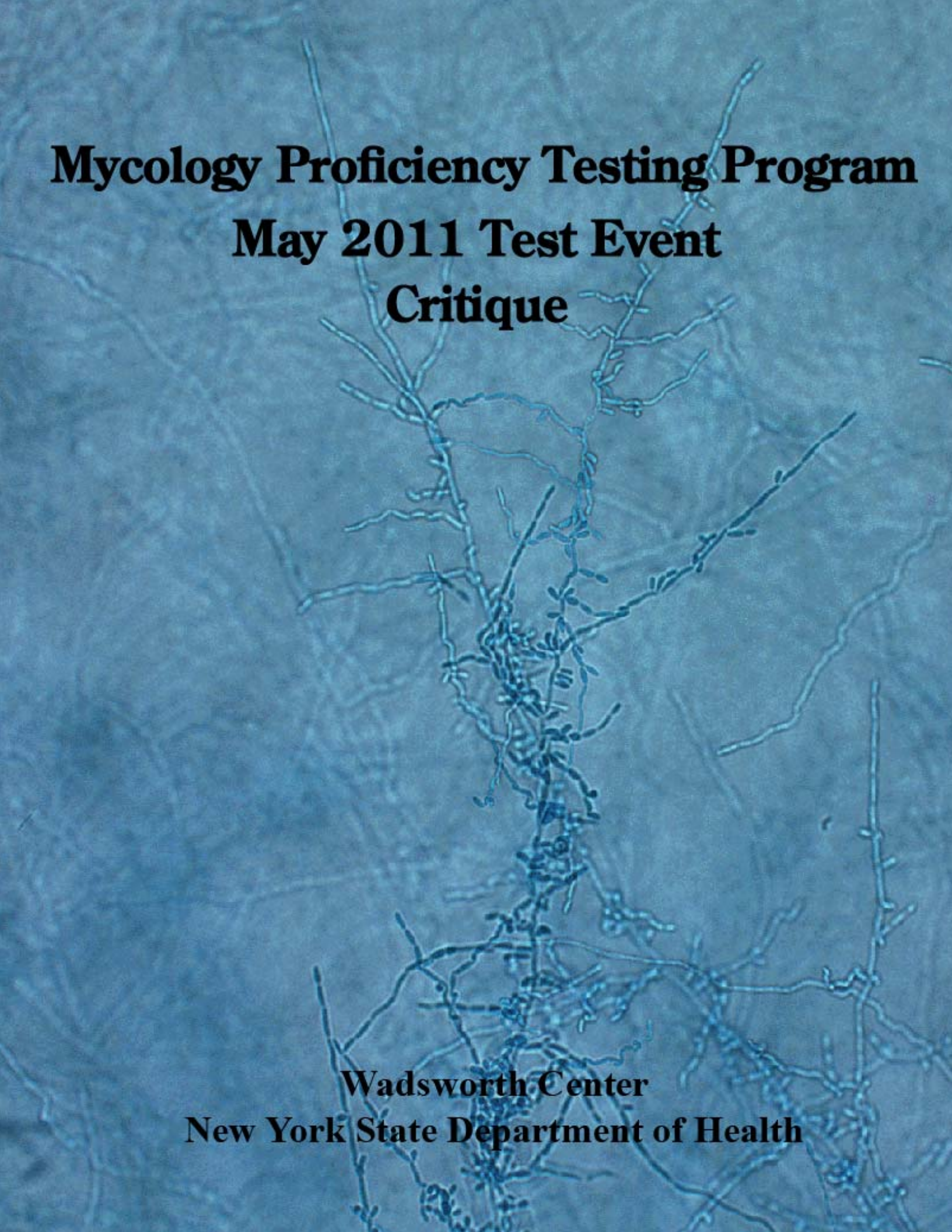
Mycology Proficiency Testing Program
May 2011 Test Event
Critique



Wadsworth Center
New York State Department of Health

Dr. Vishnu Chaturvedi, Director

Dr. Ping Ren, Proficiency Testing Program Coordinator

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: mycologypt@wadsworth.org

CONTENTS

Schedule of 2011 Mycology PT Mailouts*

CATEGORY	POSTMARK DEADLINES
Mycology Identification	**Mycology Identification**
January 26, 2011 May 25, 2011 September 27, 2011	March 11, 2011 June 17, 2011 November 14, 2011
Mycology Identification - Yeast Only	**Mycology Identification - Yeast Only**
January 26, 2011 May 25, 2011 September 27, 2011	February 18, 2011 June 17, 2011 November 14, 2011
Mycology Susceptibility	**Mycology Susceptibility**
January 26, 2011 May 25, 2011 September 27, 2011	February 18, 2011 June 17, 2011 November 14, 2011
Mycology Direct Detection	**Mycology Direct Detection**
January 26, 2011 September 27, 2011	February 11, 2011 November 14, 2011

*Mycology PT Program has a set of standard test strains, which typically represent characteristic features of the respective species. These strains will be made available to the participating laboratories for educational purposes. For practical reasons, no more than two strains will be shipped at any given time subject to a maximum of five strains per year. Preference will be given to laboratories that request test strains for remedial purposes following unsatisfactory performance.

TEST SPECIMENS AND GRADING POLICY

Test Specimens*

Two or more strains of yeast species 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 using classical approaches. Additional 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 the proposed test analyte was selected. Finally, ITS1 – ITS2 region of ribosomal genes was amplified, sequenced, and BLAST searched in two databases.

Grading Policy

A laboratory's response for each sample is compared with the response that reflects 80 percent agreement of 10 referee laboratories and/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 score in each event is established by total number of correct responses submitted by the laboratory divided by the number of organisms present plus the number of incorrect organisms reported by the laboratory multiplied by 100 as the formula shown below.

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

Acceptable results for antifungal susceptibility testing are based on consensus MIC values +/- 2 dilutions or interpretation per CLSI (NCCLS) guidelines or related, peer-reviewed publications. One yeast is to be tested against following drugs: amphotericin B, anidulafungin, caspofungin, flucytosine (5-FC), fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. The participating laboratories are allowed to select any number of antifungal drugs from the test panel based upon test practices in their facilities. A maximum score of 100 will be equally distributed to account for the drugs selected by an individual laboratory. If the result for any drug is incorrect then laboratory gets a score of zero for that particular test component or set.

A failure to attain an overall score of 80% is considered unsatisfactory performance. Laboratories receiving unsatisfactory scores in two out of three consecutive proficiency test events may be subject to 'cease testing'.

*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.

ANSWER KEY AND LABORATORY PERFORMANCE

Mycology – Identification and Identification Yeast Only

	Specimen Key	Validated Specimen	Other Acceptable Answers	Correct Responses / Total # Laboratories (%)
Y-1	*Candida albicans*	*Candida albicans*		121/122 (99)
Y-2	*Candida lipolytica*	*Candida lipolytica*		113/121 (93)
Y-3	*Candida zeylanoides*	*Candida zeylanoides*		121/121 (100)
Y-4	*Cryptococcus neoformans*	*Cryptococcus neoformans*		121/121 (100)
Y-5	*Geotrichum candidum*	*Geotrichum candidum*	*Geotrichum klebahnii* *Geotrichum penicillatum* *Geotrichum* sp.	121/121 (100)

Mycology – Antifungal Susceptibility Testing for Yeasts (S-1: *Candida albicans* M954)

Drugs	Acceptable MIC (μg/ml) Range	Acceptable Interpretation	Acceptable Responses/Total # Laboratories (%)
Amphotericin B	0.25 – 1	Susceptible / No interpretation	24/24 (100)
Anidulafungin	≤ 0.015 – 0.12	Susceptible	17/17 (100)
Caspofungin	0.03 – 0.5	Susceptible	22/22 (100)
Flucytosine (5-FC)	0.03 – 0.5	Susceptible	27/27 (100)
Fluconazole	≥ 64	Resistant	33/33 (100)
Itraconazole	0.25 – 2	Susceptible-dose dependent / Resistant	31/31 (100)
Ketoconzole	0.12 – 3	Susceptible / No interpretation	6/6 (100)
Micafungin	0.015 – 0.12	Susceptible	17/17 (100)
Posaconazole	0.25 – 2	Susceptible / No interpretation	18/18 (100)
Voriconazole	0.5 – 2	Susceptible / Susceptible-dose dependent	24/24 (100)

TEST STATISTICS

	General	Yeast Only	Antifungal Susceptibility Testing for Yeasts
Number of participating laboratories	69	53	33
Number of referee laboratories	10	10	33
Number of laboratories responding by deadline	69	53	33
Number of laboratories responding after deadline	0	0	0
Number of laboratories not responding	0	0	0
Number of laboratories successfully completing this test	69	52	33
Number of laboratories unsuccessfully completing this test	0	1	0

Number of Laboratories Using Commercial Yeast Identification System*

API 20C AUX	92
Vitek	13
Vitek2 system	55
Remel Uni-Yeast-Tek	10
Microscan	4
Other	15

Number of Laboratories Using Commercial Antifungal Susceptibility Testing System/Method*

TREK Diagnostic System Sensititre YeastOne Panel	25
Etest	5
Disk diffusion	1
Viteck 2	1
Others†	3

*Include multiple systems used by some laboratories
†Include laboratories using CLSI Microbroth dilution method

YEAST DESCRIPTIONS

Y-1 *Candida albicans*

Source: Blood / Urine / Skin

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	121
Laboratories with incorrect ID:	1
(*Candida dubliniensis*)	(1)
Outcome:	Validated

Clinical Significance: *Candida albicans* is the most common cause of candidiasis. It is ubiquitous in humans who probably encounter it initially during passage through the birth canal. The serious infections are 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 were white to cream, glossy, smooth and soft (Figure 1).
2. Microscopic morphology – On cornmeal agar with Tween 80, round blastoconidia bunched together with pseudohyphae were easily seen. Thick walled, mostly terminal chlamydospores were prominent (Figure 2).
3. Differentiation from other yeasts – By morphological criterion, *C. albicans* is difficult to distinguish from *C. dubliniensis*. However, *C. albicans* grows well at 42°C and 45°C, but *C. dubliniensis* grows poorly or not at all at 42°C or 45°C. *C. dubliniensis* generally produces more abundant chlamydospores than *C. albicans*. If the CHEOMagar was used for diagnosis, bluish green color distinguishes *C. albicans* from dark-green color of *C. dubliniensis*. The positive germ tube test for *C. albicans* distinguishes it from *C. tropicalis*.
4. *In vitro* susceptibility testing – *C. albicans* is sensitive to amphotericin B, anidulafungin, caspofungin, micafungin, fluconazole, and posaconazole. Fluconazole-resistant isolates of *C. albicans* are also reported.
5. Molecular tests – Molecular tests are available for identification of *C. albicans*. A large number of DNA typing and nucleotide sequencing methods are available for molecular epidemiology of *C. albicans* strains.

Comments: One laboratory reported this specimen as *C. dubliniensis,* which can be distinguished by use of commercially available yeast identification systems and poor to no growth at 45°C.

Sequences alignment:

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA.

```
Query  1    CATTACTGATTTGCTTAATTGCACCACATGTGTTTTTCTTTGAAACAAACTTGCTTTGGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  30   CATTACTGATTTGCTTAATTGCACCACATGTGTTTTTCTTTGAAACAAACTTGCTTTGGC  89
```

```
Query  61   GGTGGGCCCAGCCTGCCGCCAGAGGTCTAAACTTACAACCAATTTTTTATCAACTTGTCA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  90   GGTGGGCCCAGCCTGCCGCCAGAGGTCTAAACTTACAACCAATTTTTTATCAACTTGTCA  149

Query  121  CACCAGATTATTACTAATAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  150  CACCAGATTATTACTAATAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGA  209

Query  181  TGAAGAACGCAGCGAAATGCGATACGTAATATGAATTGCAGATATTCGTGAATCATCGAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  210  TGAAGAACGCAGCGAAATGCGATACGTAATATGAATTGCAGATATTCGTGAATCATCGAA  269

Query  241  TCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  270  TCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTT  329

Query  301  TCTCCCTCAAACCGCTGGGTTTGGTGTTGAGCAATACGACTTGGGTTTGCTTGAAAGACG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  330  TCTCCCTCAAACCGCTGGGTTTGGTGTTGAGCAATACGACTTGGGTTTGCTTGAAAGACG  389

Query  361  GTAGTGGTAAGGCGGGATCGCTTTGACAATGGCTTAGGTCTAACCAAAAACATTGCTTGC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  390  GTAGTGGTAAGGCGGGATCGCTTTGACAATGGCTTAGGTCTAACCAAAAACATTGCTTGC  449

Query  421  GGCGGTAACGTCTACCACGTATATCTTCAAACTTTGACCTCAAAT  465
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  450  GGCGGTAACGTCTACCACGTATATCTTCAAACTTTGACCTCAAAT  494
```

Alignment of primary sequences of the ITS1 and ITS2 regions of *Candida albicans* WM 10.98 and PT specimen *C. albicans* M954.

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.
2. Chi HW, Yang YS, Shang ST, Chen KH, Yeh KM, Chang FY, Lin JC. 2011. *Candida albicans* versus non-albicans bloodstream infections: The comparison of risk factors and outcome. *J Microbiol Immunol Infect*. [Epub ahead of print]
3. Donelli G. 2006. Vascular catheter-related infection and sepsis. *Surg Infect (Larchmt).* 7 Suppl 2:S25-7.
4. Eraso E, Moragues MD, Villar-Vidal M, Sahand IH, Gonzalez-Gomez N, Ponton J, Quindos G. 2006. Evaluation of the new chromogenic medium *Candida* ID 2 for isolation and identification of *Candida albicans* and other medically important *Candida* species. *J Clin Microbiol.* 44: 3340-3345.
5. Kim, D., Shin, W.-S., Lee, K.-H., Kim, K., and Park, J.Y. 2002. Rapid differentiation of *Candida albicans* from other *Candida* species using its unique germ tube formation at 39°C. *Yeast* 19: 957-962.
6. Krcmery, V., Huttova, M., Mateicka, F., Laho, L., Jurga, L., Ondrusova, A., Tarekova, Z., Kralinsky, K., Hanzen, J., Liskova, A., Mrazova, M., Sabo, A., Pisarcikova, M., Kovacicova, G., Chovancova, D., and Szovenyiova, Z. 2001. Breakthrough fungaemia in neonates and infants caused by *Candida albicans* and *Candida parapsilosis* susceptible to fluconazole *in vitro*. *J Antimicrob Chemother*. 8: 521-525.
7. Liguori G, Di Onofrio V, Gallé F, Lucariello A, Albano L, Catania MR, Guida M. 2010. *Candida albicans* identification: comparison among nine phenotypic systems and a

multiplex PCR. *J Prev Med Hyg.* 51: 121-124.
8. Manfredi R, Sabbatani S. 2006. Severe *Candida albicans* panophthalmitis treated with all available and potentially effective antifungal drugs: Fluconazole, liposomal amphotericin B, caspofungin, and voriconazole. *Scand J Infect Dis*. 38: 950-951.
9. Mean, M., Marchetti, O., Calandra, T. 2008. Bench-to-bedside review: *Candida* infections in the intensive care unit. *Crit Care.* 12: 204.
10. Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. 2006. A one-enzyme PCR-RFLP assay for identification of six medically important *Candida* species. *Nippon Ishinkin Gakkai Zasshi.* 47: 225-229.
11. Moudgal V, Sobel J. 2010. Antifungals to treat *Candida albicans*. *Expert Opin Pharmacother.* 2010 11: 2037-2048.
12. Odds FC. 2010. Molecular phylogenetics and epidemiology of *Candida albicans*. *Future Microbiol*. 5: 67-79.
13. Patel M, Shackleton JT, Coogan MM. 2006. Effect of antifungal treatment on the prevalence of yeasts in HIV-infected subjects. *J Med Microbiol.* 55: 1279-1284.
14. Rautemaa, R., Richardson, M., Pfaller, M.A., Perheentupa, J., and Saxén, H. 2008. Activity of amphotericin B, anidulafungin, caspofungin, micafungin, posaconazole, and voriconazole against *Candida albicans* with decreased susceptibility to fluconazole from APECED patients on long-term azole treatment of chronic mucocutaneous candidiasis. *Diagn Microbiol Infect Dis.* 62:182-185.
15. Spiess, B., Seifarth, W., Hummel, M., Frank, O., Fabarius, A., Zheng, C., Mörz, H., Hehlmann, R., Buchheidt, D. 2007. DNA microarray-based detection and identification of fungal pathogens in clinical samples from neutropenic patients. *J Clin Microbiol.* 45: 3743-3753.

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

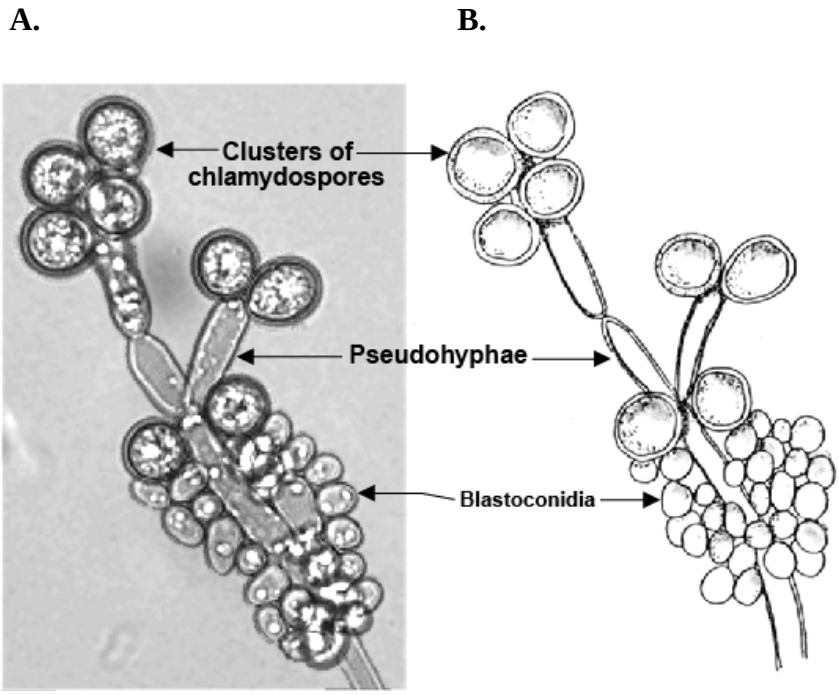


Figure 2. Microscopic morphology of *Candida albicans* on corn meal agar with Tween 80 shows terminal chlamydospores on pseudohyphae with blastoconidia (left; 400× magnification, right; line diagram not to scale).

Y-2 *Candida lipolytica*

Source: Skin / Tissue / Urine

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	113
Laboratories with incorrect ID:	8
(*Candida krusei*)	(4)
(*Candida rugosa*)	(2)
(*Trichosporon* sp.)	(2)
Outcome:	Validated

Clinical Significance: *Candida lipolytica* causes catheter-related fungemia and sinusitis in immunocompromised patients.It is also reported from traumatic ocular infections. It has been isolated as a colonizer from human vagina.

Ecology: *C. lipolytica* has been isolated from humans, lower mammals and plants.

Laboratory Diagnosis:

1. Culture – On Sabouraud's dextrose agar, after7 days at 25°C, *C. lypolytica* colony was white to cream. The surface was wrinkled (Figure 3).
2. Microscopic morphology – On corn meal agar with Tween 80, *C. lypolytica* showed abundant, multibranched true hyphae and infrequent blastoconidia along the hyphae (Figure 4). *Yarrowia lipolytica,* the teleomorph (sexual form) of *C. lypolytica*, can form ascospores on yeast malt agar in 3 to 7 days at 25°C.
3. Differentiation from other yeasts – *C. lypolytica* grows on media containing cycloheximide, grows well at 25°C, is urease positive, and negative on nitrate reactions. Sugars are not fermented by *C. lypolytica*. No growth at 42°C and positive growth on media containing cycloheximide differentiates it from *C. krusei*. Positive urease reaction and growth on media containg cycloheximide differentiates it from *C. lambia*. *C. lypolytica* is differentiated from *Geotrichum* species by negative urease reaction by the later. On the API 20C AUX, a specific assimilation biocode differentiates this organism from the Genus *Trichosporon*.
4. *In vitro* susceptibility testing – *C. lypolytica* is less susceptible to amphotericin B, but more susceptible to caspofungin. Most isolates are susceptible to azoles like fluconazole and ketoconazole and 5FC, but resistant to itraconazole.
5. Molecular tests – Comparisons of partial rRNA/rDNA sequences analysis demonstrated that *C. lypolytica* is distinctly related to selected members of Genus *Candida*. Randomly amplified polymorphic DNA (RAPD) PCR has been used for the identification of *C. lypolytica* isolates from dairy products (1, 2).

Comments: *C. lipolytica* can be differentiated from *C. krusei/inconspicua* and *C. rugosa* by its positive growth on the media containing cycloheximide. *Trichosporon* sp. produces arthroconidia, but *C. lipolytica* does not.

Sequences alignment:

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA.

```
Query  1     CATTATTGATTTTATCTATTTCTGTGGATTTCTATTCTATTACAGCGTCATTTTATCTCA  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1647  CATTATTGATTTTATCTATTTCTGTGGATTTCTATTCTATTACAGCGTCATTTTATCTCA  1706

Query  61    ATTATAACTATCAACAACGGATCTCTTGGCTCTCACATCGATGAAGAACGCAGCGAACCG  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1707  ATTATAACTATCAACAACGGATCTCTTGGCTCTCACATCGATGAAGAACGCAGCGAACCG  1766

Query  121   CGATATTTTTTGTGACTTGCAGATGTGAATCATCAATCTTTGAACGCACATTGCGCGGTA  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1767  CGATATTTTTTGTGACTTGCAGATGTGAATCATCAATCTTTGAACGCACATTGCGCGGTA  1826

Query  181   TGGCATTCCGTACCGCACGGATGGAGGAGCGTGTTCCCTCTGGGATCGCATTGCTTTCTT  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1827  TGGCATTCCGTACCGCACGGATGGAGGAGCGTGTTCCCTCTGGGATCGCATTGCTTTCTT  1886

Query  241   GAAATGGAttttttAAACTCTCAATTATTACGTCATTTCACCT  284
             ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1887  GAAATGGATTTTTTTAAACTCTCAATTATTACGTCATTTCACCT  1930
```

Alignment of primary sequences of the ITS1 and ITS2 regions of *Candida lipolytic* ATCC9773 and PT specimen *C. lipolytic* M1561.

Further Reading:

1. Andrighetto, C.E., Psomas, N., Tzanetakis, G., Suzzi, 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. Baleiras Couto, M.M., Vogels, J.T., Hofstra, H., Huis in't Veld, J.H., and van der Vossen, J.M. 1995. Random amplified polymorphic DNA and restriction enzyme analysis of PCR amplified rDNA in taxonomy: two identification techniques for food-borne yeasts. *J. Appl. Bacteriol*. 79: 525-535.
3. Barchiesi, F., Tortorano, A.M., Di Francesco, L.F., Cogliati, M., Scalise, G., and Viviani, M.A. 1999. In-vitro activity of five antifungal agents against uncommon clinical isolates of *Candida* spp. *J. Antimicrob. Chemother*. 43: 295-299.
4. Belet, N., Ciftci, E., Ince, E., Dalgic, N., Oncel, S., Guriz, H., Yagmurlu, A., Dindar, H., Dogru, U. 2006. Caspofungin treatment in two infants with persistent fungaemia due to *Candida lipolytica. Scand J Infect Dis.* 38: 559-562.
5. D'Antonio, D., Romano, F., Pontieri, E., Fioritoni, G., Caracciolo, C., Bianchini, S., Olioso, P., Staniscia, T., Sferra, R., Boccia, S., Vetuschi, A., Federico, G., Gaudio, E., and Carruba, G.2002. Catheter-related candidemia caused by *Candida lipolytica* in a patient receiving allogeneic bone marrow transplantation. *J Clin Microbiol*. 40: 1381-1386.
6. Hazen, K.C. 1995. New and emerging yeast pathogens. *Clin. Microbiol. Rev.* 8: 462-478.
7. López-Martínez R. 2010. Candidosis, a new challenge. *Clin Dermatol*. 28: 178-184.
8. Rajagopalan, B., Mathews, M.S., and Jacob, M. 1996. Vaginal colonization by *Candida lipolytica*. *Genitourin Med.* 72: 146-147.
9. Shin, J.H., Kook, H., Shin, D.H., Hwang, T.J., Kim, M., Suh, S.P., and Ryang, D.W. 2000. Nosocomial cluster of *Candida lipolytica* fungemia in pediatric patients. *Eur. J. Clin. Microbiol. Infect. Dis*. 19: 344-349.

Figure 3. Seven-day-old, white to cream colony with wrinkled surface of *Candida lypolytica* on Sabouraud's dextrose agar.

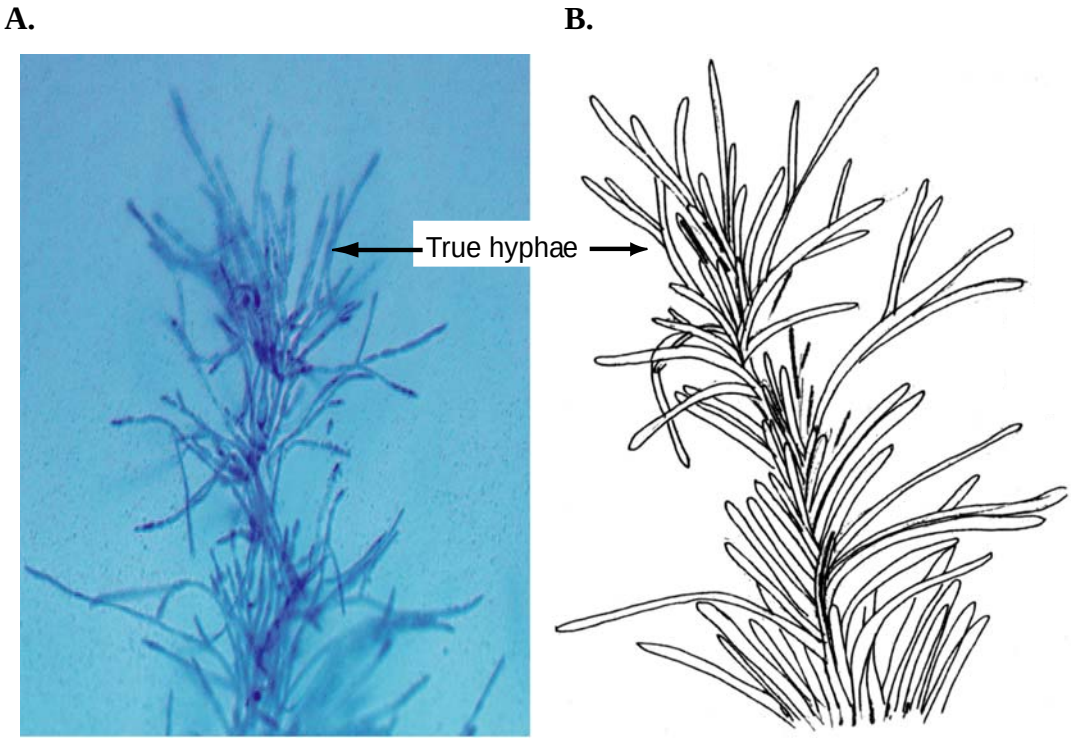


Figure 4. Microscopic morphology of *Candida lypolytica* on corn meal agar with Tween 80 showing multibranched, true hyphae, and few blastoconidia (A, 400× magnification; B, line drawing not to scale).

Y-3 *Candida zeylanoides*

Source: Nail / Urine

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	121
Laboratories with incorrect ID:	0
Outcome:	Validated

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

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

Laboratory Diagnosis:

1. Culture – On Sabouraud's dextrose agar after 7 days at 25°C, colony was smooth, cream-colored, butyrous, raised (Figure 5).
2. Microscopic morphology – On corn meal agar with Tween 80, *C. zeylanoides* formed long pseudohyphae, with verticillate, ovoid blastoconidia (Figure 6). Blastoconidia were produced in whorls around the pseudohyphae.
3. Differentiation from other yeasts – *C. zeylanoides* does not ferment any carbohydrates, grows at 37°C, grows on media containing cycloheximide, and assimilates limited carbohydrates.
4. *In vitro* susceptibility testing – *C. zeylanoides* is susceptible to amphotericin B and to the commonly used azoles.
5. Molecular tests – Multiplex PCR using ITS1 and ITS2 was reported for rapid detection and identification of yeast strains.

Comments: All of the participating laboratories correctly identified this specimen.

Sequence alignment:

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA.

```
Query  1    CATTACAGTATTCTTTTGCCAGCGCTTAATTGCGCGGCGAAAAACCTTACACACTATGtt  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  52   CATTACAGTATTCTTTTGCCAGCGCTTAATTGCGCGGCGAAAAACCTTACACACTATGTT  111

Query  61   tttttGATTTGAAACTTTTGCTTTGGTCTGACTTAGAAATGAGTTGGGCCAAAGGTTTTA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  112  TTTTTGATTTGAAACTTTTGCTTTGGTCTGACTTAGAAATGAGTTGGGCCAAAGGTTTTA  171

Query  121  TACTAAAACTTCaattttattattgaattgttaattaattatattgtcaatttgttgatt  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  172  TACTAAAACTTCAATTTTATTATTGAATTGTTAATTAATTATATTGTCAATTTGTTGATT  231

Query  181  aaattCAAAAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  232  AAATTCAAAAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGA  291
```

```
Query  241  ACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  292  ACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTG  351

Query  301  AACGCACATTGCGCCCTATGGTATTCCATAGGGCATGCCTGTTTGAGCGTCATTTCTCTC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  352  AACGCACATTGCGCCCTATGGTATTCCATAGGGCATGCCTGTTTGAGCGTCATTTCTCTC  411

Query  361  TCAAATCTTCGGATTTGGTTTTGAGTGATACTCTTAGTCAGACTAAGCGTTTGCTTGAAA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  412  TCAAATCTTCGGATTTGGTTTTGAGTGATACTCTTAGTCAGACTAAGCGTTTGCTTGAAA  471

Query  421  TGTATTGGCATGAGTGGTACTAGATAGTGCTGAACTGTTTCAATGTATTAGGTTTATCCA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  472  TGTATTGGCATGAGTGGTACTAGATAGTGCTGAACTGTTTCAATGTATTAGGTTTATCCA  531

Query  481  ACTCGTTGACCAGTATAGTATTTGTTTATTACACAGGCTCGGCCTTACAACAACAAACAA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  532  ACTCGTTGACCAGTATAGTATTTGTTTATTACACAGGCTCGGCCTTACAACAACAAACAA  591

Query  541  AGTT  544
            ||||
Sbjct  592  AGTT  595
```

Alignment of primary sequences of the ITS1 and ITS2 regions of *Candida zeylanoides* TJY13a and PT specimen *C. zeylanoides* M1564.

Further Reading:

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

Figure 5. Seven-day-old, colony creamish white, butryous, raised colony of *Candida zeylanoides* on Sabouraud's dextrose agar.

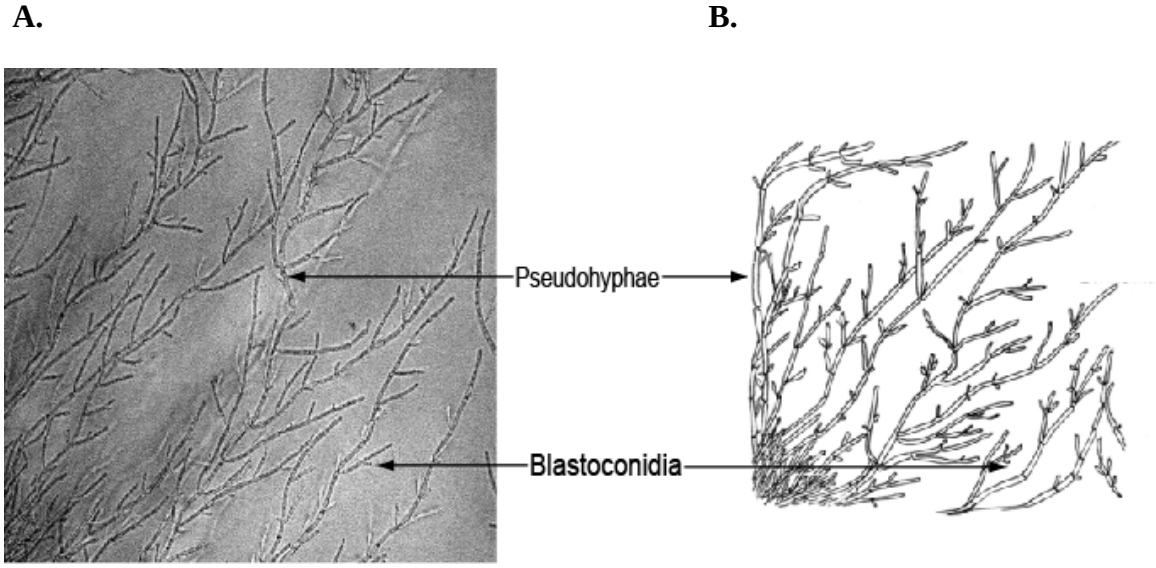


Figure 6. Microscopic morphology of *Candida zeylanoides* on corn meal agar with Tween 80, showing long pseudohyphae with verticillate, ovoid blastoconidia (left; 200x magnification, right; line drawing not to scale).

Y-4 *Cryptococcus neoformans*

Source: CSF / Skin / Urine

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	121
Laboratories with incorrect ID:	0
Outcome:	Validated

Clinical Significance: The incidence of cryptococcosis due to *Cryptococcus neoformans* infection increased with the spread of AIDS and other immunosuppressive conditions. *Cr. neoformans* var. *grubii* and var. *neoformans* mainly cause meningoencephalitis in patients with AIDS or other underlying immune dysfunctions. *Cr. neoformans* var. *neoformans* infections are more likely to have cutaneous involvement, and to infect older patients, than are infections caused by *Cr. grubii*. *Cr. gattiii* causes pulmonary cryptococcosis and systemic cryptococcosis in normal and immunocompromised hosts.

Ecology: *Cryptococcus neoformans* var. *neoformans* and var. *grubii* are commonly found in avian (pigeon) droppings. Both varieties have world-wide distributions, while *Cr. neoformans* var. *neoformans* is more common in Southern Europe. *Cr. gattii* is commonly found on *Eucalyptus* and other trees and mainly distributed in Australia, Southeast Asia, Southern California, Pacific Northwest, Vancouver Island, British Columbia, Canada, and South America.

Laboratory Diagnosis:

1. Culture – On Sabouraud's dextrose agar after 7 days at 25°C, colony was cream to tan in color, smooth, moist, and soft (Figure 7).
2. Microscopic morphology – On corn meal agar with Tween 80, *Cr. neoformans* cells were large and round, with no pseudohyphae or true hyphae. In India-ink preparation, encapsulated yeasts were seen (Figure 8).
3. Differentiation from other yeasts – *Cr. neoformans* does not ferment any carbohydrates and does not grow on media containing cycloheximide, but it grows at 37°C. *Cr. neoformans* produces dark brown colonies on niger seed agar. It produces urease enzyme and it is negative on nitrate reaction. *Cr. neoformans* and *Cr. gattii* are distinguished by 1) differential media. *Cr. gattii* growth on canavanine-glycine-bromthymol blue (CGB) agar turn the medium blue-green after 2 – 5 days of incubation at 25°C; 2) PCR technique: *Cr. gattii* can be differentiated from the other two varieties using a number of primers; 3) serotyping: *Cr. neoformans* var. *grubii* is serotype A, *Cr. neoformans* var. *neoformans* is serotype D, *Cr. gattii* is serotype B and C.
4. *In vitro* susceptibility testing – Most isolates are susceptible to amphotericin B, 5-flucytocine, and to azoles like fluconazole, itraconazole, and posaconazole. A 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 fungi. The molecular biology of this organism has revealed various virulence factors and unique genotypes among clinical strains (6).

Comments: Originally, *Cryptococcus neoformans* was described as comprising of two varieties: *Cr. neoformans* var. *neoformans* (serotypes A & D) and *Cr. neoformans* var. *gattii* (serotype B & C). Recently, *Cr. neoformans* was further subdivided into two varieties: *Cr. neoformans* var. *grubii* (serotype A) and *Cr.*

neoformans var. *neoformans* (serotype D). *Cr. neoformans* var. *gattii* was re-named as *Cr. gattii*. All the participating laboratories correctly identified this specimen.

Sequence alignment

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS2 region of rDNA.

```
Query  1    CAGTAGAGAATATTGGACTTCGGTCCATTTATCTACCCATCTACACCTGTGAACTGTTTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7    CAGTAGAGAATATTGGACTTCGGTCCATTTATCTACCCATCTACACCTGTGAACTGTTTA  66

Query  61   TGTGCTTCGGCACGTTTTACACAAACTTCTAAATGTAATGAATGTAATCTTATTATAACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  67   TGTGCTTCGGCACGTTTTACACAAACTTCTAAATGTAATGAATGTAATCTTATTATAACA  126

Query  121  ATAATAAAACTTTCAACAACGGATCTCTTGGCTTCCACATCGATGAAGAACGCAGCGAAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127  ATAATAAAACTTTCAACAACGGATCTCTTGGCTTCCACATCGATGAAGAACGCAGCGAAA  186

Query  181  TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAACTTG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  187  TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAACTTG  246

Query  241  CGCCCTTTGGTATTCCGAAGGGCATGCCTGTTTGAGAGTCATGAAAATCTCAATCCCTCG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247  CGCCCTTTGGTATTCCGAAGGGCATGCCTGTTTGAGAGTCATGAAAATCTCAATCCCTCG  306

Query  301  GGTTTTATTACCTGTTGGACTTGGATTTGGGTGTTTGCCGCGACCTGCAAAGGACGTCGG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307  GGTTTTATTACCTGTTGGACTTGGATTTGGGTGTTTGCCGCGACCTGCAAAGGACGTCGG  366

Query  361  CTCGCCTTAAATGTGTTAGTGGGAAGGTGATTACCTGTCAGCCCGGCGTAATAAGTTTCG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  367  CTCGCCTTAAATGTGTTAGTGGGAAGGTGATTACCTGTCAGCCCGGCGTAATAAGTTTCG  426

Query  421  CTGGGCCTATGGGGTAGTCTTCGGCTTGCTGATAACAACCATCTCTTTTTGTTTGACCTC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  427  CTGGGCCTATGGGGTAGTCTTCGGCTTGCTGATAACAACCATCTCTTTTTGTTTGACCTC  486

Query  481  AAATC  485
            |||||
Sbjct  487  AAATC  491
```

Alignment of primary sequences of the ITS1 and ITS2 regions of *Cr. neoformans* var *grubii* SHCZ112 and PT specimen *Cr. neoformans* M2718.

Further Reading:

1. Aller, A.I., Martin-Mazuelos, E., Lozano, F., Gomez-Mateos, J., Steele-Moore, L., Holloway, W.J., Gutierrez, M.J., Recio, F.J., and Espnel-Ingroff, A. 2000. Correlation of fluconazole MICs with clinical outcome in crytococcal infection. *Antimicrob. Agents Chemother.* 44: 1544-1548.
2. Chaturvedi, S. Rodeghier, B., Fan, J., McClelland, C.M., Wickes, B.L., and Chaturvedi, V. 2000. Direct PCR of *Cryptococcus neoformans* MATα and MATa pheromones to determine mating type, ploidy, and variety: a tool for epidemiological and molecular pathogenesis studies. *J. Clin. Microbiol*. 38: 2007-2009.

3. De Baere, T., Claeys, G., Swinne, D., Verschraegen, G., Muylaert, A., Massonet C., and Vaneechoutte, M. 2002. Identification of cultured isolates of clinically important yeast species using fluorescent fragment length analysis of the amplified internally transcribed rRNA spacer 2 region (ITS2). *BMC Microbiol.* 2: 21.
4. Dromer F, Bernede-Bauduin C, Guillemot D, Lortholary O; French Cryptococcosis Study Group. 2008. Major role for amphotericin B-flucytosine combination in severe cryptococcosis. PLoS ONE. 3: e2870.
5. Feng X, Yao Z, Ren D, Liao W. 2008. Simultaneous identification of molecular and mating types within the *Cryptococcus* species complex by PCR-RFLP analysis. *J Med Microbiol.* 57: 1481-1490.
6. Heitman, J., Kozel, T.R., Kwon-Chung, K.J., Perfect, J.R. and Casadevall A. 2010. Cryptococcus: From Human Pathogen to Model Yeast. ASM Press, Washington D.C.
7. Jarvis JN, Dromer F, Harrison TS, Lortholary O. 2008. Managing cryptococcosis in the immunocompromised host. *Curr Opin Infect Dis.* 21: 596-603.
8. Johnson E, Espinel-Ingroff A, Szekely A, Hockey H, Troke P. 2008. Activity of voriconazole, itraconazole, fluconazole and amphotericin B in vitro against 1763 yeasts from 472 patients in the voriconazole phase III clinical studies. *Int J Antimicrob Agents.* 32: 511-514.
9. Kwon-Chung, K.J., Polacheck, I., and Bennett, J.E. 1982. Improved diagnostic medium for separation of *Cryptococcus neoformans* var. *neoformans* (serotypeA and D) and *Cryptococcus neoformans* var. *gattii* (serotype B and C). *J. Clin. Microbiol.* 15: 535-537.
10. Lui, G., Lee, N., Ip, M., Choi, K.W., Tso, Y.K., Lam, E., Chau, S., Lai, R., Cockram, C.S. 2006. Cryptococcosis in apparently immunocompetent patients. *QJM*. 99:143-51.
11. Singh N, Lortholary O, Dromer F, Alexander BD, Gupta KL, John GT, del Busto R, Klintmalm GB, Somani J, Lyon GM, Pursell K, Stosor V, Munoz P, Limaye AP, Kalil AC, Pruett TL, Garcia-Diaz J, Humar A, Houston S, House AA, Wray D, Orloff S, Dowdy LA, Fisher RA, Heitman J, Wagener MM, Husain S; Cryptococcal Collaborative Transplant Study Group. 2008. Central nervous system cryptococcosis in solid organ transplant recipients: clinical relevance of abnormal neuroimaging findings. *Transplantation.* 86: 647-651.
12. Springer DJ, Chaturvedi V. 2010. Projecting global occurrence of *Cryptococcus gattii*. *Emerg Infect Dis.* 16: 14-20.
13. Steenbergen, J.N., and Casadevall. 2000. Prevalence of *Cryptococcus neoformans* var. *neoformans* (serotype D) and *Cryptococcus neoformans* var. *grubii* (serotype A) isolates in New York City. *J. Clin. Microbiol*. 38:1974-1976.
14. Tintelnot, K., Lemmer, K., Losert, H., Schar, G., and Polak, A. 2004. Follow-up of epidemiological data of cryptococcosis in Austria, Germany and Switzerland with special focus on the characterization of clinical isolates. *Mycoses.* 47: 455-64.
15. Warnatz K. 2008. Review: Cryptococcosis in HIV-negative immunodeficiency. *Clin Adv Hematol Oncol.* 6: 448-452.
16. Xiujiao, X. and Ai'e, X. 2005. Two cases of cutaneous cryptococcosis. *Mycoses.* 48: 238-241.

Figure 7. Seven-day-old, cream to tan colored, smooth, moist, and soft colony of *Cryptococcus neoformans* on Sabouraud's dextrose agar.

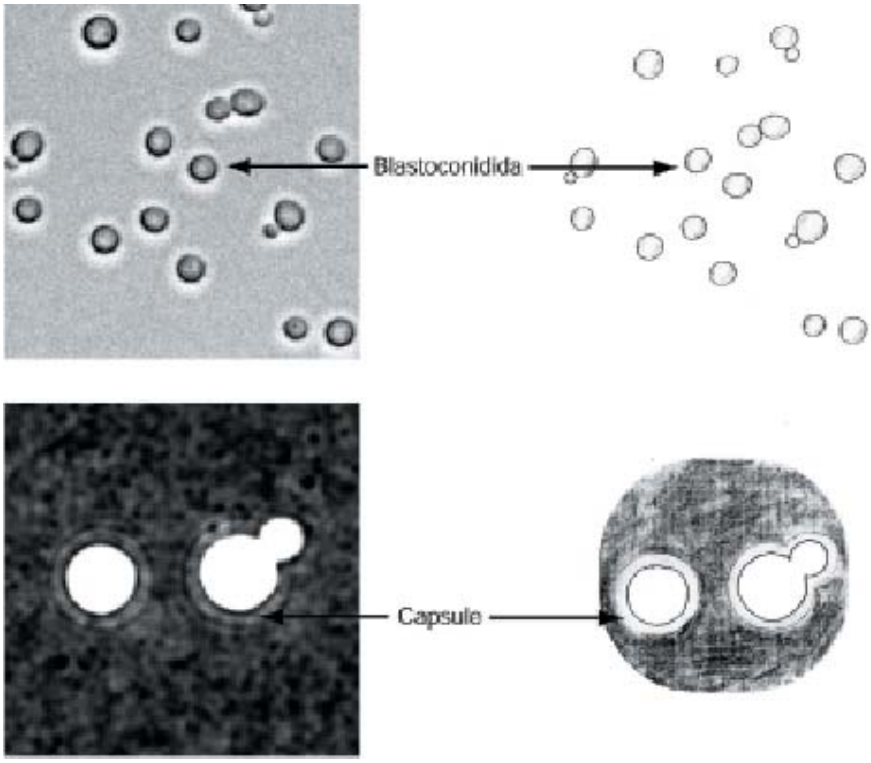


Figure 8. Microscopic morphology of *Cryptococcus neoformans* on corn meal agar with Tween 80. (Upper panel) Round, large blastoconidia. (Right, 400× magnification; Left, line drawing not to scale) (Lower panel) India-ink preparation revealing capsules (right, 1000× magnification; Left, line drawing not to scale).

Y-5 *Geotrichum candidum*

Source: Bronchial wash / Hand

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	121
Laboratories with incorrect ID:	0
Outcome:	Validated

Clinical Significance: *Geotrichum candidum* commonly causes pulmonary infections in immunocompromised patients. It also produces lesions in alimentary tract, vagina, and skin. *G. candidum* has also been reported to cause fungemia and disseminated infection.

Ecology: *G. candidum* is cosmopolitan in distribution. It has been isolated from air, water, plants, milk, and milk products. It is found as a commensal in pulmonary and gastrointestinal tract of humans.

Laboratory Diagnosis:

1. Culture – On Sabouraud's dextrose agar after 7 days at 25°C, colony grew rapidly, it was white to cream colored, fl at with aerial mycelium, (Figure 9).
2. Microscopic morphology – On corn meal agar with Tween 80, true hyphae with arthroconidia were seen (Figure 10). Arthroconidia formation was by the fragmentation of hyphae, no disjunctor cells (empty cells between the arthroconidia) and no blastoconidia were formed.
3. Differentiation from other yeasts – *G. candidum* grew on the media containing cycloheximide, negative on urease reaction, grew sparingly at 37°C. It was differentiated from *Trichosporon* species by absence of blastoconidia, no growth at higher temperatures (40, 42, & 45°C). *Blastoschizomyces capitatus* could be differentiated from *G. candidum* by the lack of growth on a medium containing D-xylose as a carbon source and its growth at 45°C. *G. candidum* was differentiated from arthroconidia forming molds by its colony morphology. Microscopically *Arthrographis* and *Odiodendron* had conidiophores while *Malbranchea* and *Coccidioides immitis* had disjunctor cells.
4. *In vitro* susceptibility testing – Limited studies suggested that most isolates were susceptible to amphotericin B and to azoles like fluconazole and itraconazole.
5. Molecular tests – Randomly amplified polymorphic DNA (RAPD) PCR had been used for the identification of *G. candidum* isolated from cheese. Using DNA/DNA reassociation techniques, de Hoog et al (1986 and 1990) found the relatedness between *G. candidum* and its teleomorph (sexual state) *Galactomyces geotrichum*.

Comments: In this test event, both *Geotrichum candidum* and *Geotrichum klebahnii* (*Geochichum penicillatum*) were accepted as correct answers since both API 20C AUX and Vitek 2 identification system could not clearly differentiate them. Mannitol assimilation test could be used for further differentiation. *G. candidum* is heterothallic. Its teleomorph is *Galactomyces candidus*.

Sequence alignment

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS2 region of rDNA.

```
Query  1    AATATTTGTGAAATTTACACAGCAAACAATAATTTTATAGTCAAAACAAAAATAATCAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  11   AATATTTGTGAAATTTACACAGCAAACAATAATTTTATAGTCAAAACAAAAATAATCAAA  70

Query  61   ACTTTTAACAATGGATCTCTTGGTTCTCGTATCGATGAAGAACGCAGCGAAACGCGATAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  71   ACTTTTAACAATGGATCTCTTGGTTCTCGTATCGATGAAGAACGCAGCGAAACGCGATAT  130

Query  121  TTCTTGTGAATTGCAGAAGTGAATCATCAGTTTTTGAACGCACATTGCACTTTGGGGTAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  131  TTCTTGTGAATTGCAGAAGTGAATCATCAGTTTTTGAACGCACATTGCACTTTGGGGTAT  190

Query  181  CCCCCAAAGTATACTTGTTTGAGCGTTGTTTCTCTCTTGGAATTGCATTGCTTTTCTAAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  191  CCCCCAAAGTATACTTGTTTGAGCGTTGTTTCTCTCTTGGAATTGCATTGCTTTTCTAAA  250

Query  241  ATTTCGAATCAAATTCGTTTGAAAAACAACACTATTCAACCTCAGATC  288
            ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  251  ATTTCGAATCAAATTCGTTTGAAAAACAACACTATTCAACCTCAGATC  298
```

Alignment of primary sequences of the ITS1 and ITS2 regions of *Geotrichum candidum* ITEM10458 and PT specimen *G. candidum* M1564.

Further Reading:

1. Andre, N., Coze, C., Gentet, J.C., Perez, R., and Bernard, J.L. 2004. *Geotrichum candidum* septicemia in a child with hepatoblastoma. *Pediatr. Infect. Dis. J*. 23: 86.
2. Bonifaz A, Vázquez-González D, Macías B, Paredes-Farrera F, Hernández MA, Araiza J, Ponce RM. 2010. Oral geotrichosis: report of 12 cases. *J Oral Sci*. 52: 477-483.
3. Depagne, C., Louerat, C., Nesme, P. 2003. Herpes simplex and *Geotrichum candidum* pneumonia in a patient with moderate renal failure. *Rev. Pneumol. Clin*. 59: 297-300.
4. De Hoog, G.S., and Amberger, A.E. 1990. Electrophoretic protein patterns of *Geotrichum* and its teleomorphs. *Antonie Van Leeuwenhoek*. 58: 101-105.
5. Pryce, T.M., Palladino, S., Kay, I.D., Coombs, G.W. 2003. Rapid identification of fungi by sequencing the ITS1 and ITS2 regions using an automated capillary electrophoresis system. *Med. Mycol.* 41: 369-81.

Figure 9. Seven-day-old, white and mold like colony of *Geotrichum candidum* on Sabouraud's dextrose agar.

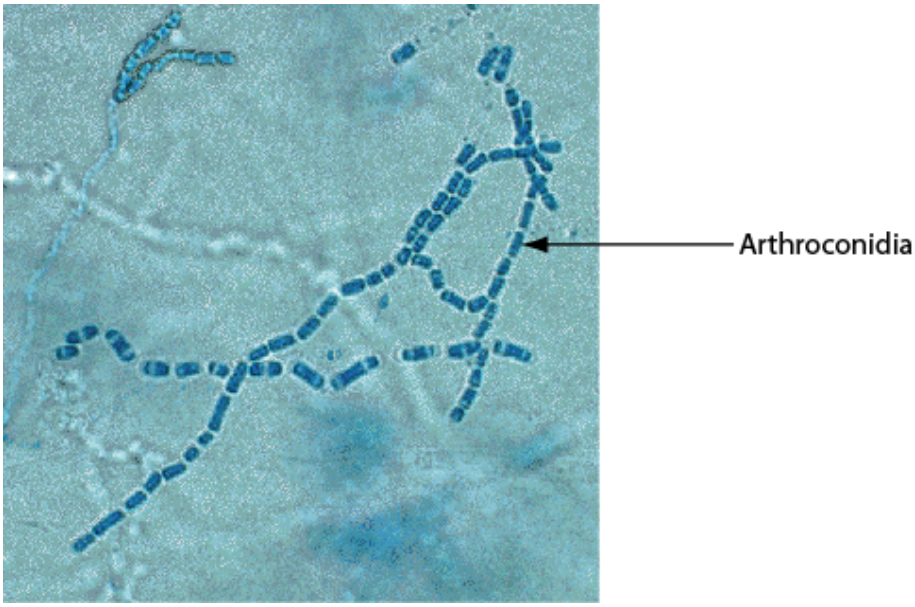


Figure 10. Microscopic morphology of *Geotrichum candidum* on corn meal agar with Tween 80 showing arthroconidia (400 × magnification).

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR YEASTS

Introduction: Documents of M27-A3 and M27-S3 published by Clinical Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards, NCCLS) is the current standard reference guide for antifungal susceptibility testing of pathogenic yeasts. FDA approved devices for antifungal susceptibility testing of yeasts includes Sensititre YeastOne Colorimetric Panel (Trek Diagnostic Systems Inc. Cleveland, OH) and Etest (AB BIODISK North America, Inc. Piscataway, NJ). The disk diffusion method approved by CLSI (M44-A) is another alternative for antifungal susceptibility testing of yeasts. There are 10 drugs in the antifungal susceptibility testing panel of NYSDOH Mycology Proficiency Test Program - amphotericin B, anidulafungin, caspofungin, flucytosine (5-FC), fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. The participating laboratories are allowed to select any number of antifungal drug(s) from the test panel for testing based upon usual practices in their facilities.

Materials & Results: *Candida albicans* (S-1) was the analyte in the May 25, 2011 antifungal proficiency testing event. Thirty laboratories participated in this event. The S-1 isolate was validated by all the participating laboratories. The acceptable results for antifungal susceptibility testings were based on consensus MIC values or interpretation per NCCLS/CLSI guidelines or other publications (Table 1).

Table 1. Interpretive Guidelines for *In Vitro* Susceptibility Testing of *Candida* spp.*

Antifungal Agent	Susceptible (S)	Susceptible-dose dependent (S-DD)	Intermediate (I)	Resistant (R)	Nonsusceptible (NS)
Amphotericin B[1]					
Anidulafungin	≤2	-	-	-	>2
Caspofungin	≤2	-	-	-	>2
Fluconazole[2]	≤8	16-32	-	≥64	-
Flucytosine (5-FC)	≤4	-	8-16	≥32	-
Itraconazole	≤0.125	0.25-0.5	-	≥1	-
Ketoconazole[3]					
Micafungin	≤2	-	-	-	>2
Posaconazole[4]					
Voriconazole	≤1	2	-	≥4	-

* Adapted from CLSI document M27-S3 (2008)

[1] **For Amphotericin B, there are no breakpoints, but > 1 is considered resistant.**

[2] **Isolates of *Candida krusei* are assumed to be intrinsically resistant to fluconazole, and their MICs should not be interpreted using this scale.**

[3] **For Ketoconazole, there is no assigned interpretative breakpoint.**

[4] **For Posaconazole, apply the voriconazole MIC interpretation as surrogate breakpoints (susceptible, ≤1 μg/ml; susceptible-dose dependent, 2 μg/ml; resistant, ≥4 μg/ml).** (Pfaller, M.A., Messer, S.A., Boyken, L., Tendolkar, S., Hollis, R.J., and Diekema, D.J. Selection of a surrogate agent (fluconazole or voriconazole) for initial susceptibility testing of posaconazole against *Candida* spp.: results from a global antifungal surveillance program. *J. Clin. Microbiol.* 2008: 46: 551-559.)

Summary:

Table 2. Antifungal MICs (µg/ml) Reported by the Participating Laboratories

S-1: *Candida albicans*

Drugs (µg/ml)	Total # of labs	≤0.015	0.03	0.06	0.12	0.19	0.25	0.38	0.5	1	1.5	2	3	≥ 32	≥64	128	256
Amphotericin B	24						2	1	19	2							
Anidulafungin	17	8	6	1	2												
Caspofungin	22		1	2	6		11		2								
Flucytosine (5-FC)	27		1	17	4	1	3		1								
Fluconazole	33												1		7	11	14
Itraconazole	31						1		15	12		1		2			
Ketoconazole	6				1				2			2	1				
Micafungin	17		13	4													
Posaconazole	18						1		5	11		1					
Voriconazole	24						1		2	18	1	2					

Table 3. Antifungal Susceptibility Interpretations Reported by the Participating Laboratories

S-1: *Candida albicans*

Antifungal Agent	Total # of labs	Susceptible	Susceptible-dose dependent	Resistant	No interpretation
Amphotericin B	24	15	1		8
Anidulafungin	17	17			
Caspofungin	22	22			
Flucytosine (5-FC)	27	27			
Fluconazole	33	33			
Itraconazole	31	2	14	15	
Ketoconazole	6	2	1		3
Micafungin	17	17			
Posaconazole	18	11	2		5
Voriconazole	24	21	2	1	

Further Reading:

1. Barry, A.L., Pfaller, M.A., Rennie, R.P., Fuchs, P.C., and Brown, S.D. 2002. Precision and accuracy of fluconazole susceptibility testing by broth microdilution, Etest, and disk diffusion methods. *Antimicrob. Agents Chemother.* 46: 1781-1784.
2. Clinical and Laboratory Standards Institute. 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard - Third Edition. CLSI document M27-A3 (ISBN 1-56238-666-2).
3. Clinical and Laboratory Standards Institute. 2008. Quality Control Minimal Inhibitory Concentration (MIC) Limits for Broth Microdilution and MIC Interpretive Breakpoints; Informational Supplement - Third Edition. CLSI document M27-S3 (ISBN 1-56238-667-0).
4. Clinical and Laboratory Standards Institute. 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard – Second Edition. CLSI document M38-A2 (1-56238-668-9).
5. Clinical and Laboratory Standards Institute. 2009. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline – Second Edition. CLSI document M44-A2 (ISBN 1-56238-703-0).
6. Clinical and Laboratory Standards Institute. 2009. Zone Diameter Interpretive Standards, Corresponding Minimal Inhibitory Concentration (MIC) Interpretive Breakpoints, and Quality Control Limits for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Informational Supplement. CLSI document M44-S3.
7. Clinical and Laboratory Standards Institute. 2010. Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Approved Guideline. CLSI document M51-A (ISBN 1-56238-725-1).
8. Clinical and Laboratory Standards Institute. 2010. Performance Standards for Antifungal Disk Diffusion Susceptibility Testing of Filamentous Fungi; Informational Supplement. CLSI document M51-S1 (ISBN 1-56238-725-1).
9. Cuenca-Estrella, M., Gomez-Lopez, A., Mellado, E., and Rodriguez-Tudela, J.L. 2005. Correlation between the procedure for antifungal susceptibility testing for *Candida* spp. of the European Committee on antibiotic susceptibility testing (EUCAST) and four commercial techniques. *Clin. Microbiol. Infect.* 11: 486-492.
10. Cuenca-Estrella M, Arendrup MC, Chryssanthou E, Dannaoui E, Lass-Florl C, Sandven P, Velegraki A, Rodriguez-Tudela JL; AFST Subcommittee of EUCAST. 2007. Multicentre determination of quality control strains and quality control ranges for antifungal susceptibility testing of yeasts and filamentous fungi using the methods of the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antimicrobial Susceptibility Testing (AFST-EUCAST) *Clin Microbiol Infect.* 13: 1018-1022.
11. Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST technical note on fluconazole. *Clin. Microbiol. Infect.* 14: 193-195.
12. Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST definitive document Edef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. *Clin. Microbiol. Infect.* 14: 398-405.
13. Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST technical note on the method for the

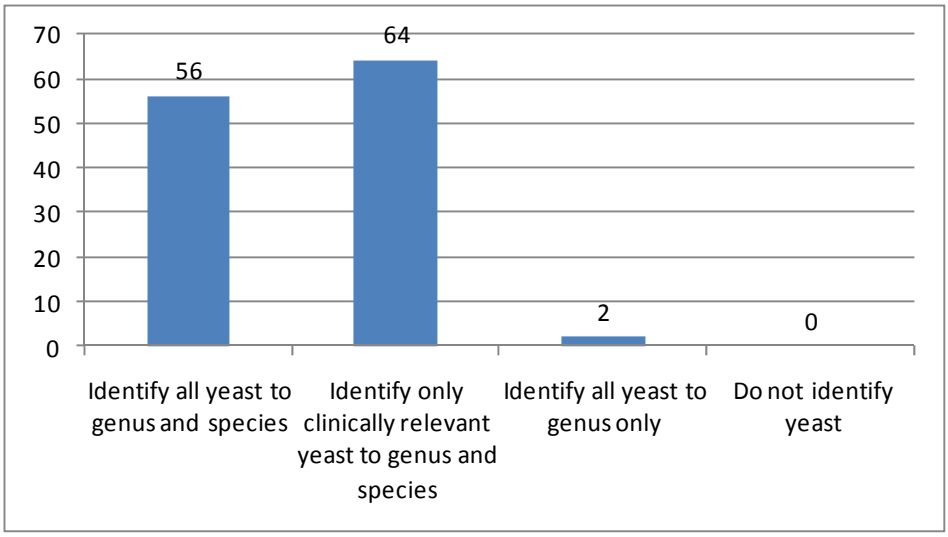
determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia–forming moulds. *Clin. Microbiol. Infect.* 14: 982-984.

14. Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST technical note on voriconazole. *Clin. Microbiol. Infect.* 14: 985-987.

MYCOLOGY PT PROGRAM
FUNGAL IDENTIFICATION SURVEY

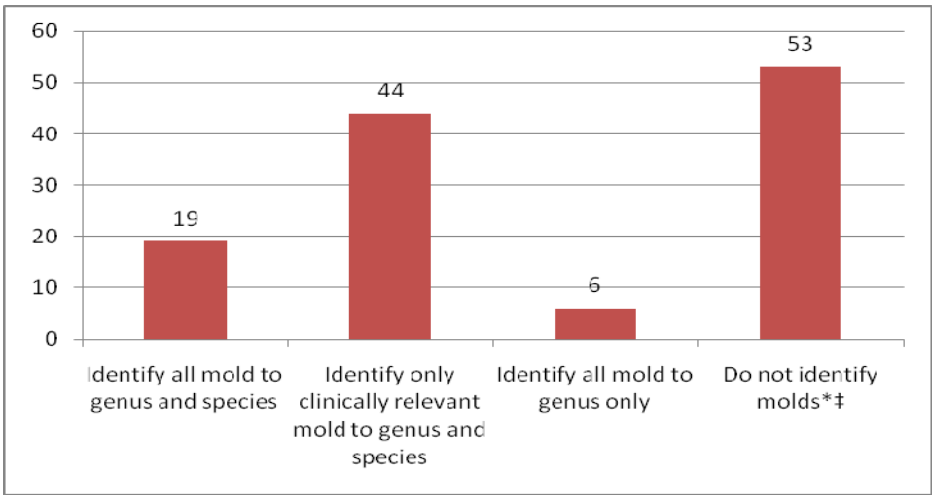
Q1. What is the level of identification provided by your laboratory for yeasts recovered from clinical specimens:

- ❑ Identify all yeast to genus and species
- ❑ Identify only clinically relevant yeast genus and species
- ❑ Identify all yeast to genus only
- ❑ Do not identify yeast



Q2. What is the level of identification provided by your laboratory for molds recovered from clinical specimens:

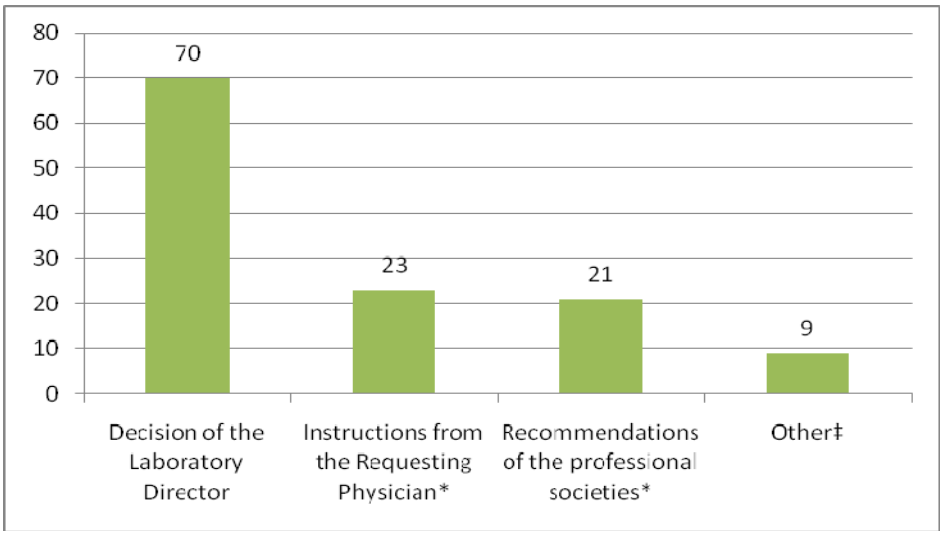
- ❑ Identify all mold to genus and species
- ❑ Identify only clinically relevant mold to genus and species
- ❑ Identify all mold to genus only
- ❑ Do not identify molds



*These are laboratories hold Mycology Identification Yeast Only permit
‡One laboratory with comprehensive permit responded in this subcategory

Q3. How is the decision made about the appropriate level of fungal identification needed for any clinical specimen:

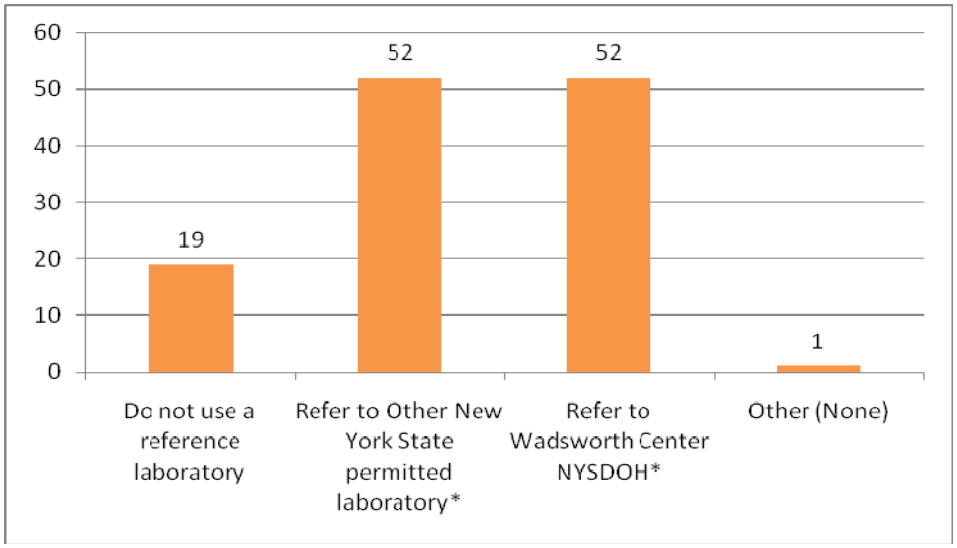
- ❑ Decision of the Laboratory Director
- ❑ Instructions from the Requesting Physician
- ❑ Recommendations of the professional societies
- ❑ Other, please specify _____



*More than one option was selected
‡Multiple options were selected

Q4. Do you use reference laboratories for further identification of fungal isolates? If yes, where these specimens are tested?

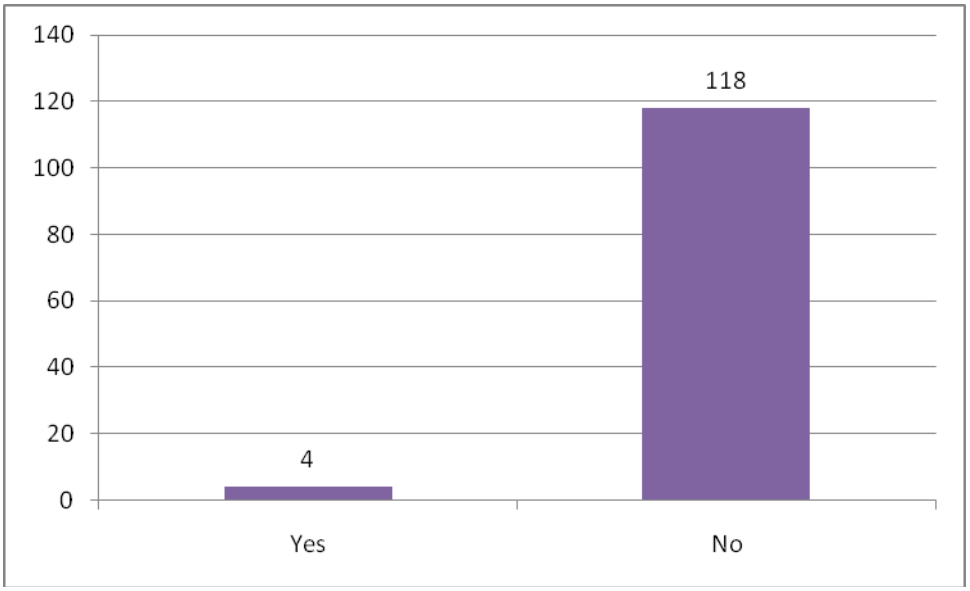
- ❑ Do not use a reference laboratory
- ❑ Refer to New York State permitted laboratory
- ❑ Refer to Wadsworth Center
- ❑ Other, please specify _____



*More than one option was selected

Q5. Do you anticipate any change in fungal identification services in near future?

- ❑ Yes ❑ No



BIBLIOGRAPHY

1. American Type Culture Collection (http://www.atcc.org/)
2. Agricultural Research Service Culture Collection, USDA (http://nrrl.ncaur.usda.gov/)
3. Arx von, J.A. 1981. The Genera of Fungi Sporulating in Pure Culture. 3rd ed. J. Cramer, Vaduz, Germany.
4. Beneke, E.S. and Rogers, A.L. 1996. Medical Mycology and Human Mycoses. Star Publishing Company.
5. Barnett, H.L. and Hunter, B.B. 1987. Illustrated Genera of Imperfect Fungi. 4th ed. Macmillan Publishing Co. New York.
6. Barnett, J.A., Payne, R.W., Yarrow, D. 2000. Yeasts: Characteristics and Identification. 3rd ed. Cambridge University Press, UK.
7. Barron, G.L. 1968. The Genera of Hyphomycetes from Soil. Williams and Wilkins Co.
8. Carmichael, J.W., Kendrick, W.B., Conners, I.L., Sigler, L. 1980. Genera of Hyphomycetes. University of Alberta Press, Edmonton.
9. The Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversiry Centre (http://www.cbs.knaw.nl/About/)
10. De Hoog, G.S., Guarro, J., Gene, J., and Figueras, M.J. 2009. Atlas of Clinical Fungi. 3rd ed (CD-ROM version). Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
11. Domsch, K.H., Gams, W., Anderson, T.H. 1980. Compendium of Soil Fungi, Vols. 1 and 2. Academic Press. New York.
12. Ellis, M.B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.
13. Ellis, M.B. 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.
14. Fisher, F. and Cook, N.B. 1998. Fundamentals of Diagnostic Mycology. W.B. Saunders Company, Philadelphia.
15. Folds, J.D., Hamilton, R.G., and Detrick, B. 2006. Manual of Molecular and Clinical Laboratory Immunology. 6th ed. ASM Press, Washington, D.C.
16. Gilman, J.C. 1957. A Manual of Soil Fungi. 2nd ed. Iowa State University Press, Ames, Iowa. Davis Company, Philadelphia.
17. Hamlin, R. 1990. Illustrated Genera of Ascomycetes. APS press. The American Phytopathological Society. St. Paul. Minnesota.
18. Japan Collection of Microorganisms (http://www.jcm.riken.go.jp/)
19. Kendrick, W.B., Carmichael, J.W. 1973. Hyphomycetes. In Ainsworth GC Sparrow FK, Sussman AS. (eds). The Fungi. Vol. IVA. Academic Press, New York, 323-509.
20. Kern, M.E. and Blevins, K.S. 1997. Medical Mycology – A Self-Instructional Text. 2nd ed. F.A. Davis, Philadelphia.
21. Kiffer, E. and Morelet, M. 1999. The Deuteromycetes – Mitosporic Fungi, Classification and Generic Keys. Science Publishers Inc. U.S.A.
22. Klich, M.A. 2002. Identification of common *Aspergillus* species. 1st ed. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
23. Kurtzman, C.P. and Fell, J.W. 1998. The Yeasts, a taxonomic study. 4th ed. Elsevier, New York, NY.
24. Kwon-Chung, K.J., Bennett, J.E. 1992. Medical Mycology. Lea & Febiger, Philadelphia.
25. Larone, D.H. 2002. Medically Important Fungi – A Guide to Identification. 4th ed, ASM Press, Washington, D.C.
26. McGinnis, M.R. 1980. Laboratory Handbook of Medical Mycology. Academic Press, New York.
27. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R.H. 2010. Manual of Clinical Microbiology. 10th ed. ASM Press, Washington, D.C.
28. New York State Department of Health Mycology Proficiency Testing Program Yeasts/Molds Master List and Instructions. January 2011.

29. Raper, K.B. and Fennell, D.I. 1973. The Genus *Aspergillus.* Robert E. Krieger Publishing Company, Huntington, New York.
30. Rebell, G. and Taplin, D. 1972. Dermatophytes - Their Recognition and Identification. University of Miami Press, Coral Gables, FL.
31. Rippon, J.W. 1988. Medical Mycology – The Pathogenic Fungi and the Pathogenic Actinomycetes. W.B. Saunders Company, Philadelphia.
32. St-Germain, G. and Summerbell, R. 2011. Identifying Fungi – A Clinical Laboratory Handbook. 2nd Edition. Star Publishing Company, Belmont, CA.
33. Sutton, D.A., Fothergill, A.W., and Rinaldi, M.G. 1998. Guide to Clinically Significant Fungi. Williams and Wilkins, A Waverly Company, Baltimore.
34. The United Kingdom National Culture Collection UKNCC (http://www.ukncc.co.uk/html/Databases/search.asp)
35. University of Alberta Microfungus Collection (http://www.devonian2.ualberta.ca/uamh/)