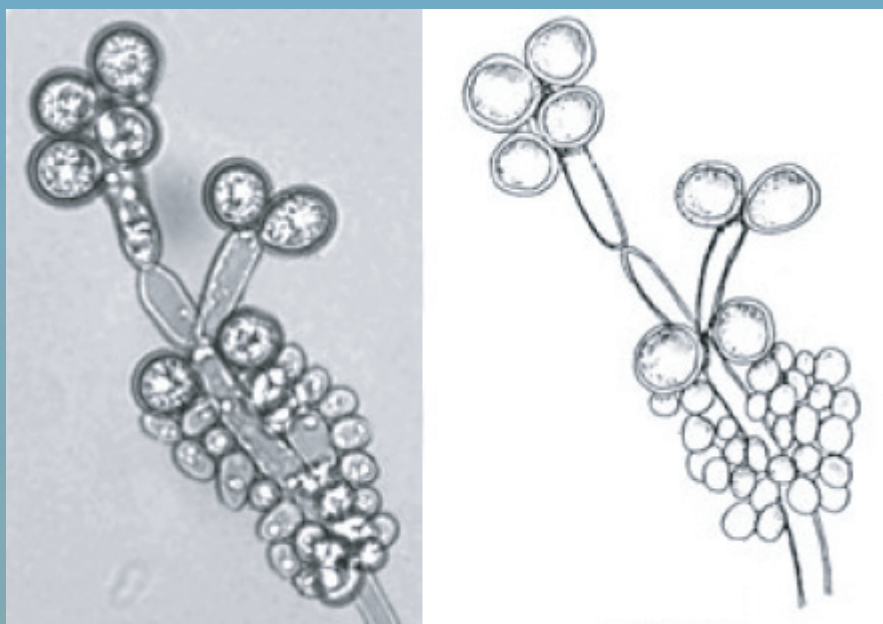


MYCOLOGY

CRITIQUE



Mycology Proficiency Testing Program
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Wadsworth Center

New York State Department of Health

Mycology PT Program

Dr. Vishnu Chaturvedi, Director

Dr. Rama Ramani, Proficiency Testing Coordinator

Contact

Mycology Laboratory
Wadsworth Center
New York State Department of Health
120 New Scotland Avenue
Albany, NY 12208

Phone: (518) 474-4177

Fax: (518) 486-7971

E-mail: mycology@wadsworth.org

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Test Specimens and Grading Policy

Test Specimens

A minimum of two strains each of the proposed mold specimens was examined for inclusion in the proficiency test event of September 2004. The colony morphology of these strains was studied on Sabouraud dextrose agar. The microscopic morphologic features were examined by potato dextrose agar slide cultures. The physiological characteristics, such as cycloheximide sensitivity and growth at higher temperatures, were investigated with the appropriate test media. The single strain that best demonstrated the morphologic and physiologic characteristics was used in the test. Similarly, two or more strains of each of the proposed yeast pathogens were examined for inclusion in the proficiency test. The colony morphology of all yeast strains was studied on corn meal agar with Tween 80 plates inoculated by Dalmau or streak-cut method. Carbohydrate assimilation was studied with the API 20C AUX identification kit. The fermentations of carbohydrates, i.e., glucose, maltose, sucrose, lactose, trehalose, and cellobiose, were also investigated. Additionally, physiologic characteristics, such as nitrate assimilation, urease activity, and cycloheximide sensitivity were investigated with the appropriate test media. The single strain that best demonstrated the morphologic and physiologic characteristics of each of the proposed yeast pathogens was used in the test.

Grading Policy

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

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

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 X 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 X 5 = 100.

For *Cryptococcus* antigen test, laboratories are evaluated on the basis of their responses and on overall performance for all the analytes tested in the Direct Detection category. Appropriate responses are determined by participant consensus requiring 80% agreement in the test. Qualitative/quantitative results are graded in relation to results given by participants for specific test kits. When the number of participants that used a specific test kit is less than 6, results are graded considering results given for the method used. Target values and acceptable ranges are mean value +/- 2 dilutions or positive or negative. When both qualitative and quantitative results are reported ten points will be deducted for each incorrect result. When only qualitative OR quantitative results are reported twenty points will be deducted from each incorrect result.

Laboratory failure to attain an overall score of at least 80% is unsatisfactory performance. Laboratories failing two out of three consecutive proficiency test events for the permit category will fail the proficiency-testing program for the category.

*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 – General & Yeast Only Specimen Key

Y-1 *Candida krusei*
 Y-2 *Candida tropicalis*
 Y-3 *Candida glabrata*
 Y-4 *Candida dubliniensis*
 Y-5 *Candida albicans/guilliermondii*
 Ed. Sp. *Cryptococcus gattii*

Validated Specimen

Candida krusei
Candida tropicalis
Candida glabrata

Other Acceptable Answers

Candida krusei/inconspicua
Torulopsis glabrata

Mycology – Antifungal Susceptibility Testing for Yeasts

Specimen Key

S-1 *C. krusei* ATCC 6258
 S-2 *C. tropicalis* ATCC 750
 S-3 *C. parapsilosis* ATCC 22019
 S-4 *C. albicans* ATCC 90028
 S-5 *C. albicans* ATCC 24433

Reference range (µg/ml)

	Amphotericin B	Fluconazole
S-1	0.5 – 2.0	16.0 – 64.0
S-2	0.5 – 2.0	1.0 – 4.0
S-3	0.25 – 1.0	2.0 – 8.0
S-4	0.5 – 2.0	0.25 – 1.0
S-5	0.25 – 1.0	0.25 – 1.0

Validated range (µg/ml)

	Amphotericin B	Fluconazole
S-1	0.25 – 4.0	32.0 – 128.0
S-2	0.25 – 4.0	0.5 – 8.0
S-3	0.12 – 2.0	1.0 – 16.0
S-4	0.25 – 4.0	0.12 – 2.0
S-5	0.12 – 2.0	0.12 – 2.0

Mycology – Direct detection (*Cryptococcus* Antigen Test)

Specimen Key

Cn-Ag-1 Positive (1:32)
 Cn-Ag-2 Negative
 Cn-Ag-3 Negative
 Cn-Ag-4 Negative
 Cn-Ag-5 Negative

Validated Specimen

Positive (1:32)
 Negative
 Negative
 Negative
 Negative

Other Acceptable Titer Range

1:8 - 1:128

Laboratory Results

Mycology - Yeast Only

	Correct Responses/ Total # Laboratories (%)	Referees (%)
Y-1 <i>Candida krusei</i>	138/140 (99)	14/14 (100)
Y-2 <i>Candida tropicalis</i>	138/140 (99)	14/14 (100)
Y-3 <i>Candida glabrata</i>	139/140 (99)	14/14 (100)
Y-4 <i>Candida dubliniensis</i>	102/140 (73)	11/14 (79)
Y-5 <i>Candida albicans/guilliermondii</i>	92/140 (66)	6/14 (43)
Ed. Sp. <i>Cryptococcus gatti</i>	7/140 (5)	0/14 (0)

Mycology - Antifungal Susceptibility Testing for Yeasts

	Correct Responses/Total # Laboratories (%)	
	Amphotericin B	Fluconazole
S- 1 <i>C. krusei</i> ATCC 6258	21/21 (100)	26/26 (100)
S- 2 <i>C. tropicalis</i> ATCC 750	20/21 (96)	26/26 (100)
S- 3 <i>C. parapsilosis</i> ATCC 22019	21/21(100)	26/26 (100)
S- 4 <i>C. albicans</i> ATCC 90028	20/21 (96)	24/26 (93)
S- 5 <i>C. albicans</i> ATCC 24433	21/21 (100)	24/26 (93)

Mycology - Direct detection (*Cryptococcus* Antigen Test)

	Correct Responses/Total # Laboratories (%)	
	Qualitative	Quantitative
Cn-Ag-1 Positive (1:32)	83/83 (100)	78/83 (94)
Cn-Ag-2 Negative	83/83 (100)	NA
Cn-Ag-3 Negative	82/83 (99)	1/83
Cn-Ag-4 Negative	82/83 (99)	1/83
Cn-Ag-5 Negative	85/85 (100)	NA

	General & Yeast Only	Antifungal Susceptibility Testing for Yeasts	Direct Detection
Number of participating laboratories	140	26	83
Number of referee laboratories	14	3	9
Number of laboratories responding by deadline	140	26	83
Number of laboratories responding after deadline	0	0	0
Number of laboratories not responding	0	0	0
Number of laboratories successfully completing this test	140	25	83
Number of laboratories unsuccessfully completing this test	0	1	0

Number of Laboratories Using Commercial Yeast Identification System*

API 20C AUX	84
AMS Vitek system	52
Remel Uni-Yeast-Tek	1
IDS Rapid System	0
Microscan	2

Number of Laboratories Using Antifungal Susceptibility System/Method

YeastOne Colorimetric microdilution method	15
Broth microdilution method	7
Etest	3
Disk diffusion method	1

Number of Laboratories Using Commercial *Cryptococcus neoformans* Antigen Detection System*

EIA method	3
Meridien Diagn.	3
Latex Agglutination method	65
Meridien Diagn.	34
Murex	18
Wampole	12
Immuno-Mycologics	1
Not specified	15

(*Includes multiple systems used by some laboratories)

Source: Catheter

Scoring:	No. Labs
Referee Labs with correct ID:	14
Labs with correct ID:	139
Labs with incorrect ID:	1
(<i>Candida lambica</i>)	(1)

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

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

Laboratory Diagnosis:

1. Culture – On Sabouraud’s dextrose agar, after 7 days at 25°C, *C. krusei* colony was dry but soft, cream to buff, glassy and wrinkled (Figure 1).
2. Microscopic morphology – On Corn meal agar with Tween 80, *C. krusei* showed branched pseudohyphae with elongated blastoconidia (Figure 2).
3. Differentiation from other yeasts – *C. krusei* ferments glucose, but not sucrose or cellobiose, and does not grow on the media containing cycloheximide. It does not assimilate sucrose, differentiating it from *C. parapsilosis* and *C. lusitanae*. It grows well at 42°C, differentiating it from *C. lambica*. It does not produce arthroconidia, thus differentiating it from *Blastoschizomyces capitatus*.
4. *In vitro* susceptibility testing – Clinical isolates are susceptible to amphotericin B and flucytosine. *C. krusei* is innately resistant to fluconazole and variably resistant to other azoles such as itraconazole and ketoconazole.
5. Molecular tests – DNA probes have been designed from the ITS regions and were incorporated into a reverse hybridization line probe assay for the detection of ITS PCR products for identification of fungal pathogens. Panfungal PCR and multiplex liquid hybridization were developed for the detection of clinically important yeasts in tissue specimens. PFGE, RFLP, and RAPD procedures were used for DNA fingerprinting and electrophoretic karyotyping of oral *C. krusei* isolates.

Comments: All labs except one identified this organism correctly. One laboratory misidentified it as *C. lambica*, which can be differentiated by sucrose assimilation test and growth at 42°C.

Further Reading:

1. Munoz, P., Sanchez-Somolinos, M., Alcalá, L., Rodríguez-Creixems, M., Peláez, T., and Bonza, E. 2005. *Candida krusei* fungaemia: antifungal susceptibility and clinical presentation of an uncommon entity during 15 years in a single general hospital. *J Antimicrobial Chemotherapy*. 55: 188 –193.
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3. Dassanayake, R.S., Samaranyake, Y.H., and Samaranyake, L.P. 2000. Genomic diversity of oral *Candida krusei* isolates as revealed by DNA fingerprinting and electrophoretic karyotyping. *APMIS*. 108: 697-704.
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5. Shemer, R., Weissman, Z., Hashman, N., and Kornitzer, D. 2001. A highly polymorphic degenerate microsatellite for molecular strain typing of *Candida krusei*. *Microbiol*. 147: 2021-2028.
6. Sancak, B., Rex, JH., Chen, E., and Marr, K. 2004. Comparison of PCR- and *Hinf*I Restriction endonuclease-based methods for typing of *Candida krusei* isolates. *J Clin Microbiol*. 42: 5889 – 5891.

Y-2 *Candida tropicalis*

Source: Blood

Scoring:	No. Labs
Referee Labs with correct ID:	14
Labs with correct ID:	139
Labs with incorrect ID:	1
<i>Candida norvegensis</i>	(1)

Clinical Significance: *Candida tropicalis* is a frequent casual agent of sepsis, wound infections, and disseminated infections in immunocompromised patients.

Ecology: *C. tropicalis* is cosmopolitan, found in water and in alimentary tract of lower mammals, and in humans.

Laboratory Diagnosis:

1. Culture – On Sabouraud's dextrose agar after 7 days at 25°C, colony was smooth to wrinkled, cream-colored, rapid-growing (Figure 3).

2. Microscopic morphology – On Corn meal agar with Tween 80, *C. tropicalis* showed long true hyphae and pseudohyphae, with either single or small clusters of blastoconidia (Figure 4).

3. Differentiation from other yeasts – *C. tropicalis* is differentiated from *C. albicans* and *C. dubliniensis* by variable growth on media containing cycloheximide, and by its fermentation of glucose, maltose, sucrose, and trehalose. Occasionally, *C. tropicalis* can produce chlamydospores on corn meal agar.

4. In vitro susceptibility testing – Few strains of *C. tropicalis* has been reported with high amphotericin B MIC. *C. tropicalis* is generally susceptible to azoles, but variably susceptible to flucytosine.

5. Molecular tests – Reverse-hybridization line probe assay combined with PCR amplification of internal transcribed-spacer (ITS) regions was used for rapid identification of clinically significant fungal pathogens including *C. tropicalis*. The combination of pan-fungal PCR and multiplex liquid hybridization of ITS regions was developed for detection and identification of fungi in tissue specimens.

Comments: All labs except one identified this organism correctly. One laboratory identified it as *Candida famata*. *C. tropicalis* ferments maltose, while *C. famata* would not ferment.

Further Reading:

1. Hendolin, P.H., Paulin, L., Koukila-Kahkola, P., Anttila, V.J., Malmberg, H., Richardson, M., and Ylikoski, J. 2000. Panfungal PCR and multiplex liquid hybridization for detection of fungi in tissue specimens. *J. Clin. Microbiol.* 38: 4186-4192.
2. Dawson, N.L., Robles, H.A., and Alvarez, S. 2005. Recurrent *Candida tropicalis* meningitis. *Clinical Neurology and Neurosurgery.* 107: 243 – 245.
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6. Sim, JPY., kho, BCS., Liu, HSY., Yung, R., Chan, JCW. 2005. *Candida tropicalis* arthritis of the knee in a patient with acute lymphoblastic leukaemia: successful treatment with caspofungin. *Hong Kong Med J.* 11: 120 – 123.

Source: Urine

Scoring:	No. Labs
Referee Labs with correct ID:	14
Labs with correct ID:	139
Labs with incorrect ID:	1
(Yeast)	(1)

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*

Epidemiology: 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 5).

2. Microscopic morphology – On Corn meal agar with Tween 80, *C. glabrata* blastoconidia are tiny, round or elliptical shaped (Figure 6).

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*.

Comments: All the labs except one identified this organism correctly. One laboratory did not speciate this specimen as they only indentify *C. albicans* from urine.

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. 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.

3. Malani, A., Hmoud, J., Chiu, L., Carver, PL., Bielaczyc, A., kauffman, CA. 2005. *Candida glabrata* fungemia: experience in a tertiary care center. Clin Infect Dis. 41: 975 – 981.

4. Oliveira, ER., Fothergill, AW., Kirkpatrick, WR., Coco, BJ., Patterson, TF., Redding SW. 2005. In vitro interaction of posaconazole and caspofungin against clinical isolates of *Candida glabrata*. Antimicrobial agents Chemotherapy. 49: 3544 – 3545.

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Y-4 *Candida dubliniensis*

Source: Sputum

Scoring:	No. Labs
Referee Labs with correct ID:	11
Labs with correct ID:	102
Labs with incorrect ID:	37
<i>Candida albicans</i>	(37)

History: *Candida dubliniensis* is a chlamydo-spores-positive, germ tube-positive species of *Candida*, which is closely related to *Candida albicans*. It was first described in 1995 by Sullivan et al. from Dublin, Ireland.

Clinical Significance: Isolates 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.

Pathogenicity: The preliminary data suggests that *C. dubliniensis* produces higher levels of proteinase and has more pronounced adherence to the buccal epithelial cells than *C. albicans*. On the contrary, comparisons in mouse model showed that virulence of *C. dubliniensis* is lower than that of *C. albicans*.

Epidemiology: Even though this organism was first isolated and described in Ireland, it is reported from many parts of world including Israel, the Netherlands, USA, etc.

Laboratory Diagnosis:

1. Culture – Phenotypically, *C. dubliniensis* is practically indistinguishable from *C. albicans* (Fig. 7).
2. Microscopic morphology – It has been reported that *C. dubliniensis* produces abundant chlamydo-spores, often in contiguous pairs or triplets but at least one study has not found this to be consistent, and therefore, the relative abundance of chlamydo-spores may not be a definite criterion. As illustrated in the (Fig. 8), the test isolate produced chlamydo-spores in clusters.
3. Differentiation from other yeasts especially *C. albicans* - One physiologic feature that does appear to be fairly stable is that *C. dubliniensis* grows poorly or not at all at 42°C while *C. albicans* grows well at this temperature. Some commercial yeast identification kits such as the API 20C AUX or the ID 32C have the codes for *C. dubliniensis* included in the databases.
4. *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.
5. Molecular tests - Genetically, *Candida 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.

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 and in June 2002 PT event. Although many more labs reported correct ID this time, there were still about 27% labs that failed to identify *C. dubliniensis*. Previously, in June 2002 PT event, 42% labs failed to identify this organism. 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.

Further Reading:

1. Mirhendi, H., Makimura, K., Zomorodian, K., Maeda, N., Ohshima, T., Yamaguchi, H. 2005. Differentiation of *Candida albicans* and *Candida dubliniensis* using a single enzyme PCR-RFLP method. Jpn. J Infect Dis. 58: 235 – 237.
2. Graf, B., Trost, A., Eucker, J., Gobel, UB., Adam, T. 2004. Rapid and simple differentiation of *C. dubliniensis* from *C. albicans*. Diagnostic Microbiology & Infect Dis. 48: 149 – 151.
3. Sullivan, DJ., Moran, GP., Pinjon, E., Al-Mosaid, A., Stokes, C., Vaughan, C., Coleman, DC. 2004. Comparison of the epidemiology, drug resistance mechanisms and virulence of *Candida dubliniensis* and *Candida albicans*. FEMS Yeast Research. 4: 369 – 376.
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5. Jabra-Rizk, MA., Johnson, JK., Forrest, G., Mankers, K., Meiller, TF, Venezia, RA. 2005. Prevalence of *Candida dubliniensis* fungemia at a large teaching hospital. Clinical Infect Dis. 41: 1064 – 1067.
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Source: Bronchial lavage

Scoring:	No. Labs	
Referee Labs with correct ID:	6	
Labs with correct ID:	92	
Labs with incorrect ID:	48	
(<i>Candida guilliermondii</i> only)	(36)	(<i>Candida parapsilosis</i>) (2)
(<i>Candida albicans</i> only)	(45)	(<i>Trichosporon mucoides</i>) (2)
(<i>Candida tropicalis</i>)	(21)	(<i>Candida pelliculosa</i>) (1)
(<i>Candida famata</i>)	(6)	Yeast (3)
(<i>Candida intermedia</i>)	(3)	

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 9).
2. Microscopic morphology – On Cornmeal agar with Tween 80, round blastoconidia bunched together with pseudohyphae are easily seen. Thick walled, mostly terminal chlamydo spores are prominent (Figure 10).
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 chlamydo spores 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:

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Source: Bronchial lavage

Scoring:	No. Labs	
Referee Labs with correct ID:	6	
Labs with correct ID:	92	
Labs with incorrect ID:	48	
(<i>Candida guilliermondii</i> only)	(36)	(<i>Candida parapsilosis</i>) (2)
(<i>Candida albicans</i> only)	(45)	(<i>Trichosporon mucoides</i>) (2)
(<i>Candida tropicalis</i>)	(21)	(<i>Candida pelliculosa</i>) (1)
(<i>Candida famata</i>)	(6)	Yeast (3)
(<i>Candida intermedia</i>)	(3)	

Clinical Significance: *Candida guilliermondii* is a frequent causal agent of nosocomial fungemia in immunosuppressed patient. Also, infrequent casual agent of urinary tract infections, brain abscess, and ocular infections.

Ecology: *C. guilliermondii* 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 11).

2. Microscopic morphology – On Corn meal agar with Tween 80, few short pseudohyphae with clusters of blastoconidia, were seen (Figure 12).

3. Differentiation from other yeasts – *C. guilliermondii* ferments glucose, sucrose, and trehalose, grows at 37°C, and on media containing cycloheximide. It does not form pink pigment thereby differentiating it from *Rhodotorula* species. It does not produce true hyphae, which differentiates it from *Candida visferrii* and *Trichosporon beigelii*. Unlike *Candida lusitaniae*, it is unable to grow at 45°C.

4. *In vitro* susceptibility testing – Most clinical isolates are susceptible to amphotericin B, 5-flucytosine, and azoles such as fluconazole, ketoconazole, and itraconazole. 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 *C. guilliermondii* from *C. famata/Debaryomyces hansenii* complex. Isolates of *C. guilliermondii* were identified using PCR to amplify ribosomal DNA, followed by restriction digestion of the PCR product.

Comments: Y5A and Y5B were sent in as mixed cultures. Forty-three percent referee laboratories and sixty-six percent participating laboratories correctly identified the mixed cultures. Unfortunately, the sample was not validated. It was disconcerting to note that many laboratories reported unrelated species from this specimen.

Further Reading:

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Source: CSF

Clinical Significance: Prior to AIDS epidemic, cryptococcosis was commonly reported from patients with underlying cancer. *Cr. neoformans* consisted of two species: *Cr. neoformans* var. *neoformans* (serotypes A & D) and *Cr. gattii* (serotypes B & C). Recently, *Cr. neoformans* var. *neoformans* was further subdivided to two varieties: *Cr. neoformans* var. *neoformans* (D) and *Cr. neoformans* var. *grubii* (A) (4). *Cr. gattii* causes meningoencephalitis and pulmonary cryptococcosis in patients with normal immune status. *Cr. neoformans* var. *neoformans* and *Cr. neoformans* var. *grubii* infect patients with AIDS or other underlying immune dysfunctions. *Cr. neoformans* var. *neoformans* infections are more likely to have cutaneous involvement, and infect older patients as compared to infections caused by *Cr. neoformans* var. *grubii* (9).

Ecology: *Cr. neoformans* var. *neoformans* and *Cr. neoformans* var. *grubii* are commonly found in avian (pigeon) droppings while *Cr. gattii* is commonly found on various plants and trees such as *Eucalyptus camaldulensis* (red river gum tree).

Epidemiology: Infections caused by *Cr. neoformans* var. *neoformans* are more common in Europe. *Cr. neoformans* var. *grubii* and *Cr. gattii* is worldwide in distribution.

Laboratory Diagnosis:

1. Culture – At 25^o C, on Sabouraud's dextrose agar, mucoid, smooth, moist, initially cream later turning tan colonies seen in 3 – 5 days.
2. Microscopic morphology – On Corn meal agar with Tween – 80, round yeast cells with no pseudohyphae and in India-ink preparation encapsulated yeasts are seen (Fig. 13).
3. Differentiation from other yeasts - *Cr. neoformans* does not ferment any carbohydrates, does not grow on the media containing cycloheximide, and grows at 37^o C. *Cr. neoformans* produces dark brown colonies on niger seed agar. It produces urease enzyme and it is negative on nitrate reaction. On the API 20C AUX, a specific assimilation biocode identifies this organism. The three varieties are differentiated:
 - 1) Growth and color change by *Cr. gattii* on canavanine-glycine-bromthymol blue (CGB) medium to blue-green after 2 – 5 days at 25^o C and on Glycine-Cycloheximide Phenol-red agar (GCP) medium to red after 2-5 days at 25^o C
 - 2) PCR technique can differentiate *Cr. gattii* from the other two varieties (3).
4. *In vitro* susceptibility testing – Most isolates are susceptible to amphotericin B, 5 FC, and to azoles like fluconazole, itraconazole, etc. Few isolates with high MIC to fluconazole have been isolated from AIDS patients.
5. Molecular tests - *Cr. neoformans* is one of the most intensely studied pathogenic fungus. Molecular biology of this organism has revealed various virulence factors. Recently, a direct PCR technique has been developed to differentiate the three varieties of this pathogen (4).

Comments: The organism sent in this testing event was *Cr. gattii*. Out of 140 participating labs, only 7 labs identified it as *Cr. gattii*, while all the other labs identified it as *Cr. neoformans*. This is because many laboratories do not use CGB/BCP media to differentiate between *Cr. neoformans* and *Cr. gattii*.

Further Reading:

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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 on 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-six microbiology laboratories within the United States and one reference laboratory each from Canada and United Kingdom participated in this event. Two NCCLS quality control strains, *Candida krusei* ATCC 6258 (S-1) and *Candida parapsilosis* ATCC 22019 (S-3), three NCCLS reference strains, *Candida albicans* ATCC 90028 (S-4), ATCC 24433 (S-5), and *Candida tropicalis* ATCC 750 (S-2) were included in the May 25, 2005 antifungal proficiency testing event. These isolates have been well characterized, and their MIC ranges against amphotericin B and fluconazole have been published. MICs within ± 2 dilutions of the reference result (range of MICs for a particular yeast described in NCCLS, M27-A2) are the acceptable results in this event.

Results: A total of 26 laboratories participated in this antifungal susceptibility testing event. The performances of all laboratories except one were satisfactory. Of the 26 participating laboratories, 7 laboratories used the broth microdilution method, 15 laboratories used YeastOne Colorimetric microdilution method, and 3 laboratories used Etest and 1 laboratory used disk diffusion method. The supplementary information on antifungal susceptibility testing procedures is summarized in Table 1. The MIC results submitted by the 26 participants are illustrated in Figure 15. Good performance was noted for all 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 98% against fluconazole for all isolates.

Further Reading:

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S-1 *Candida krusei* ATCC 6258

Summary	NCCLS Reference Range	Expanded Range
Amphotericin B	0.5-2.0 µg/ml	0.12-2.0 µg/ml
Fluconazole	16.0-64.0 µg/ml	8.0-128.0 µg/ml

Amphotericin B values and fluconazole values were reported within NCCLS reference range by all the participating laboratories.

S-2 *Candida tropicalis* ATCC 750

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

Amphotericin B values were reported within NCCLS reference range by 20 participating laboratories and one laboratory reported values out of range. Fluconazole values were reported within NCCLS reference range by 26 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 and fluconazole values were reported within NCCLS reference range by all the participating laboratories.

S-4 *Candida albicans* ATCC 90028

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

Amphotericin B values were reported within NCCLS reference range by 20 participating laboratories, and one laboratory reported out of range. Fluconazole values were reported within NCCLS reference range by 21 laboratories, and within the expanded values by 3 laboratories. Fluconazole MIC values higher than the expended range were reported by 2 laboratories.

S-5 *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

Amphotericin B values were reported within NCCLS reference range by 21 participating laboratories. Fluconazole values were reported within NCCLS reference range by 23 laboratories, and within the expanded values by 1 laboratory. Fluconazole MIC values higher than the expended range were reported by 2 laboratories.

Further Reading: (cont'd)

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*A*ntifungal susceptibility testing

Test Method*	No. Participant Laboratories
NCCLS broth microdilution	7
Sensititre YeastOne Colorimetric	15
Etest	3
Disk diffusion	1
Medium employed*	
RPMI 1640	13
RPMI 1640 w / alamar blue	7
Antibiotic medium 3	1
Sabouraud dextrose	2
YeastOne broth	7
Mueller-Hinton Agar + 2% glucose + 0.5 g/ml Methylene Blue	1
Inoculum preparation*	
Spectrophotometric	6
MacFarland	20
Inoculum size (CFU/ml)	
$0.5-2.5 \times 10^3$	8
$1.5-8 \times 10^3$	12
$0.5-1.0 \times 10^4$	3
2.5×10^6	1
MacFarland 0.5	2
Incubation temperature*	
35°C	24
37°C	2
Incubation duration*	
24 hr	16
48 hr	10
Endpoint reading*	
Visual	16
Colorimetric	11
Spectrophotometric	1
Biomic	1
Scoring endpoint¹*	
100% inhibition	10
95% inhibition	1
80% inhibition	5
50% inhibition	4
Color change	9
Prominent decrease	1
QC organism	
NCCLS recommended strains	26
Unknown	0

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

* More than one value reported by individual laboratories

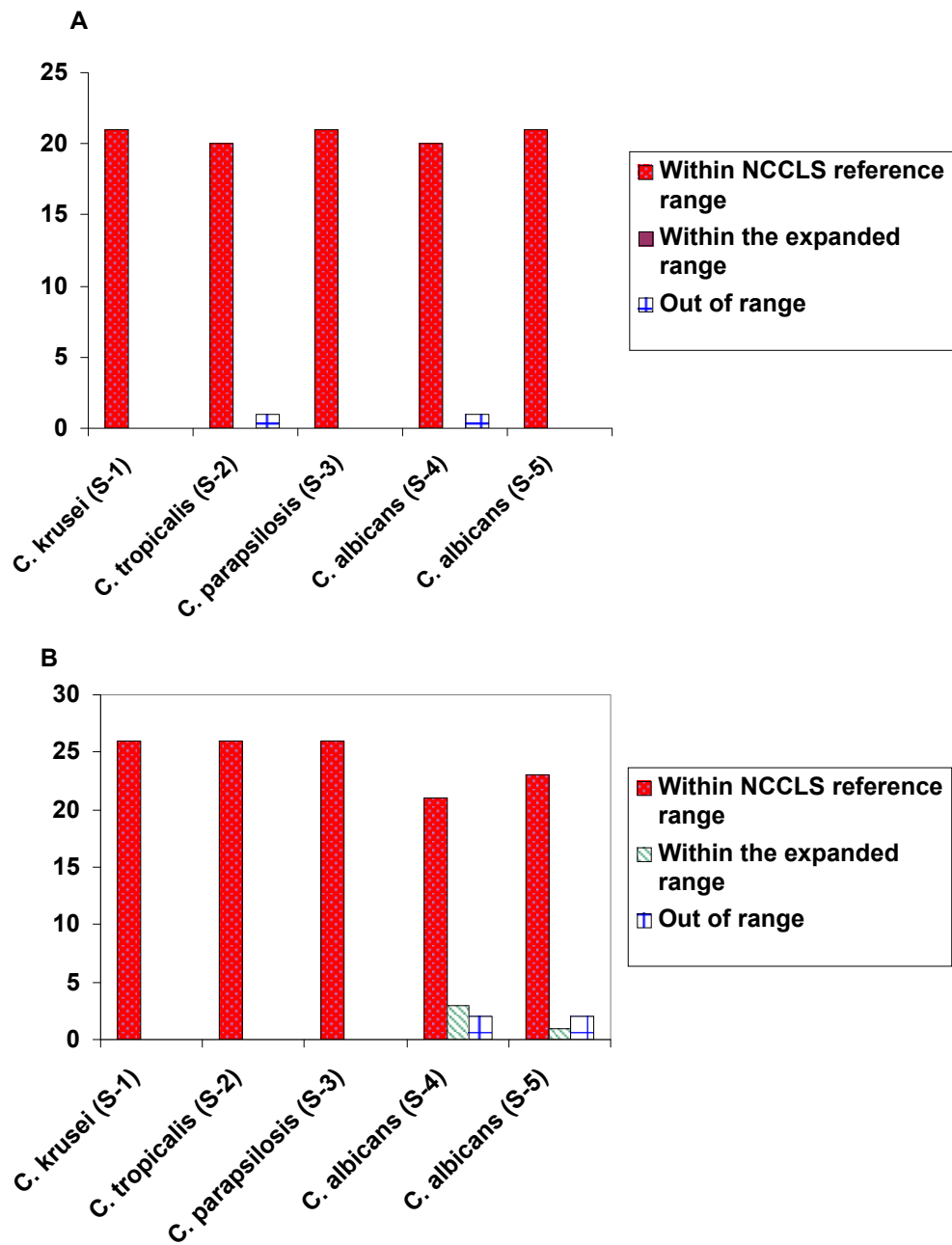


Figure 15. Summary of the results submitted by the participating laboratories for 5 isolates, for amphotericin B (A) and fluconazole (B).

Introduction: A simple, sensitive latex test capable of detecting the capsular polysaccharide of *C. neoformans* in CSF and serum was described, and proven to be superior in sensitivity to the India ink mount (1, 2). Clinical studies established the prognostic value of the test (3, 5, 6 and 7), and showed it to be a valuable aid in establishing a diagnosis when culture was negative (4). Paired serum and CSF specimens allowed detection of antigen in confirmed cases (7). Parallel serologic studies for both antigen and antibody are recommended to ensure detection of extrameningeal cryptococcosis. Newly emerging disease states and therapies have been shown to increase the opportunity for nonspecific interference in some serum specimens. Pretreatment of serum specimens with pronase prior to utilization of the latex agglutination test reduces nonspecific interference and enhances the detection of capsular polysaccharide antigens of *Cryptococcus neoformans*.

Materials & Methods: Eighty-five laboratories within United States participated in this event. Two positive serums for Cryptococcal antigen were included in the May 25, 2004 direct detection antigen testing event. The titer of the serum was 1:32. Titers within ± 2 dilutions of the reference result are the acceptable results in this event.

Results: A total of 85 laboratories participated in this *Cryptococcus neoformans* Antigen Test. The performances of all 85 laboratories were satisfactory. Of the 85 participating laboratories, 74 laboratories used latex agglutination method, 3 laboratories used EIA method, and 8 laboratories did not specify the test method they use. The supplementary information on qualitative and quantitative assays on *Cryptococcus neoformans* antigen test are summarized in Table 2 and 3. Good performance was noted for all specimens irrespective of the methodology used by the laboratories.

Further Reading:

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2. Bloomfield, N., Gordon, M.A., and F-Elmendorf, D., Jr. 1963. Detection of *Cryptococcus neoformans* antigen in body fluids by latex particle agglutination. Proc. Soc. Exp. Bio. Med. 114: 64-67.
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Table 2. Summary of qualitative assay

Method (<i>Manufacture</i>)	Sample Total Number of labs	Cn-Ag- 1 N/P*	Cn-Ag- 2 N/P	Cn-Ag- 3 N/P	Cn-Ag- 4 N/P	Cn-Ag- 5 N/P
EIA (<i>Meridien Diagn.</i>)	3	0/3	3/0	3/0	3/0	3/0
Latex Agglutination (<i>Meridien Diagn.</i>)	65	0/65	65/0	64/1	64/1	65/0
(<i>Murex</i>)	34	0/34	34/0	34/0	33/1	34/0
(<i>Wampole</i>)	18	0/18	18/0	18/0	18/0	18/0
(<i>Immuno-Mycologics</i>)	12	0/12	12/0	11/1	12/0	12/0
	1	0/1	1/0	1/0	1/0	1/0
Not specified	15	0/15	15/0	15/0	15/0	15/0

* N/P: number of laboratories reported Negative vs. number of laboratories reported Positive

Table 3. Summary of quantitative assay

The number of laboratories that reported titers is listed for positive test samples Cn-Ag-1.

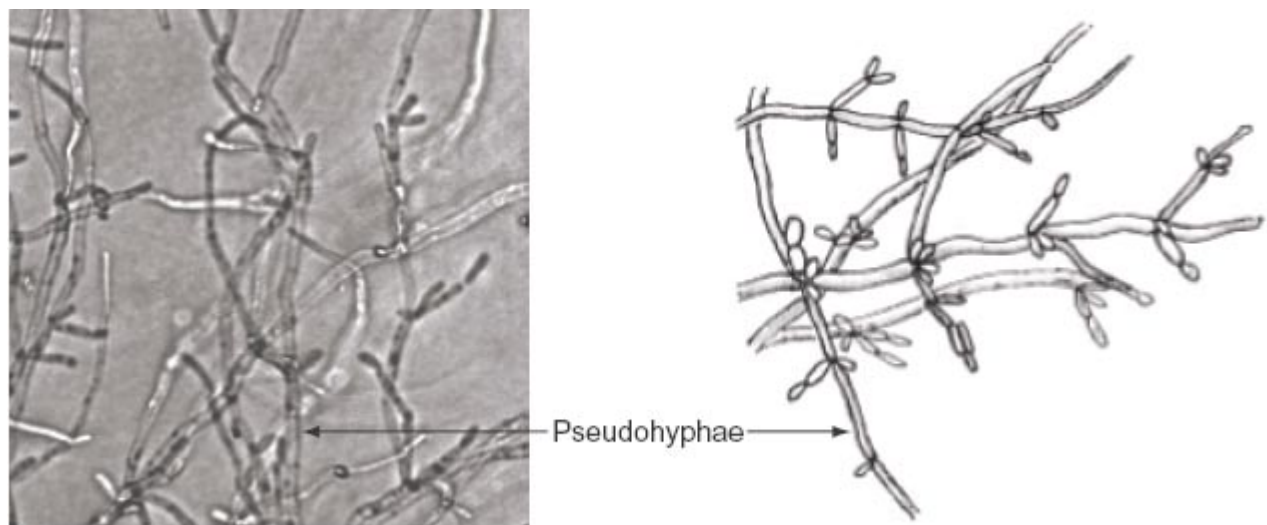
Method (<i>Manufacture</i>)	Sample Total # of labs	Cn-Ag-1 Titers						
		4	8	16	32	64	128	256
EIA (<i>Meridien Diagn.</i>)	3				3			
Latex Agglutination (<i>Meridien Diagn.</i>)	34	1	2	7	18	6		
(<i>Murex</i>)	18		6	2	4	2	4	
(<i>Wampole</i>)	12	1	2	4		1	2	2
(<i>Immuno-Mycologics</i>)	1			1				
Not specified	15	1	2		3	7	2	

Figure 1



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

Figure 2



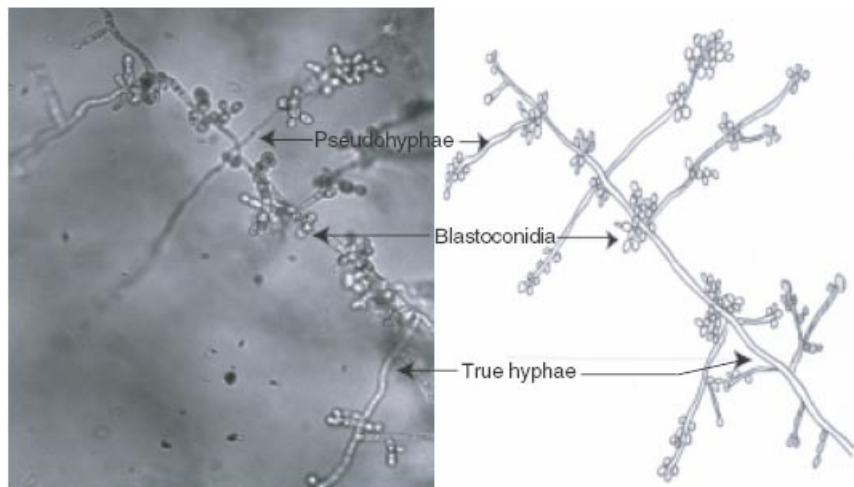
Microscopic morphology of *Candida krusei* on corn meal agar showing long, branched pseudohyphae with oval blastoconidia (left; 200X magnification, right; line drawing not to scale).

Figure 3



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

Figure 4



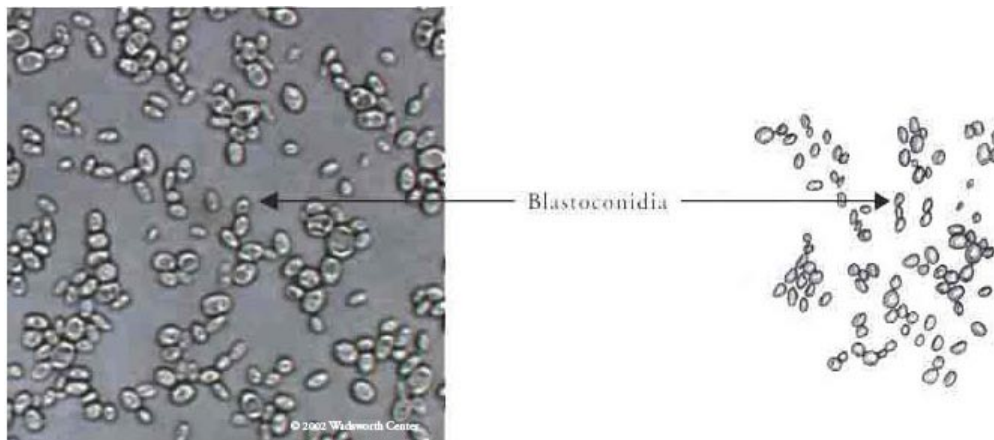
Microscopic morphology of *Candida tropicalis*, on corn meal agar with Tween 80, shows long true hyphae and pseudohyphae with clusters of blastoconidia (left; 400X magnification, right; line drawing not to scale).

Figure 5



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

Figure 6



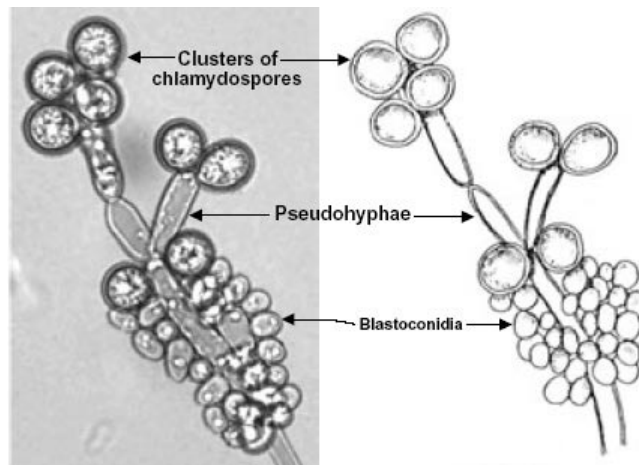
Microscopic morphology of *Candida glabrata* on corn meal agar with Tween 80 shows elliptical shaped blastoconidia (left; 400 X magnification, right; line diagram not to scale).

Figure 7



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

Figure 8



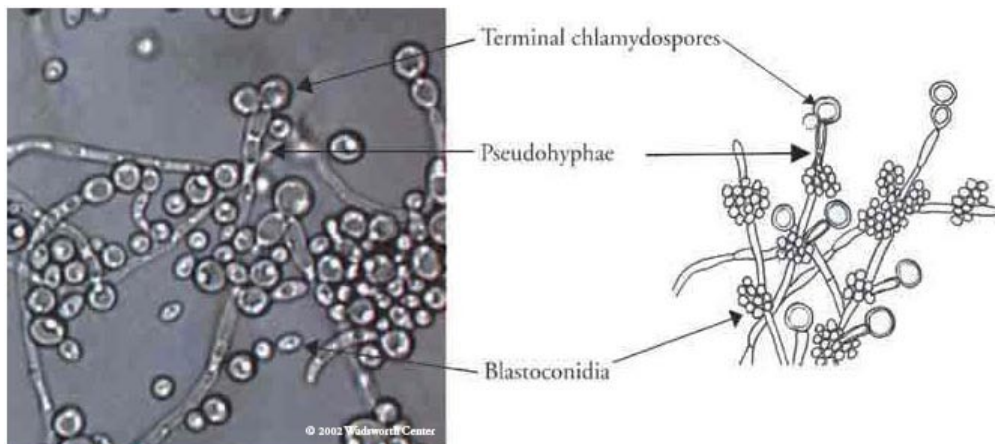
C. dubliniensis on cornmeal agar showing clusters of chlamyospores and blastoconidia (left, 400X magnification; line drawing on right not to scale).

Figure 9



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

Figure 10



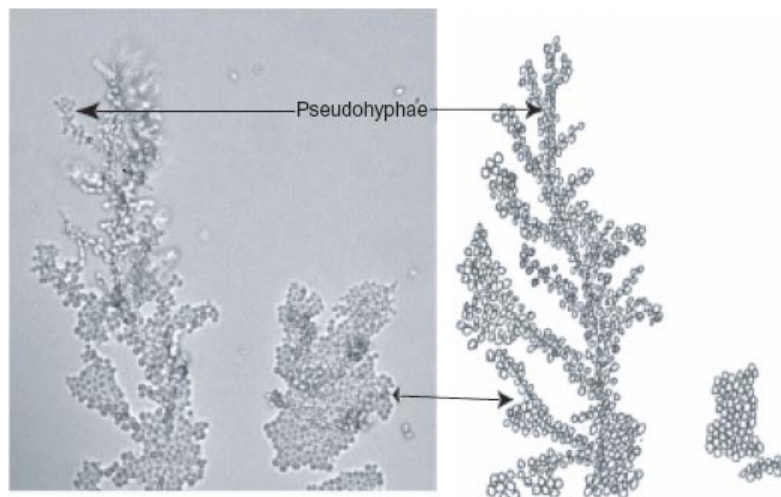
Microscopic morphology of *Candida albicans* on corn meal agar with Tween 80 shows terminal chlamydoconidia on pseudohyphae with blastoconidia (left; 400 X magnification, right; line diagram not to scale).

Figure 11



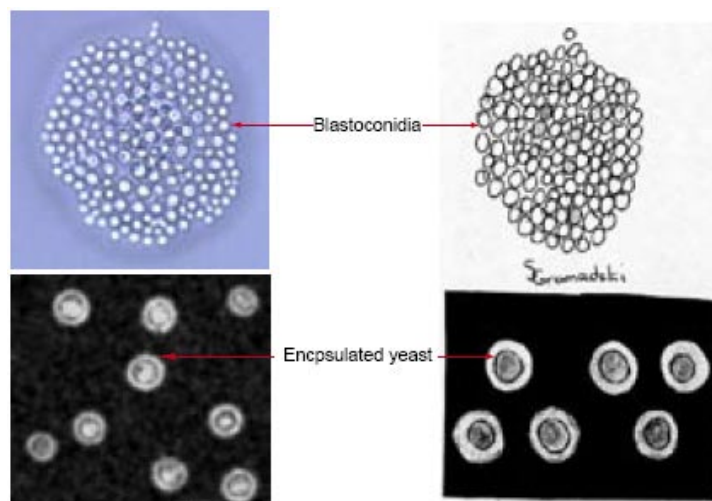
Seven-day-old, flat, smooth, cream-yellow colony of *Candida guilliermondii* on Sabouraud's dextrose agar.

Figure 12



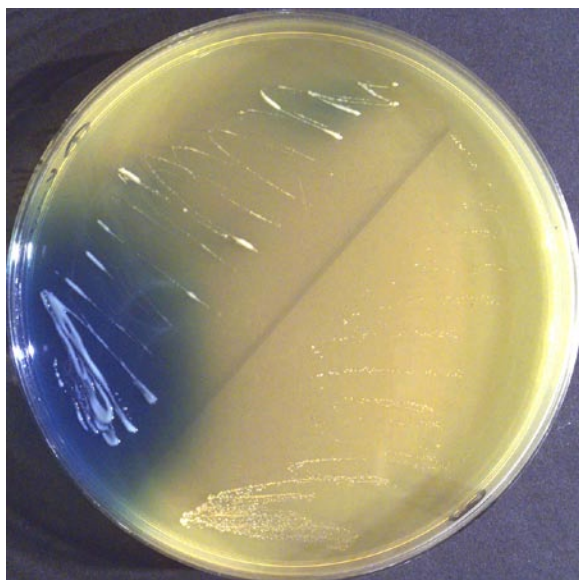
Microscopic morphology of *Candida guilliermondii*. On corn meal agar with Tween 80 culture, short pseudohyphae with clusters of blastoconidia are seen (left; 200X magnification, right; line drawing not to scale).

Figure 13

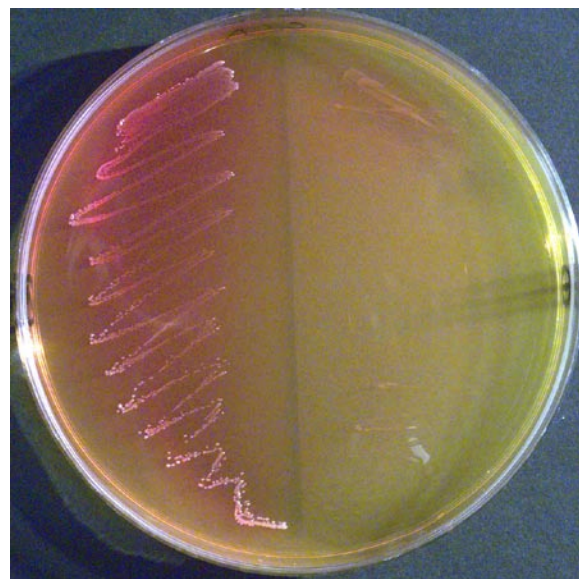


Microscopic morphology of *Cryptococcus gattii* on cornmeal agar showing round blastoconidia (left; 200X magnification, right; line drawing not to scale) and India-ink preparation demonstrating capsule (400X magnification).

Figure 14



One half of plate illustrating two day old colonies of *Cryptococcus gattii* on CBG agar, turning the media blue-green, while the other half illustrating colonies of *Cryptococcus neoformans* var. *grubii* not turning the media color.



One half of plate illustrating two day old colonies of *Cryptococcus gattii* on GCP agar, turning the media red, while the other half illustrating colonies of *Cryptococcus neoformans* var. *grubii* not turning the media color.

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