTGGAAAA AAAACATCCA ATGAAAAGGC CAGCAATTTT AAGTTAACTC
CTTTGGAAAA AAAACATCCA ATGAAAAGGC CAGCAATTTT AAGTTAACTC

251
CAAAGAGTAT CACTCACTAC CAAACAGAAT GTTTGAGAAG GAAATGACGC
CAAAGAGTAT CACTCACTAC CAAACAGAAT GTTTGAGAAG GAAATGACGC

301
TCAAACAGGC ATGCCCCCTG GAATACCAAG GGGCGCAATG TCGGTTCAAA
TCAAACAGGC ATGCCCCCTG GAATACCAAG GGGCGCAATG TCGGTTCAAA

351
GATTCGATGA TTCACGGAAT TCTGCAATTC ACATTACGTA TCGCATTTCG
GATTCGATGA TTCACGGAAT TCTGCAATTC ACATTACGTA TCGCATTTCG

401
CTGCGTTCTTC ATCGATGC
CTGCGTTCTTC ATCGATGC

```

Figure 8. Alignment of primary sequences of the ITS2 region of *Saccharomyces cerevisiae* UWFP-382 and PT specimen *S. cerevisiae* UAMH196. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

**Comments:** Only one lab reported this specimen as *Pichia obmeri*, which can be differentiated from *S. cerevisiae* by assimilation of glycerol and 2-keto-D-gluconate.

Source: Toenail

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	136
Labs with incorrect ID:	7
( <i>Saccharomyces cerevisiae</i> )	(2)
( <i>Candida</i> sp.)	(1)
( <i>Cryptococcus humicolus</i> )	(1)
( <i>Geotrichum penicillatum</i> )	(1)
( <i>Schizosaccharomyces</i> sp.)	(1)
( <i>Trichosporon pullulous</i> )	(1)

**Clinical Significance:** *Candida kiferrii* is the causative agent of onychomycosis. It can be isolated from ears, skin, nails, and eyes.

**Ecology:** *C. kiferrii* is found in soil.

**Laboratory Diagnosis:**

1. **Culture** – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was white to cream, yellowish, wrinkled (Figure 9).
2. **Microscopic morphology** – On corn meal agar with Tween 80, pseudohyphae and true hyphae were seen. Blastoconidia were born laterally on denticles and may form short chains (Figure 10).
3. **Differentiation from other yeasts** – *C. kiferrii* was able to grow on the media containing cycloheximide and at 42°C, it is both urease and nitrate negative.
4. **In vitro susceptibility testing** – *C. kiferrii* was susceptible to amphotericin B, 5 fluorocytosine (5 FC), and itraconazole but resistant to fluconazole.
5. **Molecular tests** – No information available.

**Comments:** *C. kiferrii* is distinguished from *Saccharomyces cerevisiae* by its ability to grow on the media containing cycloheximide. *Cryptococcus humicolus* is urease positive, but *C. kiferrii* is urease negative. *Trichosporon pullulous* is both urease and nitrate positive but *C. kiferrii* is negative for both reactions.

**Further Reading:**

1. De Gentile, L., Bouchara, J.P., Le Clec’h, C., Cimon, B., Symoens, F., and Chabasse, D. 1995. Prevalence of *Candida kiferrii* in elderly patients with tropic disorders of the legs. *Mycopathologia*. 131: 99-102.
2. De Gentile, L., Bouchara, J.P., Cimon, B., and Chabasse, D. 1991. *Candida kiferrii*: clinical and microbiological features of an emerging pathogen. *Mycoses*. 34: 125-128.
3. Furman, R.M. and Ahearn, D.G. 1983. *Candida kiferrii* and *Candida chiropterorum* isolated from clinical specimens. *J. Clin. Microbiol.* 18: 1252–1255.
4. Gunsilius, E., Lass-Florl, C., Kahler, C.M., Gastl, G., and Petzer, A.L. 2001. *Candida kiferrii*, a new fluconazole-resistant yeast causing systemic mycosis in immunocompromised patients. *Ann. Hematol.* 80: 178-179.

Source: Mouth wash

Scoring:	No. Labs
Referee Labs with correct ID:	8
Labs with correct ID:	121
Labs with incorrect ID:	22
( <i>Candida glabrata</i> )	(5)
( <i>Candida colliculosa</i> )	(4)
( <i>Hansenula polymorpha</i> )	(3)
( <i>Candida norvegensis</i> )	(2)
( <i>Candida</i> sp.)	(2)
( <i>Blastoschizomyces capitatus</i> )	(1)
( <i>Candida kefyr</i> )	(1)
( <i>Candida parapsilosis</i> )	(1)
( <i>Candida sphaerica</i> )	(1)
( <i>Candida zeylanoides</i> )	(1)
( <i>Hansenula anomala</i> )	(1)
( <i>Sporobolomyces salmonicolor</i> )	(1)

**Clinical Significance:** *Candida magnoliae* was implicated in one case of human disease.

**Ecology:** *C. magnoliae* is usually isolated from flowers of *Magnolia* sp., and from bumble bee's gut.

**Laboratory Diagnosis:**

1. **Culture** – On Sabouraud's dextrose agar after 7 days at 25°C, colony was white to cream colored, soft, and smooth (Figure 11).
2. **Microscopic morphology** – On corn meal agar with Tween 80, cells were globose to oval, single or budding. No filaments was seen (Figure 12).
3. **Differentiation from other yeasts** – *C. magnoliae* did not grow on the media containing cycloheximide, and was negative on urease reaction and positive on nitrate reaction. It fermented glucose and sucrose only.
4. **In vitro susceptibility testing** – No information available.
5. **Molecular tests** – No information available.

**Comments:** This specimen was not validated. A majority of participating laboratories reported this specimen as *C. glabrata*, which only ferments glucose but not sucrose. Four laboratories reported it as *C. colliculosa*, which has variable fermentation responses on many carbohydrates such as galactose, maltose sucrose, trehalose, etc.

**Further Reading:**

1. Lane, J.E., Lee, M.A., and Stephens, J.L. 2001. Tenosynovitis secondary to *Candida magnoliae* in an immunocompetent host: *Candida Magnoliae* tenosynovitis. *The Internet Journal of Infectious Diseases*. Volume 1 Number 2. (<http://www.ispub.com/osita/index.php?xmlFilePath=journal/s/ijid/vol1n2/magno.xml>)

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

**Materials & Methods:** Twenty-five microbiology laboratories within the United States and one reference laboratory each from Canada and United Kingdom participated in this event. Two NCCLS quality control strains, *Candida parapsilosis* ATCC 22019 (S-3) and *Candida krusei* ATCC 6258 (S-5), three NCCLS reference strains, *Candida albicans* ATCC 24433 (S-1), *Candida parapsilosis* ATCC 90028 (S-2), and *Candida parapsilosis* ATCC 90018 (S-4) were included in the June 2, 2004 antifungal proficiency testing event. These isolates have been well characterized, and their MIC ranges against amphotericin B and fluconazole have been published. MICs within  $\pm 2$  dilutions of the reference result (range of MICs for a particular yeast described in NCCLS, M27-A2) are the acceptable results in this event.

**Results:** A total of 27 laboratories participated in this antifungal susceptibility testing event. The performances of all participating laboratories were satisfactory. Of the 27 participating laboratories, 9 laboratories used the broth microdilution method, 15 laboratories used YeastOne Colorimetric microdilution method, 3 laboratories used Etest, and 1 laboratory each used the broth macrodilution and disk diffusion method. The supplementary information on antifungal susceptibility testing procedures is summarized in Table 1. The MIC results submitted by the 27 participants are illustrated in Figure 13. *Candida albicans* ATCC 24433 (S-1) was not validated, because it appeared to be a mix culture of *C. albicans* and *C. guilliermondii*. It is not clear how this mixed culture was shipped out, but this incident is currently under investigation. Good performance was noted for all validated specimens irrespective of the methodology used by the laboratories for both amphotericin B and fluconazole. Overall, agreement with the NCCLS reference ranges was 99% against amphotericin B and fluconazole for all four validated isolates.

## Further Reading:

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## S-1 *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

Not validated. This is a mix culture of *C. albicans* and *C. guilliermondii*.

## 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

Amphotericin B values were reported within NCCLS reference range by 20 laboratories, and within expanded range by 2 laboratories. Fluconazole values were reported within NCCLS reference range by 20 laboratories, and within the expanded values by 7 laboratories.

## S-3 *Candida parapsilosis* ATCC 22019

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

Amphotericin B values were reported within NCCLS reference range by 21 laboratories, and within expanded range by 1 laboratory. Fluconazole values were reported within NCCLS reference range by all participating laboratories.

## S-4 *Candida parapsilosis* ATCC 90018

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

Amphotericin B values were reported within NCCLS reference range by 21 laboratories, and within expanded range by 1 laboratory. Fluconazole values were reported within NCCLS reference range by 22 laboratories, and within the expanded values by 5 laboratories.

## S-5 *Candida krusei* ATCC 6258

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

Amphotericin B values were reported within NCCLS reference range by all participating laboratories. Fluconazole values were reported within NCCLS reference range by 26 laboratories, and 1 laboratory did not report any value.

## Further Reading: (cont'd)

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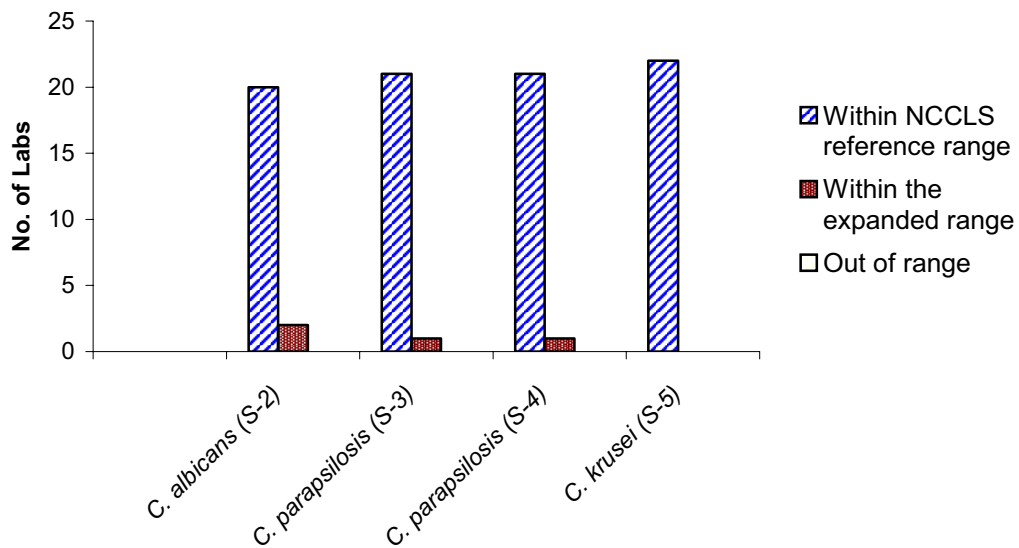
# *A*ntifungal susceptibility testing

<b>Test Method*</b>	<b>No. Participant Laboratories</b>
NCCLS broth microdilution	9
NCCLS broth macrodilution	1
Sensititre YeastOne Colorimetric	15
Etest	3
Disk diffusion	1
<b>Medium employed*</b>	
RPMI 1640	13
RPMI 1640 w / alamar blue	2
Antibiotic medium 3	1
Sabouraud dextrose	5
YeastOne broth	7
Mueller-Hinton Agar + 2% glucose + 0.5 g/ml Methylene Blue	1
<b>Inoculum preparation*</b>	
Spectrophotometric	10
MacFarland	19
<b>Inoculum size (CFU/ml)</b>	
$0.5-2.5 \times 10^3$	9
$1.5-8 \times 10^3$	13
$0.5-1.0 \times 10^4$	2
$2.5 \times 10^6$	1
MacFarland 0.5	2
<b>Incubation temperature*</b>	
35°C	29
37°C	3
<b>Incubation duration*</b>	
24 hr	18
48 hr	11
<b>Endpoint reading*</b>	
Visual	17
Colorimetric	11
Biomlic	1
<b>Scoring endpoint<sup>1</sup>*</b>	
100% inhibition	11
95% inhibition	1
80% inhibition	6
50% inhibition	4
Color change	11
<b>QC organism</b>	
NCCLS recommended strains	27
Unknown	0

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

\* More than one value reported by individual laboratories

(A)



(B)

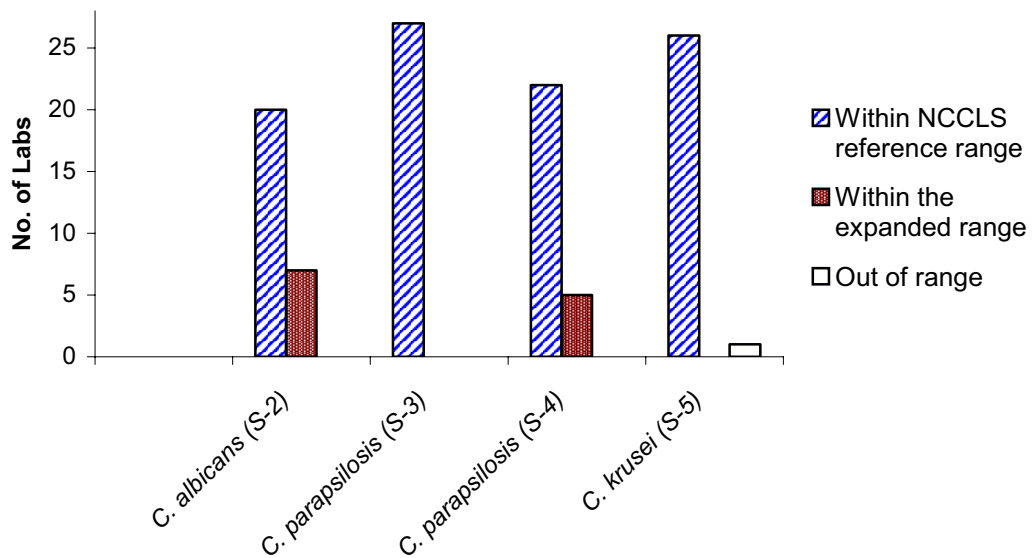


Figure 13. Summary of the results submitted by the participating laboratories for 4 isolates, for amphotericin B (A) and fluconazole (B).

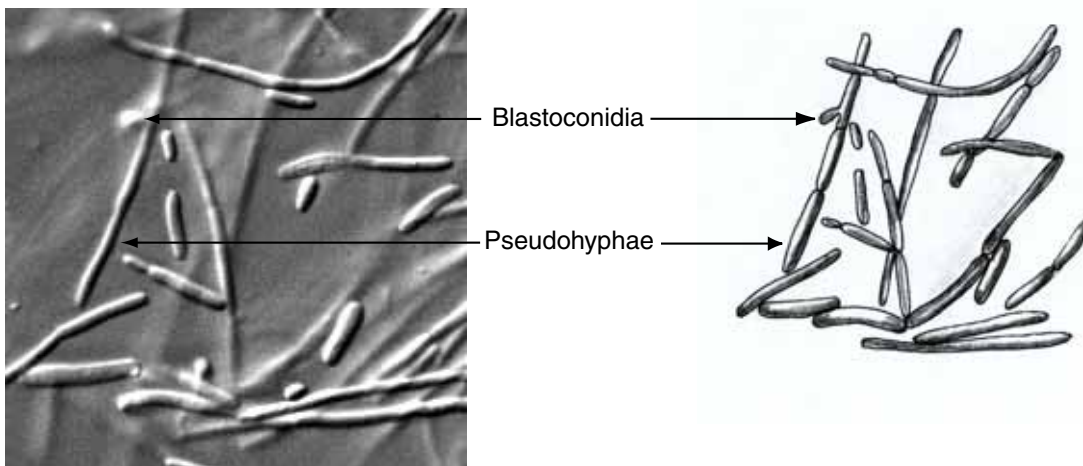
Figure 1



Seven-day-old, white, and wrinkled colony of *Candida boidinii* on Sabouraud's dextrose agar.

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Figure 2



Microscopic morphology of *Candida boidinii* on cornmeal agar showing pseudohyphae consisting of long branched chains of cells with blastoconidia (left; 400× magnification, right; line drawing not to scale).

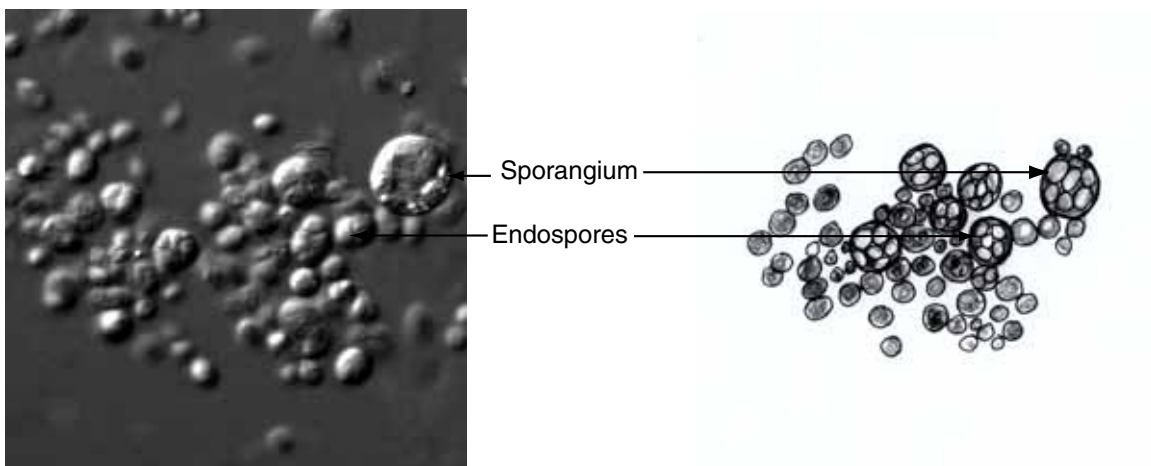
Figure 3



Seven-day-old, moist, cream-colored colony of *Prototheca wickerhamii* on Sabouraud's dextrose agar.

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Figure 4



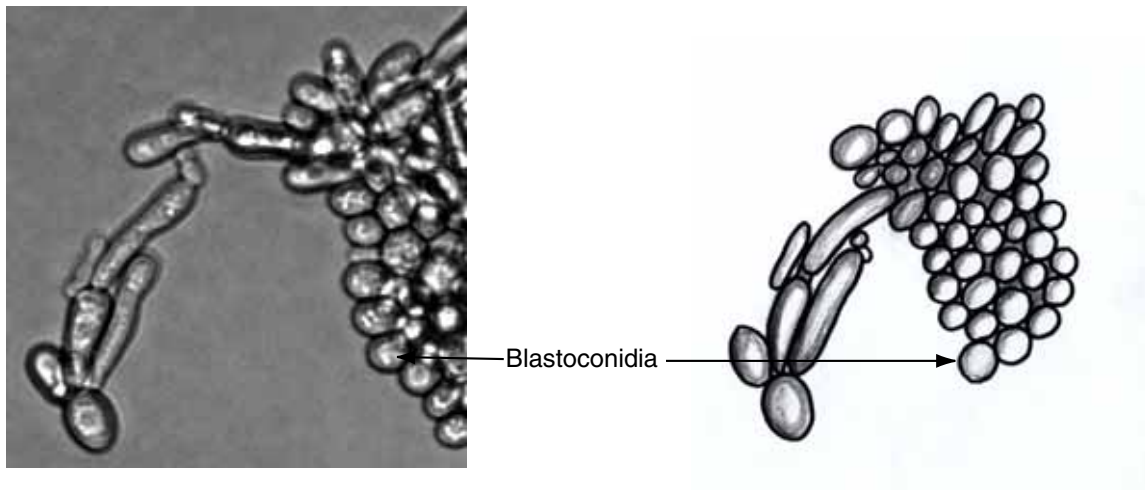
Microscopic morphology of *Prototheca wickerhamii* on cornmeal agar showing sporangia of various sizes, some filled with sporangiospores (endospores) (left; 400× magnification, right; line drawing not to scale).

Figure 5



Seven-day-old, cream, smooth, dull butyrous colony of *Saccharomyces cerevisiae* on Sabouraud's dextrose agar.

Figure 6



Microscopic morphology of *Saccharomyces cerevisiae* on cornmeal agar showing round to oval blastoconidia (left; 400× magnification, right; line drawing not to scale).

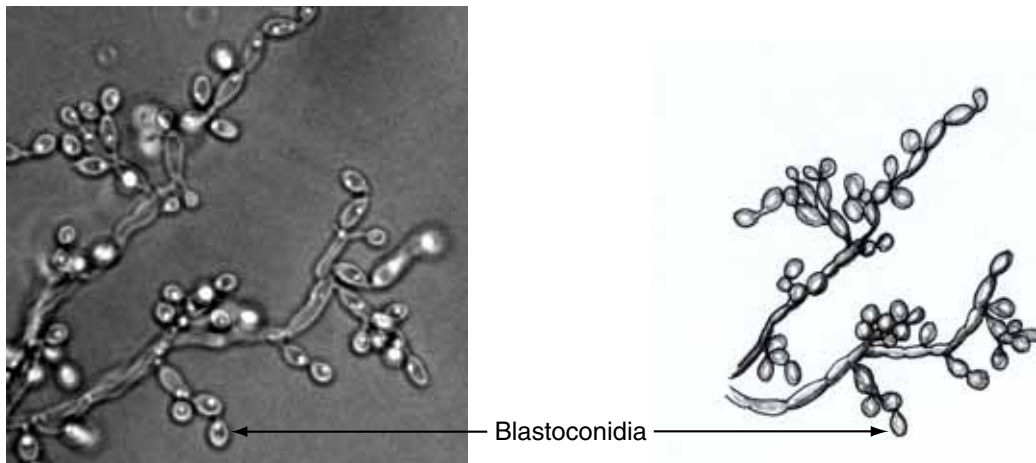
Figure 9



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

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Figure 10



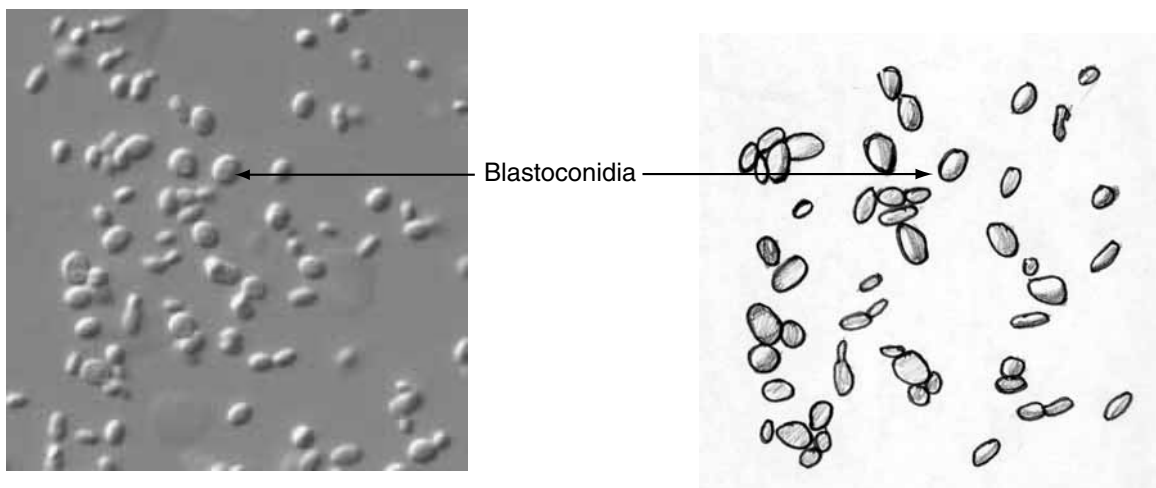
Microscopic morphology of *Candida ciferrii* on cornmeal agar showing blastoconidia forming short chains (left; 400× magnification, right; line drawing not to scale).

Figure 11



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

Figure 12



Microscopic morphology of *Candida magnoliae* showing globose to oval blastoconidia (left; 400× magnification, right; line diagram not to scale).

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