

Mycology Proficiency Testing Program
May 2010 Test Event
Critique



Wadsworth Center
New York State Department of Health

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Schedule of 2010 Mycology PT Mailouts*[‡]

GENERAL

January 27, 2010
May 26, 2010
September 29, 2010

GENERAL POSTMARK DEADLINES

March 12, 2010
June 18, 2010
November 12, 2010

YEASTS ONLY

January 27, 2010
May 26, 2010
September 29, 2010

YEASTS ONLY POSTMARK DEADLINES

February 19, 2010
June 18, 2010
October 22, 2010

DIRECT DETECTION TESTING

January 27, 2010
September 29, 2010

DIRECT DETECTION TESTING POSTMARK DEADLINES

February 12, 2010
October 15, 2010

ANTIFUNGAL SUSCEPTIBILITY FOR YEASTS

January 27, 2010
May 26, 2010
September 29, 2010

ANTIFUNGAL SUSCEPTIBILITY FOR YEASTS POSTMARK DEADLINES

February 19, 2010
June 18, 2010
October 22, 2010

*Please provide us with your email information so we could inform you when a new critique is posted online.

[‡]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 each yeast species were examined for inclusion in the proficiency test. These strains were tested for colony morphology on corn meal agar with Tween 80, for carbohydrate assimilation using API 20C AUX identification kit, and for carbohydrates fermentation using classical approaches. Additional physiologic characteristics including nitrate assimilation, urease activity, and cycloheximide sensitivity were also carried out with the appropriate test media. The single strain that best demonstrated the morphologic and physiologic characteristics of the proposed test analyte was selected for PT mail out.

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 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 based on consensus MIC values +/- 2 dilutions or interpretation per CLSI (NCCLS) guidelines or other 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 testing practices in their facilities. A maximum score of 100 will be equally divided among the drugs selected by the individual laboratory. If a result 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 subjected to 'cease testing' of clinical specimens.

*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 – Yeast Only

	Specimen Key	Validated Specimen	Other Acceptable Answers	Correct Responses / Total # Laboratories (%)
Y-1	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>		121/122 (99)
Y-2	<i>Candida lusitaniae</i>	<i>Candida lusitaniae</i>		120/122 (98)
Y-3	<i>Blastoschizomyces capitatus</i>	<i>Blastoschizomyces capitatus</i>	<i>Geotrichum capitatum</i>	102/122 (84)
Y-4	<i>Candida kefyr</i>	<i>Candida kefyr</i>		122/122 (100)
Y-5	<i>Cryptococcus uniguttulatus</i>	<i>Cryptococcus uniguttulatus</i>		122/122 (100)

Mycology – Antifungal Susceptibility Testing for Yeasts (S-1: *Candida glabrata* M1409)

Drugs	Acceptable MIC (µg/ml) Range	Acceptable Interpretation	Acceptable Responses/Total # Laboratories (%)
Amphotericin B	0.12 – 1.0	Susceptible / No interpretation	26/26 (100)
Anidulafungin	0.015 – 0.12	Susceptible	17/17 (100)
Caspofungin	0.03 – 0.5	Susceptible	22/22 (100)
Flucytosine (5-FC)	0.015 – 0.12	Susceptible	25/26 (96)
Fluconazole	≥ 64	Resistant	31/32 (97)
Itraconazole	≥ 8	Resistant	29/29 (100)
Ketoconazole	2 – 32	No interpretation	8/8 (100)
Micafungin	0.008 – 0.03	Susceptible	17/17 (100)
Posaconazole	8 – 32	Resistant	18/18 (100)
Voriconazole	2.0 – 8.0	Susceptible-dose dependent / Resistant	25/25 (100)

TEST STATISTICS

	General	Yeast Only	Antifungal Susceptibility Testing for Yeasts
Number of participating laboratories	71	51	32
Number of referee laboratories	10	10	32
Number of laboratories responding by deadline	71	51	32
Number of laboratories responding after deadline	0	0	0
Number of laboratories not responding	0	0	0
Number of laboratories successfully completing this test	70	51	32
Number of laboratories unsuccessfully completing this test	1	0	0

Number of Laboratories Using Commercial Yeast Identification System*

BioMerieux API 20C AUX	78
BioMerieux Vitek	13
BioMerieux Vitek2 System	51
Remel RapID Yeast Plus System	6
Remel Uni-Yeast-Tek	9
Dade Behring MicroScan Rapid Yeast Identification System	5
Molecular sequencing	4

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

YeastOne Colorimetric microdilution method	25
Etest	4
Disk diffusion method	1
Others [†]	5

(*Include multiple systems used by some laboratories)

([†]Include laboratories using CLSI Microbroth dilution method)

YEAST DESCRIPTIONS

Y-1 *Saccharomyces cerevisiae*

Source: Sputum / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

(*Saccharomyces* sp.)

Outcome:

No. Laboratories

10

121

1

(1)

Validated

Clinical Significance: *Saccharomyces cerevisiae*, the baker's yeast, causes disseminated infection in immunocompromised hosts.

Epidemiology: It is cosmopolitan in distribution on food materials especially fruits.

Laboratory Diagnosis:

1. Culture – At 25°C, colonies on Sabouraud's dextrose agar are creamy, smooth, dull butyrous or buttery texture after 3 –5 days of incubation (Figure 1).
2. Microscopic morphology – On corn meal agar with Tween – 80, round to oval yeast cells with no pseudohyphae or rudimentary pseudohyphae (Figure 2). On special media like V – 8 agar or malt agar, characteristic ascospores encased in asci are seen.
3. Differentiation from other yeasts - *Saccharomyces cerevisiae* ferments glucose, maltose and sucrose, does not grow on the media containing cycloheximide, and grows at 37°C. On the API 20C AUX, a specific assimilation biocode is obtained for identification of this organism.
4. Molecular tests - *Saccharomyces cerevisiae* is the most intensely studied model organism also being the first eukaryote to have its entire genome sequenced and mapped.
5. *In vitro* susceptibility testing –Most isolates are susceptible to amphotericin B, 5-FC, and to azoles like fluconazole, miconazole, voriconazole. etc.

Comments: All of the participating laboratories except one correctly identified this specimen. Speciation is required for this specimen.

Further Reading:

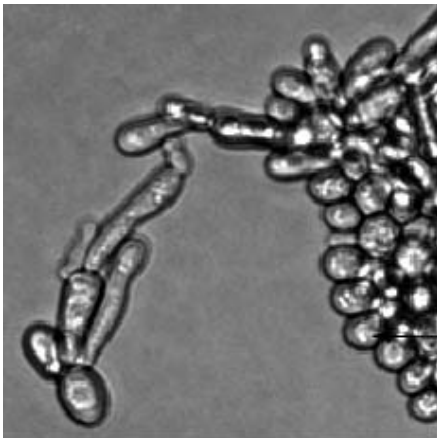
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6. Konecny P, Drummond FM, Tish KN, Tapsall JW. 1999. *Saccharomyces cerevisiae* oesophagitis in an HIV – Infected patient. *International J STD & AIDS*. 10: 821 –822.

7. Ren P, Sridhar S, Chaturvedi V. 2004. Use of paraffin-embedded tissue for identification of *Saccharomyces cerevisiae* in a baker's lung nodule by fungal PCR and nucleotide sequencing. *J Clin Microbiol.* 42: 2840 - 2842.
8. Munoz P, Bouza E, Cuenca-Estrella M, Eiros JM, Perez MJ, Sanchez-Somolinos M, Rincon C, Hortal J, Pelaez T. 2005. *Saccharomyces cerevisiae* fungemia: an emerging infectious disease. *Clin Infect Dis.* 40: 1625-1634.
9. Posteraro B, Sanguinetti M, Masucci L, Romano L, Morace G, Fadda G. 2000. Reverse cross blot hybridization assay for rapid detection of PCR – amplified DNA from *Candida* species, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae* in clinical samples. *J Clin Microbiol.* 38: 1609 – 1614.
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Figure 1. Seven-day-old, creamy, smooth, dull butyrous colony of *Sacchromyctes cerevisiae* on Sabouraud's dextrose agar.

A.



B.

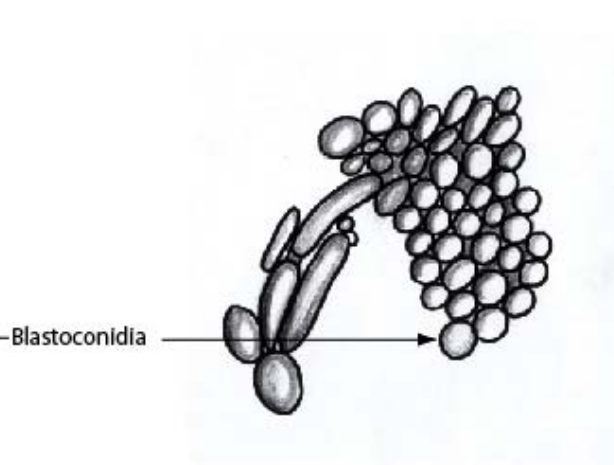


Figure 2. Microscopic morphology of *Saccharomycetes cerevisiae* on cornmeal agar showing round to oval blastoconidia (A, 400× magnification; B, line drawing not to scale).

Y-2 *Candida lusitaniae*

Source: Blood / Skin / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

(*Candida famata*)

(*Candida guilliermondii*)

Outcome:

No. Laboratories

10

120

2

(1)

(1)

Validated

Clinical Significance: *Candida lusitaniae* is recovered from blood, urine, and respiratory tract of immunocompromised and debilitated patients with cancer, diabetes, or asthma, and also from neonates in intensive care. The major clinical manifestations are fungemia and sepsis.

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

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar, at 25°C for 5 days, *C. lusitaniae* colony was white to creamish, shiny, slightly raised in the center (Figure 3).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *C. lusitaniae* produced many short, branched (“bushy”) pseudohyphae. Along the length of the pseudohyphae, elongated blastoconidia formed in short chains (Figure 4).
3. **Differentiation from other yeasts** – *C. lusitaniae* and *C. parapsilosis* both are able to grow at 37°C, but not on the media containing cycloheximide. Both are able to assimilate glucose, maltose, sucrose, galactose, and xylose. They do not hydrolyze urea and assimilate nitrate. However, *C. lusitaniae* is able to ferment and assimilate cellobiose, which differentiates it from *C. parapsilosis*.
4. **In vitro susceptibility testing** – Some *C. lusitaniae* strains are reported to be inherently resistant to amphotericin B. Amphotericin B susceptible strains are also known to develop resistance during the

course of treatment with this drug. *C. lusitaniae* also has been reported more susceptible to voriconazole than fluconazole.

5. **Molecular tests** – Specific nucleic acid probes targeting the large subunit rRNA genes have been developed for identification of *C. lusitaniae*. Three pulsed-field electrophoretic methods and a random amplified polymorphic DNA (RAPD) method were also reported to delineate strains of *C. lusitaniae*.

Comments: *Clavispora lusitaniae* is the perfect or teleomorphic state of *C. lusitaniae*. One laboratory each reported this specimen as *Candida famata* or *Candida guilliermondii*, which are able to grow in the media with cycloheximide, but *C. lusitaniae* cannot.

Further Reading:

1. Alberth, M., Majoros, L., Kovalecz, G., Borbas, E., Szegedi, I., J Marton, I., Kiss, C. 2006. Significance of oral *Candida* infections in children with cancer. *Pathol Oncol Res.* 12: 237-241.
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3. Bariola, J.R. and Saccente, M. 2008. *Candida lusitaniae* septic arthritis: case report and review of the literature. *Diagn Microbiol Infect Dis.* 61: 61-63.
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- chronic granulomatous disease. *Pediatr Infect Dis J.* 25: 758-759.
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 6. Lehmann, P.F., Lin, D., and Lasker, B.A. 1992. Genotypic identification and characterization of species and strains within the genus *Candida* by using random amplified polymorphic DNA. *J Clin Microbiol.* 30: 3249-3254.
 7. McClenny, N.B., Fei, H., Baron, E.J., Gales, A.C., Houston, A., Hollis, R.J., and Pfaller, M.A. 2002. Change in colony morphology of *Candida lusitanae* in association with development of amphotericin B resistance. *Antimicrob. Agents Chemother.* 46: 1325-1328.
 8. Minari, A., Hachem, R., and Raad, I. 2001. *Candida lusitanae*: a cause of breakthrough fungemia in cancer patients. *Clin. Infect. Dis.* 32: 186-190.
 9. Parentin, F., Liberali, T., Perissutti, P. 2006. Polymicrobial keratomycosis in a three-year-old child. *Ocul Immunol Inflamm.* 14: 129-131.
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Figure 3. Four-day-old, white, smooth colony of *Candida lusitanae* on Sabouraud's dextrose agar.

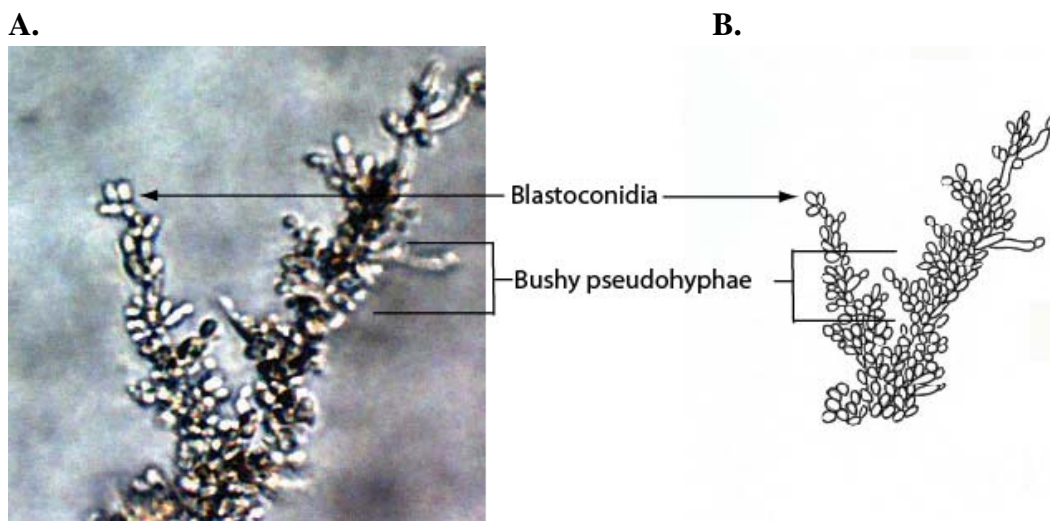


Figure 4. Microscopic morphology of *Candida lusitanae* on corn meal agar with Tween 80 showing bushy pseudohyphae and blastoconidia (A, 400× magnification; B, line drawing not to scale).

Y-3 *Blastoschizomyces capitatus*

Source: Stool / Bone lesions / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

(*Geotrichum* sp.)

(*Candida lipolytica*)

(*Geotrichum candidum*)

(*Candida utilis*)

(*Trichosporon* sp.)

Outcome:

No. Laboratories

10

102

20

(9)

(6)

(3)

(1)

(1)

Validated

Clinical Significance: *Blastoschizomyces capitatus* is an opportunistic pathogen in neutropenic patients.

Ecology: *B. capitatus* is frequently isolated from sputum, and it is a minor component of the normal skin flora. The fungus is also found in wood pulp and poultry droppings.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was smooth to wrinkled, raised, and hyaline (Figure 5).
2. **Microscopic morphology** – On corn meal agar with Tween 80, true hyphae were produced. Anelloconidia emerged from the annellides (A percurrent, indeterminate conidiogenous cell in which the first conidium is holoblastic and each successive conidium is enteroblastic. Annellations occur with the release of annelloconidia. Annellides become longer and narrower with the production of each new conidium.) (Figure 6), and might undergo schizolytic division (Barnett, H.L. and Hunter, B.B. 1987). The resulting conidia simulated the appearance of arthroconidia, as seen in the genus *Trichosporon* and *Geotrichum*.
3. **Differentiation from other yeasts** – *B. capitatus* can be differentiated from *G. candidum* by the lack of growth on a medium containing D-xylose as a carbon source. It can be differentiated from *T. beigelii* by lack

of urease and its growth at 45°C. *B. capitatus* is included in the database of commercial yeast identification systems.

4. **In vitro susceptibility testing** – *B. capitatus* is susceptible to amphotericin B; fluconazole resistant strains have been reported from cancer patients.
5. **Molecular tests** – Primers for large ribosomal subunit DNA sequences were used in PCR to differentiate between *C. famata* and *C. guilliermondii*. The amplification of 340 bp of the large rDNA led to rapid and specific identification of *C. famata*. RAPD-PCR analysis was applied to identify *C. famata* in dairy product.

Comments: The correct taxonomic name of the fungus is *Geotrichum capitatum*. Thus, *B. capitatus* or *Trichosporon capitatum* will be regarded as a synonym. However, all three answers were accepted as correct answers in this testing event due to prevailing nomenclature in semiautomated yeast ID systems. However, *G. candidum* reported by three laboratories is different from *B. capitatus* based on xylose assimilation test. *G. candidum* can not grow on the medium containing xylose as a carbon source, but *B. capitatus* can. *B. capitatus* is urease negative, which can be used to differentiate it from *Candida lipolytica*. This specimen was required to identify to species level.

Further Reading:

1. Bouza, E and Munoz, P. 2004. Invasive infections caused by *Blastoschizomyces capitatus* and *Scedosporium* spp. *Clin. Microbiol. Infect.* 1:76-85.
2. Buchta, V., Zak, P., Kohout, A., and Otcenasek, M. 2001. Case report. Disseminated infection of *Blastoschizomyces capitatus* in a patient with acute myelocytic leukaemia. *Mycoses.* 44: 505-512.
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Figure 5. Seven-day-old, white, smooth to slightly wrinkled, raised colony of *Blastoschizomyces capitatus* on Sabouraud's dextrose agar.

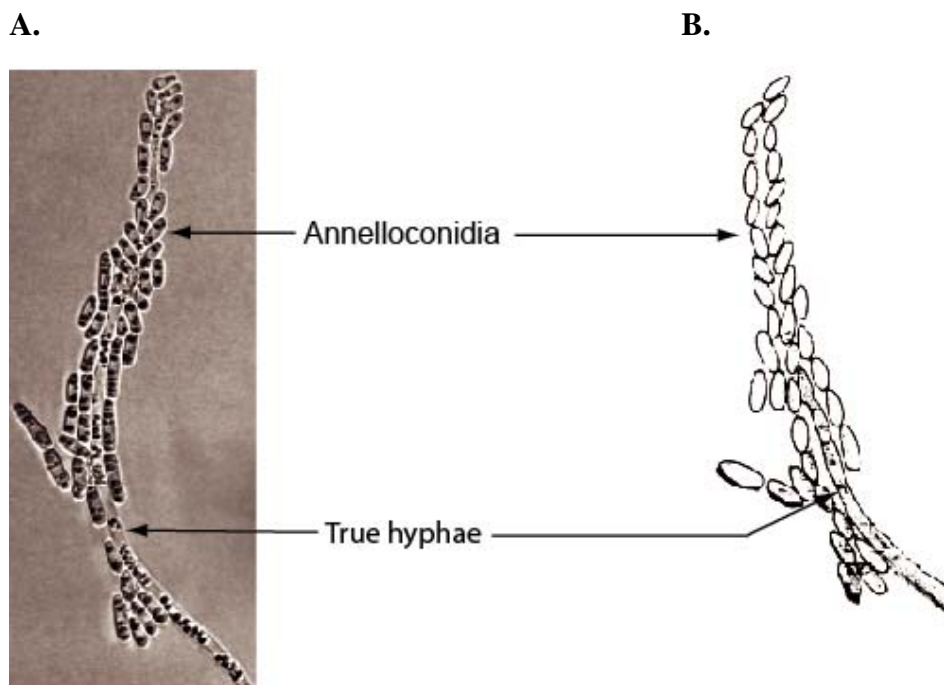


Figure 6. Microscopic morphology of *Blastoschizomyces capitatus* on corn meal agar with Tween 80, shows annelloconidia formed from true hyphae (A, 200× magnification; B, line drawing not to scale).

Y-4 *Candida kefyr*

Source: Vaginal swab / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

Outcome:

No. Laboratories

10

122

0

Validated

Clinical Significance: *Candida kefyr* is a rarely isolated *Candida* species in the clinical laboratory. The infections are reported from the reproductive and digestive tracts and the mucous linings.

Ecology: *C. kefyr* is isolated from milk, other dairy products, grains, and some mammals.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar at 25°C for 3 to 5 days, colonies appeared smooth, creamy, and soft (Figure 7).
2. **Microscopic morphology** – On corn meal agar with Tween 80, microscopic morphology of *C. kefyr* showed plenty of long pseudohyphae, and oval to elongated blastoconidia (Figure 8). Ascospores in asci were observed when *C. kefyr* was cultured on V-8 or malt extract agar.
3. **Differentiation from other yeasts** – *C. kefyr* grows at 45°C and on the cycloheximide containing media. *C. kefyr* ferments glucose, sucrose, lactose, galactose, but not maltose, trehalose, and cellobiose, which differentiates it from other medically important *Candida* species.
4. **In vitro susceptibility testing** – *C. kefyr* showed susceptibility to amphotericin B, caspofungin, different azoles, and 5-fluorocytosine.
5. **Molecular tests** – Randomly amplified polymorphic DNA-polymorase chain reaction (RADP-PCR) was applied for the identification of *C. kefyr*.

Comments: All participating laboratories correctly identified this specimen.

Further Reading:

1. Alberth, M., Majoros, L., Kovalecz, G., Borbás, E., Szegedi, I., J Márton, I., and Kiss, C. 2006. Significance of oral *Candida* infections in children with cancer. *Pathol Oncol Res.* 12: 237-241.
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6. Gil-Lamaignere, C. and Muller, F.M.2004. Differential effects of the combination of caspofungin and terbinafine against *Candida albicans*, *Candida dubliniensis* and *Candida kefyr*. *Int J Antimicrob Agents.* 23: 520-523.
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Figure 7. Five-day-old, creamy, smooth, and soft colony of *Candida kefyr* on Sabouraud's dextrose agar.

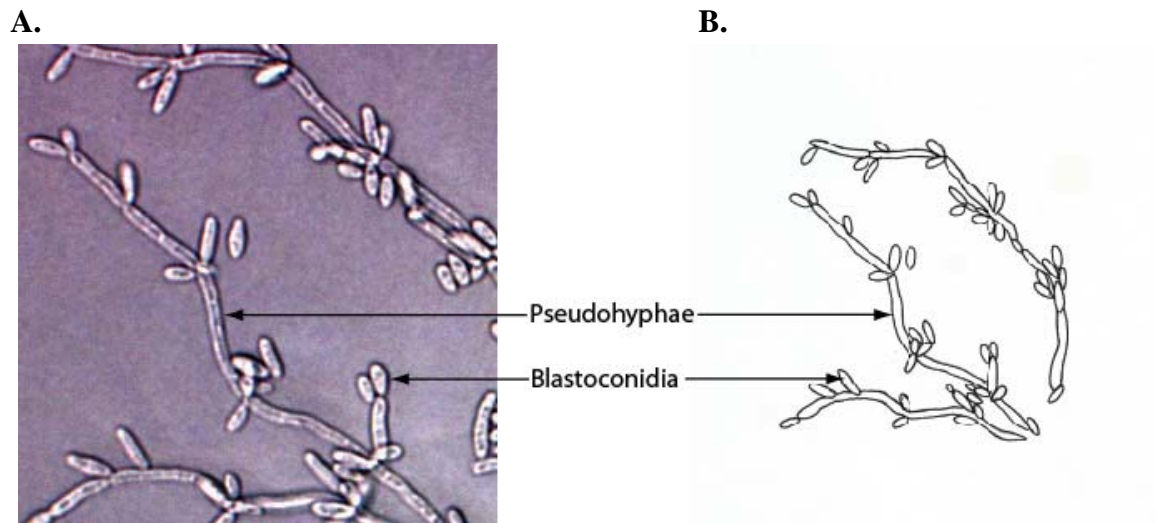


Figure 8. Microscopic morphology of *Candida kefyr* on corn meal agar with Tween 80 showing long, pseudohyphae with oval to elongated blastoconidia (A, 400× magnification; B, line drawing not to scale).

Y-5 *Cryptococcus uniguttulatus*

Source: Catheter / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

Outcome:

No. Laboratories

10

122

0

Validated

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

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

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar, after 7 days at 25°C, *C. uniguttulatus* colony was smooth, dull, cream colored (Figure 9).
2. **Microscopic morphology** – On corn meal agar with Tween 80, round blastoconidia were seen (Figure 10). No pseudo- or true hyphae were formed.
3. **Differentiation from other yeasts** – *C. uniguttulatus* does not ferment any carbohydrate, does not grow at 37°C or on the media containing cycloheximide. It produces urease enzyme. It does not form brown colonies on caffeic seed agar, thus differentiating it from *C. neoformans*. It does not assimilate nitrate, differentiating from *C. albidus*. *C. laurentii* assimilates lactose and dulcitol, but *C. uniguttulatus* does not assimilate these carbohydrates.
4. **In vitro susceptibility testing** – A single clinical isolate was susceptible to amphotericin B and itraconazole.
5. **Molecular tests** – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA was reported to differentiate several *Cryptococcus* species including *C. uniguttulatus*.

Comments: All participating laboratories correctly identified this specimen.

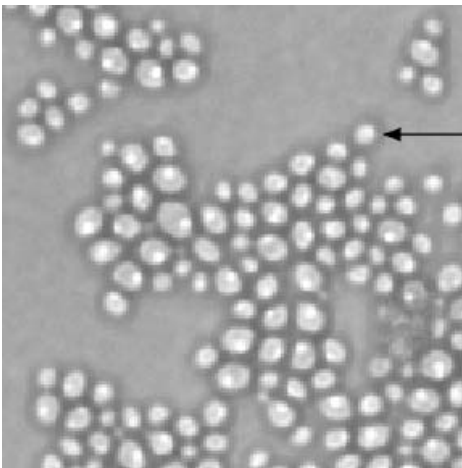
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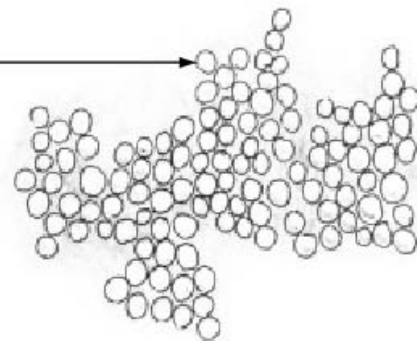


Figure 9. Seven-day-old, soft and smooth colony of *Cryptococcus uniguttulatus* on Sabouraud's dextrose agar.

A.



B.



Blastoconidia

Figure 10. Microscopic morphology of *Cryptococcus uniguttulatus* on corn meal agar with Tween 80 showing round blastoconidia (A, 200× magnification; B, line drawing not to scale).

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 based upon usual test practices in their facilities.

Materials & Results: *Candida glabrata* M1409 (S-1) was the analyte in the May 26, 2010 antifungal proficiency testing event. Thirty-two 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 2).

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 draft document M27-S3 (December 2007)

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. Summary of Laboratory Performance, Antifungal Susceptibility Testing for Yeast Only, May 2010 PT Event

Acceptable Responses/Total # Laboratories (%)	
S- 1: <i>Candida glabrata</i> M1409	
Amphotericin B	26/26 (100)
Anidulafungin	17/17 (100)
Caspofungin	22/22 (100)
Flucytosine (5-FC)	25/26 (96)
Fluconazole	31/32 (97)
Itraconazole	29/29 (100)
Ketoconazole	8/8 (100)
Micafungin	17/17 (100)
Posaconazole	18/18 (100)
Voriconazole	25/25 (100)

Table 3. Distribution of Antifungal MIC values (µg/ml) Reported by Participating Laboratories

S-1: *Candida glabrata* M1409

Drugs (µg/ml)	Total # of labs	0.008	0.015	≤0.03	≤0.06	0.12	0.25	0.38	0.5	0.75	1	2	4	6	8	16	32	≥64	≥128	≥256
Amphotericin B	26					2		1	17	1	5									
Anidulafungin	17		1	9	6	1														
Caspofungin	22			2	10	6	2		2											
Flucytosine (5-FC)	26		1	6	17	2														
Fluconazole	32																1	4	7	20
Itraconazole	29											2			1	23	3			
Ketoconazole	8											2		1	2	2	1			
Micafungin	17	1	12	4																
Posaconazole	18											1			14	2	1			
Voriconazole	25											8	14	1	2					

Table 4. Distribution of Antifungal Susceptibility Interpretations Reported by Participating Laboratories

S-1: *Candida glabrata* M1409

Antifungal Agent	Total # of labs	Susceptible	Susceptible -dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	26	14					12
Anidulafungin	17	17					
Caspofungin	22	22					
Flucytosine (5-FC)	26	25			1		
Fluconazole	32		1		30		1
Itraconazole	29				29		
Ketoconazole	8	1			1		6
Micafungin	17	17					
Posaconazole	18				11		7
Voriconazole	25		5	1	18		1

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR MOLDS (EDUCATIONAL)

Introduction: Eight laboratories participated in this educational test event. The document of M38-A2 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 molds. The following 10 drugs were included 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.

Materials & Results: *Aspergillus fumigatus* M2040 was used. Laboratories were free to choose any number of drugs and preferred test method. Six out of twelve laboratories used CLSI Microdilution method, three laboratories used YeastOne Colorimetric method, and three laboratories used Etest. The acceptable ranges of MIC_{80/100} (µg/ml) values are listed in the Table 5. The MIC values and interpretations based upon yeast breakpoints criteria were reported by all the participating laboratories (Table 6 and Table 7).

Discussion: This maiden event for antifungal susceptibility testing for molds was a success. Seven out of thirty laboratories, which hold antifungal susceptibility testing for yeasts permit, participated in this event. Six references laboratories were invited to perform this test as well. Acceptable results for antifungal susceptibility testing for molds were the consensus results for any single drug. All the participating laboratories except one reported the MIC values within the acceptable ranges for anidulafungin, caspofungin, 5-flucytosine, and voriconazole. All the participating laboratories reported the MIC values within the acceptable ranges for amphotericin B, fluconazole, itraconazole, ketoconazole, and posaconazole, respectively. The consensus values for micafungin could not be generated since too few laboratories tested for these drugs. There are no widely agreed breakpoints for molds although one group has proposed initial breakpoints for itraconazole, voriconazole, and posaconazole (Verweij, et al. 2009).

Future plan: Additional educational events will be offered to assess degree of consensus among participating laboratories for mold antifungal susceptibility testing.

Table 5. Acceptable range of MIC values for Mold Antifungal Susceptibility Educational Sample: *Aspergillus fumigatus* M2040.

Acceptable Ranges of MIC (µg/ml) values	
Educational specimen: <i>Aspergillus fumigatus</i> M2040	
Amphotericin B	0.125 – 2.0
Anidulafungin	0.008-0.03
Caspofungin	≤ 0.5
Flucytosine (5-FC)	≥ 32
Fluconazole	≥ 64
Itraconazole	≥ 8
Ketoconazole	≥ 8
Micafungin	Not Available
Posaconazole	0.25 – 2.0
Voriconazole	0.25 – 2.0

Table 6. MIC ($\mu\text{g/ml}$) Values of Mold Antifungal Susceptibility Educational Sample: *Aspergillus fumigatus* M2040

Drugs ($\mu\text{g/ml}$)	Total # of labs	0.008	0.015	0.03	0.094	0.12	0.19	0.25	0.5	1.0	2.0	4.0	≥ 8	≥ 16	≥ 32	≥ 64	≥ 256
Amphotericin B	11						1		6	3	1						
Anidulafungin	8	1	4	2									1				
Caspofungin	9	1	1	1	1	1		1	2				1				
Flucytosine (5-FC)	10								1						2	6	1
Fluconazole	11															5	6
Itraconazole	10												2	7	1		
Ketoconazole	4												1	1	1	1	
Micafungin	9	2	3	1		1							2				
Posaconazole	8							1	2	1	4						
Voriconazole	10							1	2	2	4	1					

Colors represent the testing method used:

- CLSI microdilution method
- YeastOne Colorimetric method
- Etest
- Multiple methods

Table 7. Distribution of Interpretation Reported by Participating Laboratories for Mold Antifungal Susceptibility Educational Sample: *Aspergillus fumigatus* M2040*

Antifungal Agent	Total # of labs	Susceptible	Intermediate	Resistant	Non-Susceptible	No interpretation
Amphotericin B	11	3				8
Anidulafungin	8	2			1	5
Caspofungin	9	2			1	6
Flucytosine (5-FC)	10			4		6
Fluconazole	11			4		7
Itraconazole	10			3		7
Ketoconazole	4			3		1
Micafungin	9	2			1	6
Posaconazole	8	2	1	1		4
Voriconazole	11	2	2			7

*Based upon yeast interpretation.

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