

Mycology Proficiency Testing Program
September 2009 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*

At least two strains of each mold specimen were examined for inclusion in the proficiency test event of January 2009. 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 appropriate test media. The single strain that best demonstrated the morphologic and physiologic characteristics typical of the species was used as a test analyte. Similarly, 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.

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.

For *Cryptococcus neoformans* 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 80% agreement in participant responses. Target values and acceptable ranges are mean value +/- 2 dilutions; positive or negative answers will be acceptable from laboratories that do not report titers. 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.

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' 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

Mycology – General

	Specimen Key	Validated Specimen	Other Acceptable Answers
M-1	<i>Curvularia</i> sp.	<i>Curvularia</i> sp.	
M-2	<i>Aspergillus terreus</i>	<i>Aspergillus terreus</i>	<i>Aspergillus terreus</i> species complex
M-3	<i>Fusarium</i> sp.	<i>Fusarium</i> sp.	
M-4	<i>Aspergillus clavatus</i>	<i>Aspergillus clavatus</i>	
M-5	<i>Trichophyton mentagrophytes</i>	<i>Trichophyton mentagrophytes</i>	
M-Edu.	<i>Sepedonium</i> sp.		

Mycology – Yeast Only

	Specimen Key	Validated Specimen	Other Acceptable Answers
Y-1	<i>Candida albicans</i>	<i>Candida albicans</i>	
Y-2	<i>Candida dubliniensis</i>	<i>Candida dubliniensis</i>	
Y-3	<i>Candida lipolytica</i>	<i>Candida lipolytica</i>	<i>Yarrowia lipolytica</i>
Y-4	<i>Candida zeylanoides</i>	<i>Candida zeylanoides</i>	
Y-5	<i>Cryptococcus albidus</i>	<i>Cryptococcus albidus</i>	
Y-Edu.	<i>Kodamaea ohmeri</i>		<i>Pichia ohmeri</i>

Mycology – Antifungal Susceptibility Testing for Yeasts (S-1: *Candida parapsilosis* M956)

Drugs	Consensus MIC (µg/ml)	Consensus Interpretation
Amphotericin B	0.12 – 1.0	Susceptible
Anidulafungin	1.0 – 2.0	Susceptible
Caspofungin	0.5 – 2.0	Susceptible
Flucytosine (5-FC)	0.03 – 0.25	Susceptible
Fluconazole	0.25 – 2.0	Susceptible
Itraconazole	0.03 – 0.25	Susceptible
Ketoconazole	0.016 – 0.12	No interpretation
Micafungin	0.5 – 4.0	Susceptible
Posaconazole	0.03 – 0.25	Susceptible
Voriconazole	0.008 – 0.06	Susceptible

Mycology – Direct detection (*Cryptococcus* Antigen Test)

	Specimen Key	Validated Specimen	Acceptable Titer Range
Cn-Ag-1	Negative	Negative	
Cn-Ag-2	Positive (1:256)	Positive (1:256)	1:64 – 1:2048
Cn-Ag-3	Negative	Negative	
Cn-Ag-4	Positive (1:16)	Positive (1:16)	1:4 – 1:64
Cn-Ag-5	Negative	Negative	

LABORATORY PERFORMANCE SUMMARY

Mycology – General

	Correct Responses/ Total # Laboratories (%)	Referees (%)
M - 1 <i>Curvularia</i> sp.	74/74(100)	10/10 (100)
M - 2 <i>Aspergillus terreus</i>	73/74 (99)	10/10 (100)
M - 3 <i>Fusarium</i> sp.	73/74 (99)	10/10 (100)
M - 4 <i>Aspergillus clavatus</i>	72/74 (97)	10/10 (100)
M - 5 <i>Trichophyton mentagrophytes</i>	60/75 (80)	9/10 (90)

Mycology – Yeast Only

	Correct Responses/ Total # Laboratories (%)	Referees (%)
Y - 1 <i>Candida albicans</i>	46/52 (88)	9/10 (90)
Y - 2 <i>Candida dubliniensis</i>	44/52 (85)	9/10 (90)
Y - 3 <i>Candida lipolytica</i>	48/52 (93)	10/10 (100)
Y - 4 <i>Candida zeylanoides</i>	51/52 (98)	10/10 (100)
Y - 5 <i>Cryptococcus albidus</i>	51/52 (98)	10/10 (100)

Mycology – Antifungal Susceptibility Testing for Yeasts

Acceptable Responses/Total # Laboratories (%)

S- 1: *Candida parapsilosis* M957

Amphotericin B	26/26(100)	Itraconazole	28/28 (100)
Anidulafungin	16/16 (100)	Ketoconazole	6/6 (100)
Caspofungin	21/21 (100)	Micafungin	15/15 (100)
Flucytosine (5-FC)	25/25 (100)	Posaconazole	17/17 (100)
Fluconazole	31/31 (100)	Voriconazole	23/23 (100)

Mycology – Direct detection (*Cryptococcus* Antigen Test)

	Correct Responses/Total # Laboratories (%)	
	Qualitative	Quantitative
Cn-Ag-1 Negative	70/70 (100)	NA
Cn-Ag-2 Positive (1:256)	70/70 (100)	64/64 (100)
Cn-Ag-3 Negative	69/70 (99)	NA
Cn-Ag-4 Positive (1:16)	70/70 (100)	62/64 (97)
Cn-Ag-5 Negative	70/70 (100)	NA

TEST STATISTICS

	General	Yeast Only	Antifungal Susceptibility Testing for Yeasts	Direct Detection
Number of participating laboratories	75	52	31	70
Number of referee laboratories	10	10	31	70
Number of laboratories responding by deadline	75	52	31	70
Number of laboratories responding after deadline	0	0	0	0
Number of laboratories not responding	0	0	0	0
Number of laboratories successfully completing this test	73	48	31	70
Number of laboratories unsuccessfully completing this test	2	4	0	0

Number of Laboratories Using Commercial Yeast Identification System*

API 20C AUX	39
AMS Vitek / Vitek2 system	29
Remel Uni-Yeast-Tek	0
IDS Rapid System	0
Microscan	2

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

YeastOne Colorimetric microdilution method	25
Etest	4
Disk diffusion method	0
Others [†]	4

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

EIA method	1
<i>Meridien Diagnostic</i>	1
Latex Agglutination method	69
<i>Immuno-Mycologics</i>	4
<i>Meridien Diagnostic</i>	43
<i>Remel</i>	3
<i>Wampole</i>	17

(*Include multiple systems used by some laboratories)

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

MOLD DESCRIPTIONS

M-1 *Curvularia* sp.

Source: Eye

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	74
Laboratories with incorrect ID:	0

Clinical Significance: An infrequent cause of sinusitis, keratitis, endocarditis, mycetoma, and cerebral abscess. Few cases of disseminated infection have been reported in immunocompromised patients.

Ecology: Cosmopolitan, pathogen of tropical and subtropical plants.

Laboratory Diagnosis:

1. Culture – *Curvularia* grew fast on Sabouraud's dextrose agar. After 5 days, colonies were initially white, later becoming brownish black, wooly in texture (Figure 1).
2. Microscopic morphology – Lactophenol cotton blue or Calcofluor mounts showed brown septate hyphae: conidiophores brown, geniculate with poroconidia (a holoblastic conidium produced through a pore or channel in the cell wall of the conidiophore or conidiogenous cell) slightly curved, brown, 3-4 transverse septations, central cell larger and darker than the other cells (Figure 2).
3. Differentiation from other fungi – *Curvularia* species could be easily differentiated from other dark, muriform (both longitudinal and vertical septa present) fungi by its rapid growth. Microscopically, multicellular, subtly curved conidia with large and dark central cell. In *Bipolaris* species and *Drechslera* species, the conidia are distoseptate (multicellular conidia in which the cells are contained with sacks rather than separated by septa), while in *Curvularia* species, the conidia are transversely septate.
4. In vitro susceptibility testing – Most clinical isolates are susceptible to amphotericin B, itraconazole, miconazole and ketoconazole, but resistant to flucytosine and fluconazole.
5. Molecular tests – Analysis of genes coding for small subunit rRNA sequences of dematiaceous fungal pathogens provided means of assessing relationships of pathogenic and non-pathogenic forms, and accurate identification. Electrophoretic karyotyping of *Curvularia lunata* demonstrated that there are 12 chromosomes, size ranging from 1.4 to 4.0 Mb.

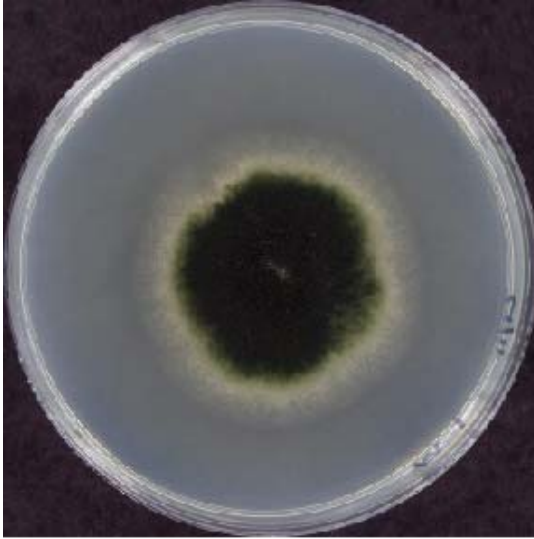
Comments: All the laboratories reported correct identification.

Further Reading:

1. Carter, E. and Boudreaux, C. 2004. Fatal cerebral phaeohyphomycosis due to *Curvularia lunata* in an immunocompetent patient. *J Clin Microbiol.* 42: 5419-5423.
2. Fan, Y.M., Huang, W.M., Li, S.F., Wu, G.F., Li, W., and Chen, R.Y. 2009. Cutaneous phaeohyphomycosis of foot caused by *Curvularia clavata*. *Mycoses.* 52: 544-546.
3. Hiromoto, A., Nagano, T., and Nishigori, C. 2008. Cutaneous infection caused by *Curvularia* species in an immunocompetent patient. *Br J Dermatol.* 158: 1374-1375.

4. Pfaller, M.A., Messer, S.A., Boyken, L., Hollis, R.J., and Diekema, D.J. 2003. *In vitro* susceptibility testing of filamentous fungi: comparison of Etest and reference M38-A microdilution methods for determining posaconazole MICs. *Diagn Microbiol Infect Dis.* 45: 241-244.
5. Pimentel, J.D., Mahadevan, K., Woodgyer, A., Sigler, L., Gibas, C., Harris, O.C., Lupino, M., and Athan, E. 2005. Peritonitis due to *Curvularia inaequalis* in an elderly patient undergoing peritoneal dialysis and a review of six cases of peritonitis associated with other *Curvularia* spp. *J Clin Microbiol.* 43: 4288-4292.
6. Tessari, G., Forni, A., Ferretto, R., Solbiati, M., Faggian, G., Mazzucco, A., and Barba, A. 2003. Lethal systemic dissemination from a cutaneous infection due to *Curvularia lunata* in a heart transplant recipient. *J Eur Acad Dermatol Venereol.* 17: 440-442.
7. Thomas PA. 2003. Fungal infections of the cornea. *Eye.* 17: 852-862.
8. Vachharajani, T.J., Zaman, F., Latif, S., Penn, R., and Abreo, K.D. 2005. *Curvularia geniculata* fungal peritonitis: a case report with review of literature. *Int Urol Nephrol.* 37: 781-784.

A.



B.

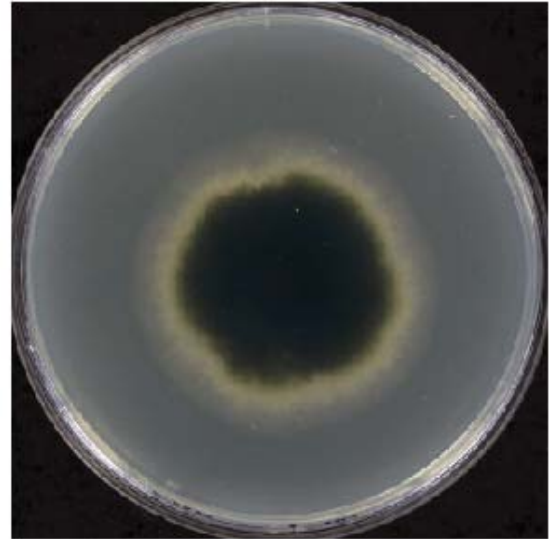
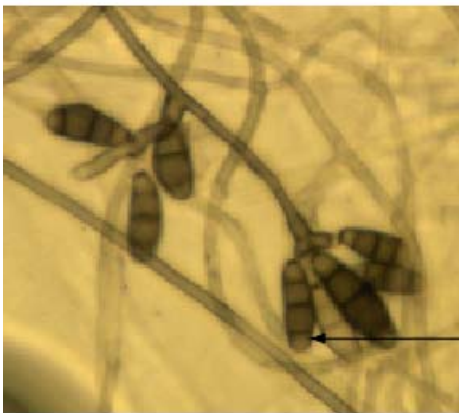
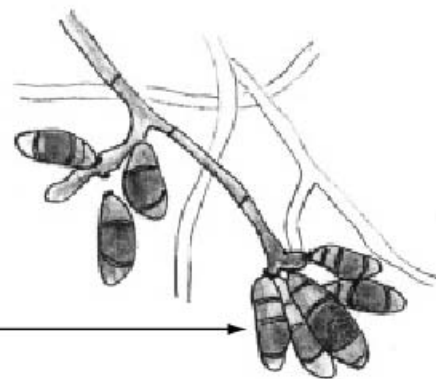


Figure 1. (A) Three-day-old, wooly, brownish black colony of *Curvularia* sp. on Sabouraud's dextrose agar. (B) The reverse of three-day-old colony of *Curvularis* sp. on Sabouraud's dextrose agar.

A.



B.



Curved conidia

Figure 2. Microscopic morphology of *Curvularia* sp. showing hyphae and poroconidia formed sympodially, slightly curved with transverse septations (A, 400X magnification; B, line drawing not to scale).

M-2 *Aspergillus terreus*

Source: Bronchial wash

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	73
Laboratories with incorrect ID:	1
(<i>Aspergillus fumigatus</i>)	(1)

Clinical Significance: *Aspergillus terreus* is an uncommon pathogen that causes allergic broncho-pulmonary aspergillosis or invasive aspergillosis. It is also reported from cutaneous, ophthalmic, pulmonary, and disseminated infections. Keratitis, arthritis, spondylodiscitis, and suppurative otitis are also reported to be caused by *A. terreus*. This pathogen is among very few fungi that are reported to exhibit intrinsic amphotericin B resistance.

Ecology: *A. terreus* is worldwide in distribution and often isolated from stored crops.

Laboratory Diagnosis:

1. **Culture** – *A. terreus* grew rapidly. The colony was cinnamon to brown, powdery on Sabouraud's dextrose agar, at 25°C after 7 days (Figure 3A). The reverse was pale yellow to brown (Figure 3B).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed hyaline hyphae with solitary aleurioconidia attached (Figure 4A) and phialides biseriate and conidial head in the form of compact column (Figure 4B). Conidia were in chains, round, and smooth walled.
3. **Differentiation from other molds** – *A. terreus* forms cinnamon to brown colony, which differentiates it from *A. fumigatus* (dark greenish to gray colony), *A. niger* (black colony), *A. flavus* (yellow to green colony), *A. versicolor* (yellow, tan, to green colored colony) *A. nidulans* (usually green colony), *A. glaucus* (green colony), and *A. clavatus* (blue-green colony). *A. terreus* phialides are biseriate, but *A. fumigatus*, *A. glaucus*, and *A. clavatus* are uniseriate. *A. flavus* phialides are both uniseriate and biseriate. Cleistothecia are usually present in *A. nidulans* and *A. glaucus*, but not in *A. terreus*. Conidial head is loosely radiate and covers most of vesicle for *A. versicolor*, but it is more compact and phialides limited mainly to the upper part of the vesicle in *A. terreus*. Please refer to Table 1 for these details.
4. **In vitro susceptibility testing** – *A. terreus* is resistant to fluconazole and 5 fluorocytosine (5 FC) but susceptible to itraconazole, ketoconazole, and caspofungin.
5. **Molecular tests** – Molecular typing with the random amplified polymorphic DNA (RAPD) technique was used to discriminate between patients isolates. Nested PCR with a mixture of specific primers to DNA topoisomerase II gene was also reported to identify pathogenic *Aspergillus* species including *A. terreus*.




























Comments: One laboratory reported it as *A. fumigatus*, which can grow very well at 45°C and appears as dark greenish to gray colony, while *A. terreus* is cinnamon to brown.

Further Reading:

1. Baddley, J.W., Pappas, P.G., Smith, A.C., and Moser, S.A. 2003. Epidemiology of *Aspergillus terreus* at a university hospital. *J. Clin. Microbiol.* 41: 5525-5529.
2. Balajee SA. 2009. *Aspergillus terreus* complex. *Med Mycol.* 47 Suppl 1:S42-6.

3. Bowman, J.C., Abruzzo, G.K., Flattery, A.M., Gill, C.J., Hickey, E.J., Hsu, M.J., Kahn, J.N., Liberator, P.A., Misura, A.S., Pelak, B.A., Wang, T.C., Douglas, C.M. 2006. Efficacy of caspofungin against *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus nidulans*. *Antimicrob Agents Chemother.* 50: 4202-4205
4. Caston, J.J., Linares, M.J., Gallego, C., Rivero, A., Font, P., Solis, F., Casal, M., Torre-Cisneros, J. 2007. Risk factors for pulmonary *Aspergillus terreus* infection in patients with positive culture for filamentous fungi. *Chest.* 131: 230-6.
5. Kanbe, T., Yamaki, K., and Kikuchi, A. 2002. Identification of the pathogenic *Aspergillus* species by nested PCR using a mixture of specific primers to DNA topoisomerase II gene. *Microbiol. Immunol.* 46: 841-848.
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7. Martin-Mazuelos, E., Peman, J., Valverde, A., Chaves, M., Serrano, M.C., and Canton, E. 2003. Comparison of the Sensititre YeastOne colorimetric antifungal panel and Etest with the NCCLS M38-A method to determine the activity of amphotericin B and itraconazole against clinical isolates of *Aspergillus* spp. *J. Antimicrob. Chemother.* 52: 365-370.
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9. Ozer B, Kalaci A, Duran N, Dogramaci Y, Yanat AN. 2009. Cutaneous infection caused by *Aspergillus terreus*. *J Med Microbiol.* 58: 968-970.
10. Walsh, T.J., Petraitis, V., Petraitiene, R., Field-Ridley, A., Sutton, D., Ghannoum, M., Sein, T., Schaufele, R., Peter, J., Bacher, J., Casler, H., Armstrong, D., Espinel-Ingroff, A., Rinaldi, M.G., Lyman, C.A. 2003. Experimental pulmonary aspergillosis due to *Aspergillus terreus*: pathogenesis and treatment of an emerging fungal pathogen resistant to amphotericin B. *J. Infect. Dis.* 188: 305-319.

Table 1. Scheme for differentiation of *Aspergilli* most commonly involved in human diseases.

	A. flavus	A. fumigatus	A. nidulans	A. niger	A. terreus	A. versicolor
Colony	Yellow - gree	Blue - gree	Dark- green	Black	Tan - buff	Pale - green
Conidiophores						
Vesicle						
Sterigmata						
Conidia						
Other Structures						

A.



B.



Figure 3. (A) Seven-day-old, pale yellow colony of *Aspergillus terreus* on Sabouraud's dextrose agar. (B) The reverse of seven-day-old *Aspergillus terreus* colony on Sabouraud's dextrose agar

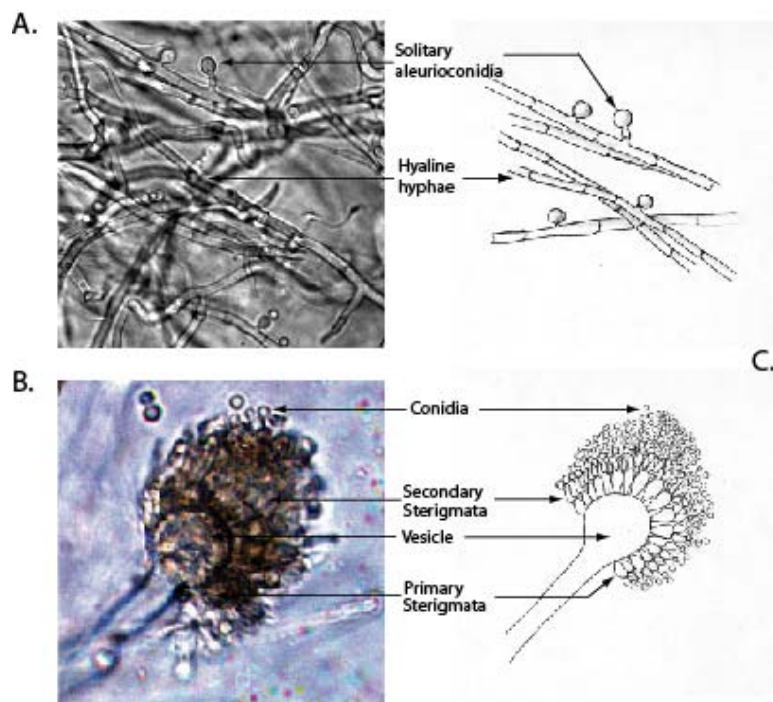


Figure 4. Microscopic morphology of *Aspergillus terreus* showing hyaline hyphae with solitary aleurioconidia attached (A) and typical compact column formed conidial head with biseriata phialides and round, smooth conidia (B) (400× magnification). Line drawing not to scale (C).

M-3 *Fusarium* sp.

Source: Blood

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	73
Laboratories with incorrect ID:	1
(<i>Acremonium</i> sp.)	(1)

Clinical Significance: A frequent casual agent of keratitis, endophthalmitis, and onychomycosis in healthy individuals. It has been reported from peritonitis and disseminated infection in immunocompromised patients. Most common etiologic agents of human infections are *F. oxysporum* species complex (FOSC) and *F. solani* species complex (FSSC).

Ecology: Cosmopolitan in soil and plants. Some species of *Fusarium* are major plant pathogens.

Laboratory Diagnosis:

1. Culture – *Fusarium* grew fast on Sabouraud’s dextrose agar. After 5 days, colony was white, pinkish, to purplish in color, wooly with orange, to red–violet reverse (Figure 5).
2. Microscopic morphology – Lactophenol cotton blue or Calcofluor mounts showed septate hyphae, with short or long phialides. Microconidia were ovoid, and macroconidia were septate and curved-boat/banana-shaped (Figure 6). Chlamydospores were seen.
3. Differentiation from other molds – *Fusarium* species produce curved, septate macroconidia along with single-cell microconidia, which distinguish them from other hyphomycetes, especially *Acremonium* species.
4. In vitro susceptibility testing – Most clinical isolates are susceptible to amphotericin B. Some isolates are variably susceptible to azoles.
5. Molecular tests – PCR method for rapid detection and identification of *Fusarium* species from culture and clinical samples was described. Pan-fungal PCR, followed by nested PCR with species-specific primers was reported for rapid detection of *Fusarium* DNA in ocular samples.

Comments: One participating laboratory reported this specimen as *Acremonium* sp. possibly because macroconidia of *Fusarium* were not observed.

Further Reading:

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A.



B.

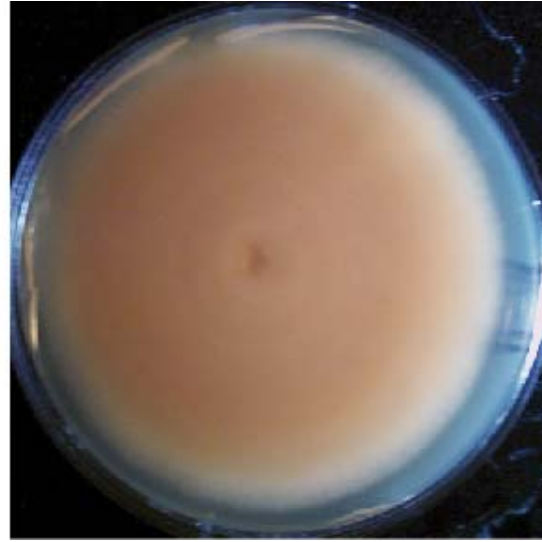


Figure 5. (L) Seven-day-old, woolly, orange to pinkish colony of *Fusarium* sp. on Sabouraud's dextrose agar. (R) The reverse side of seven-day-old *Fusarium* sp. colony on Sabouraud's dextrose agar.

A.



B.

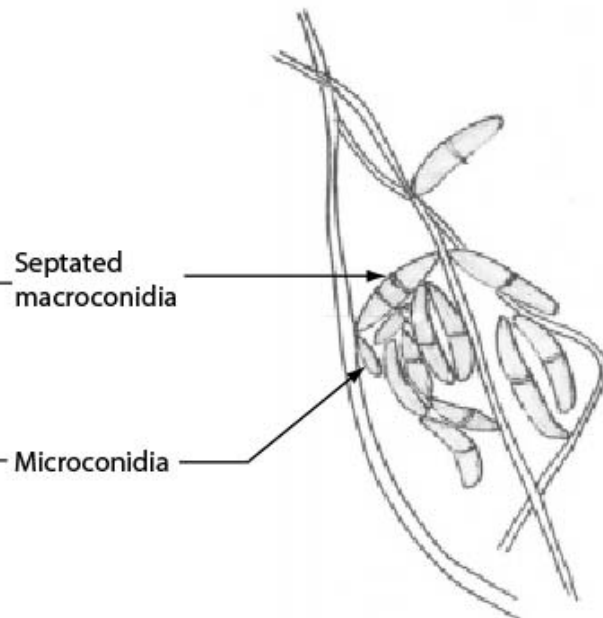


Figure 6. Microscopic morphology of *Fusarium* sp. showing curved, septate microconidia and elongated macroconidia (A, 200× magnification; B: line drawing not to scale)

M-4 *Aspergillus clavatus*

Source: Bone Marrow

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	72
Laboratories with incorrect ID:	2
(<i>Aspergillus fumigatus</i>)	(1)
(<i>Aspergillus versicolor</i>)	(1)

Clinical Significance: *Aspergillus clavatus* causes occasional pulmonary infections and less common cases of otomycosis. It produces patulin in certain foodstuff like cereal that can cause mycotoxicosis. It is also one of the causal agents of allergic aspergillosis.

Ecology: *A. clavatus* is found in soil and widely distributed in food and indoor environments.

Laboratory Diagnosis:

1. Culture – *A. clavatus* grew very fast. On Sabouraud's dextrose agar, after 5 days at 25°C, the colony was bluish-green, powdery (Figure 7A). The reverse was pale to yellowish (Figure 7B).
2. Microscopic morphology – Lactophenol cotton blue mount showed septate hyphae with colorless conidiophores. Conidiophores terminated in vesicle, which was clavate and the entire surface was covered (radiating) with one series of sterigmata (uniseriate). Conidia smooth, ellipsoidal (Figure 8).
3. Differentiation from other *Aspergillus* species – *A. clavatus* was differentiated from other *Aspergilli* by distinctive large, club shaped vesicle. Conidial head is radiate and uniseriate.
4. In vitro susceptibility testing – There was a report that *A. clavatus* was resistant to itraconazole.
5. Molecular tests – A PCR based amplification of ITS1 and ITS 2 regions has been used.

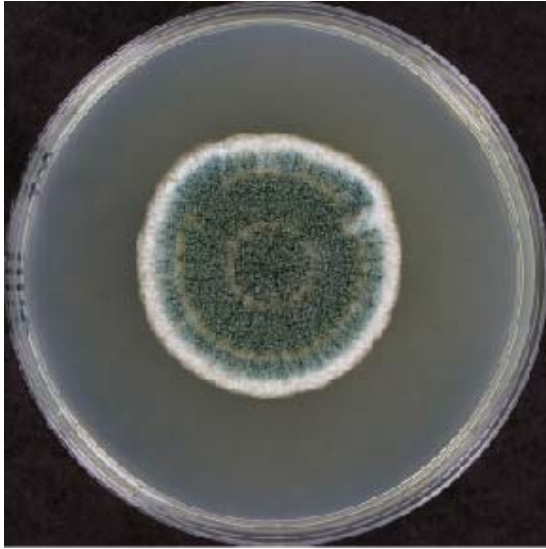
Comments: One laboratory each identified this specimen as *A. fumigatus* or *A. versicolor*. *A. clavatus* can be distinguished from other *Aspergillus* species by its clavate shape of vesicle.

Further Reading:

1. Gilmour JS, Inglis DM, Robb J, Maclean M. 1989. A fodder mycotoxicosis of ruminants caused by contamination of a distillery by-product with *Aspergillus clavatus*. *Vet Rec.* 124: 133-135.
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A.



B.

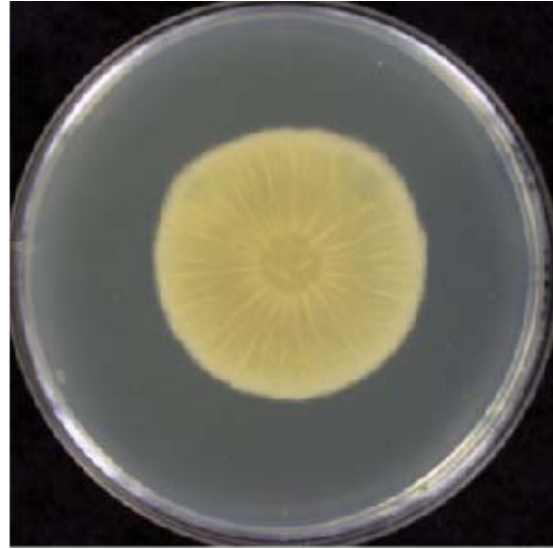


Figure 7. (A) Five-day-old, Green-bluish colony of *Aspergillus clavatus* on Sabouraud's dextrose agar. (B) The reverse of five-day-old *Aspergillus clavatus* colony on Sabouraud's dextrose agar.

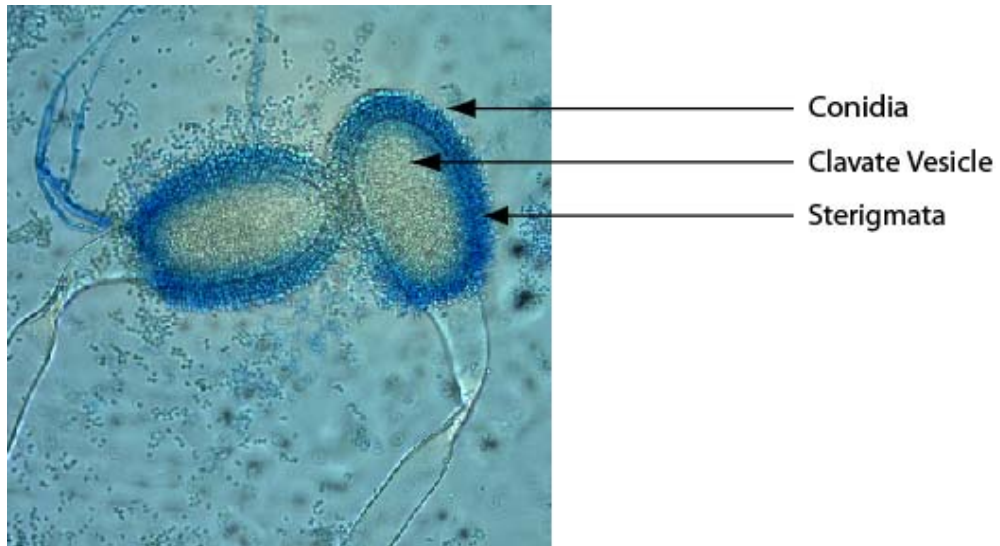


Figure 8. Microscopic morphology of *Aspergillus clavatus* depicting typical radiate heads with clavate vesicle, uniseriate sterigmata, and round conidia (200× magnification).

M-5 *Trichophyton mentagrophytes*

Source: Skin

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	9
Laboratories with correct ID:	60
Laboratories with incorrect ID:	15
(<i>Trichophyton rubrum</i>)	(6)
(<i>Microsporum persicolor</i>)	(4)
(<i>Trichophyton</i> sp.)	(3)
(<i>Trichophyton tonsurans</i>)	(2)

Clinical Significance: *Trichophyton mentagrophytes* frequently causes chronic infections of the feet, nails, hair, and groin. Anthropophilic isolates, such as *T. mentagrophytes* var. *interdigitale*, cause chronic infection of nails, feet, and groin. Zoophilic isolates, such as *T. mentagrophytes* var. *mentagrophytes*, cause infection of skin, scalp, and beard.

Ecology: *T. mentagrophytes* is a cosmopolitan dermatophyte either anthropophilic (primarily associated with humans) or zoophilic (primarily associated with animals). Zoophilic isolates are mainly isolated from rodents, rabbits, hedgehogs, and other small animals.

Laboratory Diagnosis:

1. **Culture** – *Trichophyton mentagrophytes* grew rapidly on Sabouraud's dextrose agar. After 7 days at 25°C, the colony showed white to cream color and powdery surface with yellowish to tan reverse (Figure 9).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed hyaline septate hyphae with both macro- and micro-conidia. Macroconidia were club-shaped with multiseptation and thin walls. Microconidia were round and clustered on conidiophores (Figure 10). Spiral hyphae were often seen.
3. **Differentiation from other dermatophytes** – Microscopically, *T. mentagrophytes* is differentiated from *T. rubrum* by its round conidia produced in clusters, and the presence of spiral hyphae. *T. mentagrophytes* is urease-positive, hair perforation-positive, and has no specific growth requirements. It is differentiated from *Microsporum persicolor* by alkaline reaction on BCP-milk-glucose agar. It is differentiated from *T. terrestre* by good growth at 37°C. It has round-shaped microconidia, which distinguished it from *T. tonsurans* which has diverse shapes and sizes of microconidia.
4. **In vitro susceptibility testing** – Susceptibility testing using the CLSI protocol (M38-P) indicated that common clinical isolates are susceptible to terbinafine, itraconazole, and voriconazole.
5. **Molecular tests** - ITS1 sequences of clinical isolates are species-specific. Species-specific primers of chitin synthase 1 gene have been used to differentiate the *T. mentagrophytes* complex in specimens from humans and animals. The random amplified polymorphic DNA (RAPD) method has been used to study the genetic diversity of clinical isolates.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 rDNA. The sequence is deposited in GenBank under the accession number AY222457.

CBS558.66 (TMZ99001)	1						50
NFI 0105 (AY222457)	AAGTAAAAGT	CGTAACAAGG	TTTCCGTAGG	TGAAC-TGCG	GAAGGATCAT		
			TCCGTAGG	TGAACCTGCG	GAAGGATCAT		

```

51                                                    100
TAGCGCGCAG GCCGGAGGCT GGCCCCCCAC GATAGGGCCA AACGTCCGTC
TAGCGCGCAG GCCGGAGGCT GGCCCCCCAC GATAGGGCCA AACGTCCGTC

101                                                    150
AGGGGTGAGC AGATGTGCGC CGGCCGTACC GCCCCATTCT TGTCTACATT
AGGGGTGAGC AGATGTGCGC CGGCCGTACC GCCCCATTCT TGTCTACATT

151                                                    200
ACTCGGTTGC CTCGGCGGGC CGCGCTCTCC CAGGAGAGCC GTTCGGCGAG
ACTCGGTTGC CTCGGCGGGC CGCGCTCTCC CAGGAGAGCC GTTCGGCGAG

201                                                    250
CCTCTCTTTA GTGGCTAAAC GCTGGACCGC GCCCGCCGGA GGACAGACGC
CCTCTCTTTA GTGGCTAAAC GCTGGACCGC GCCCGCCGGA GGACAGACGC

251                                                    300
AAAAAAATTC TTTCAGAAGA GCTGTCAGTC TGAGCGTTAG CAAGCAAAAA
AAAAAAATTC TTTCAGAAGA GCTGTCAGTC TGAGCGTTAG CAAGCAAAAA

301                                                    350
TCAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCGGCA TCGATGAAGA
TCAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCGGCA TCGATGAAGA

351                                                    400
ACGCAGCGAA ATGCGATAAG TAATGTGAAT TGCAGAATTC CGTGAATCAT
ACGCAGC

```

Alignment of primary sequences of the ITS1 regions of *T. mentagrophytes* CBS 558.66 and PT specimen *T. mentagrophytes* NFI 0105. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Comments: Many participating laboratories were unable to correctly identify this specimen. *T. mentagrophytes* can be differentiated from *T. rubrum* and *Microsporium persicolor* by using BCP-milk-glucose agar. Alkalinization can be observed on BCP-milk-glucose agar for *T. mentagrophytes*, but no change in pH for *T. rubrum* and *M. persicolor*. *T. tonsurans* is distinguished from *T. mentagrophytes* by its microconidia of varying shapes and sizes, and by the stimulating effect on media with thiamine.

Further Reading:

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A.



B.

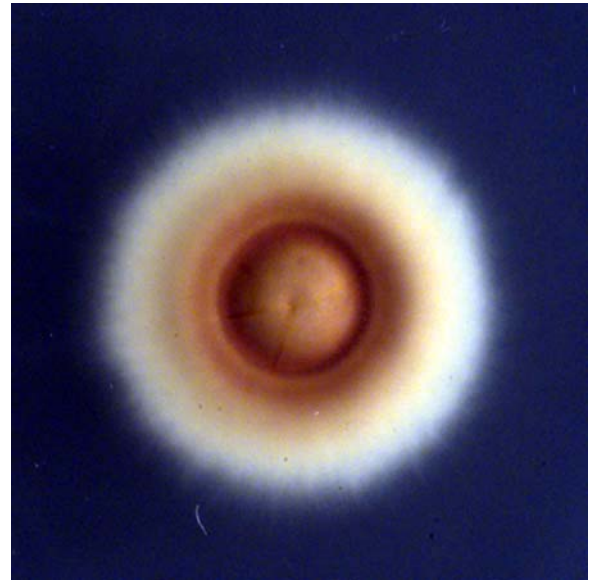


Figure 9. (A) Nine-day-old, white to cream colored powdery colony of *Trichophyton mentagraphytes* on Sabouraud's dextrose agar. (B) The reverse of nine-day-old *T. mentagraphytes* colony on Sabouraud's dextrose agar.

A.



B.



Round, clustered
Microconidia

Figure 10. Microscopic morphology of *Trichophyton mentagraphytes*. Round microconidia clustered on conidiophores are seen (A, 200× magnification; B, line drawing not to scale).

M-Edu. *Sepedonium* sp.

Source: Nail

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	7
Laboratories with correct ID:	52
Laboratories with incorrect ID:	23
(<i>Chrysosporium</i> sp.)	(15)
(<i>Conidiobolus</i> sp.)	(2)
(<i>Emmonsia</i> sp.)	(2)
(<i>Emmonsia parva</i>)	(1)
(<i>Scedosporium</i> sp.)	(1)
(<i>Thielavia</i> sp.)	(1)

Clinical Significance: It is considered a common laboratory contaminant.

Ecology: *Sepedonium* sp. is a soil saprophytic fungus distributed all over the world. It is also found as a parasite on higher basidiomycetes.

Pathogenicity: No information available.

Laboratory Diagnosis:

1. **Culture** – It was a moderately fast growing mold. At 25°C, initial growth was white, becoming fluffy and golden yellow (Figure 11). Very little or no growth is seen when cultures are incubated at 37°C. The fungus is sensitive to cycloheximide.
2. **Microscopic morphology** – Lactophenol Cotton Blue mount showed hyaline, septate hyphae with simple or branched conidiophores, large, thick walled, tuberculate (warty projections) macroconidia, and thin walled, egg shaped microconidia produced along the hyphae (Figure 12).
3. **Differentiation from other molds** – The microscopic morphology of *Sepedonium* species at 30°C resembles the mold phase of *Histoplasma capsulatum*. This fungus can be differentiated from *H. capsulatum* by: a) Failure to grow on media containing cycloheximide; b) Failure to convert to yeast phase at 35 - 37°C; c) Negative result with exoantigen test specific for *H. capsulatum*; d) Negative DNA probe results with *H. capsulatum* specific test.
4. **In vitro susceptibility testing** – No information available
5. **Molecular tests** – No information available

Comments: Many laboratories reported this specimen as *Chrysosporium* sp., which produces arthroconidia intercalated within the conidiogenous hyphae or in short chains. *Sepedonium* sp. usually produces unicellular, rather rounded, thick-walled, and often echinulate conidia. Two laboratories reported this specimen as *Conidiobolus*, which has villose sporangiole very similar to the echinulate conidia from *Sepedonium* sp. However, *Conidiobolus*, a zygomycete, has the free sporangiole with the prominent papilla, which is distinguishable from *Sepedonium* sp. Some laboratories called this specimen as *Emmonsia* spp., which have the conidia that can transform into adiaspores when incubated at 37°C or 40°C.

Further reading:

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6. Piecková E, Jesenská Z. 1999. Occurrence of itraconazole-tolerant micromycetes in the soil and food products. *Folia Microbiol (Praha).* 44: 677-682.
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9. Sutton, B.C. 1975. Two undescribed hyphmycetes from Malaysia. *Antonie van Leeuwenhoek.* 41: 56 – 62.

A.



B.

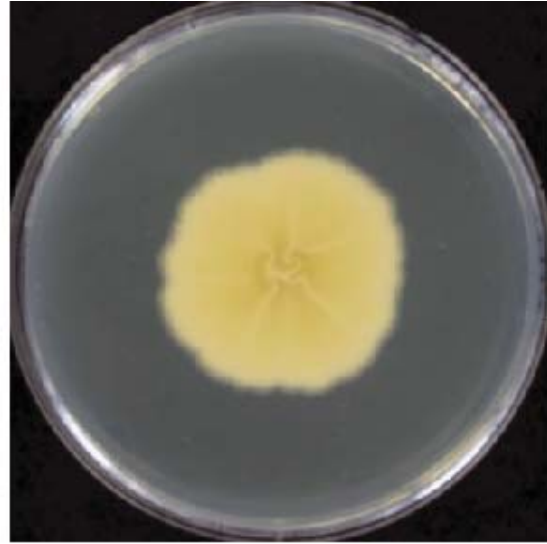
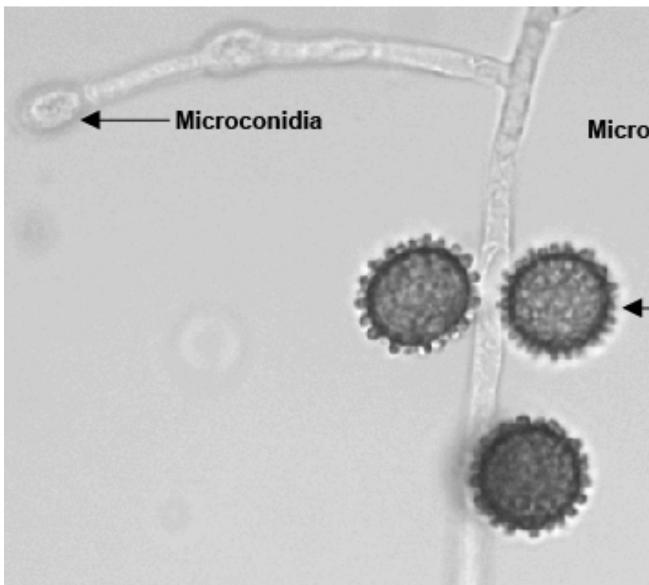


Figure 11. (A) Seven-day-old, white powdery colony of *Sepedonium* sp. on Sabouraud's dextrose agar. (B) The reverse of seven-day-old *Sepedonium* sp. colony on Sabouraud's dextrose agar.

A.



B.

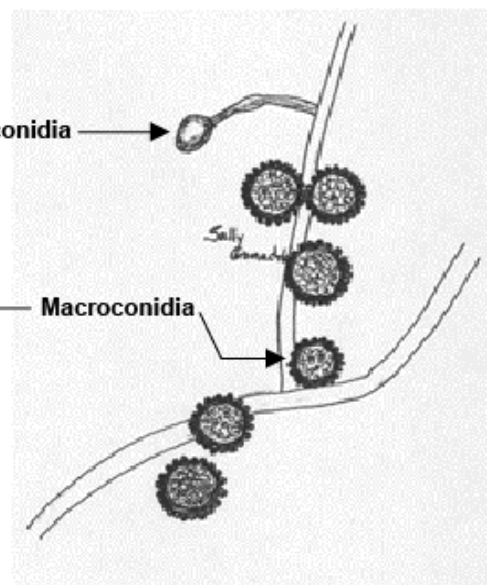


Figure 12. Microscopic morphology of *Sepedonium* species with hyaline hyphae, macroconidia, and microconidia (A, 400X magnification; B, line drawing not to scale).

YEAST DESCRIPTIONS

Y-1 *Candida albicans*

Source: Blood / Urine / Tissue

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	9
Laboratories with correct ID:	46
Laboratories with incorrect ID:	6
(<i>Candida rugosa</i>)	(3)
(<i>Candida dubliniensis</i>)	(2)
(<i>Candida parapsilosis</i>)	(1)

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 13).
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 14).
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 for *C. albicans* rather than dark-green color for *C. dubliniensis*. The positive germ tube test for *C. albicans* distinguishes it from *C. tropicalis*.
4. In vitro susceptibility testing – Both fluconazole-resistant and -sensitive isolates of *C. albicans* were reported. However, in general, *C. albicans* is sensitive to amphotericin B, anidulafungin, caspofungin, micafungin, fluconazole, and posaconazole.
5. Molecular tests – Molecular tests are available for identification of *C. albicans*. Combination of RFLP generated by different restriction digestion of the PCR products of the V3 region of the 25S rDNA gene (rDNA) or from ITS were reportedly able to differentiate *Candida albicans* subgroups, *C. dubliniensis*.

Comments: Three laboratories reported this specimen as *C. rugosa* and one laboratory reported it as *C. parapsilosis*, which are both sensitive to cycloheximide, do not form chlamydospores, and germ tubes negative. Two laboratories identified this specimen as *C. dubliniensis*, which can be easily distinguished by use of commercial available yeast identification systems.

Sequences alignment:

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

```

1
ATCC10231 (AY939786) TCCGTAGGTG AACCTGCGGA AGGATCATT A CTGATTTGCT TAATTGCACC 50
NYSDOH 0602 TCCGTAGGTG AACCTGCGGA AGGATCATT A CTGATTTGCT TAATTGCACC

51
ACATGTGTTT TTCTTTGAAA CAAACTTGCT TTGGCGGTGG GCCCAGCCTG 100
ACATGTGTTT TTCTTTGAAA CAAACTTGCT TTGGCGGTGG GCCCAGCCTG

101
CCGCCAGAGG TCTAAACTTA CAACCAATTT TTTATCAACT TGTCACACCA 150
CCGCCAGAGG TCTAAACTTA CAACCAATTT TTTATCAACT TGTCACACCA

151
GATTATTACT AATAGTCAAA ACTTTCAACA ACGGATCTCT TGGTTCTCGC 200
GATTATTACT AATAGTCAAA ACTTTCAACA ACGGATCTCT TGGTTCTCGC

201
ATCGATGAAG AACGCAGC
ATCGATGAAG AACGCAGC

```

Alignment of primary sequences of the ITS1 regions of *C. albicans* ATCC10231 and PT specimen *C. albicans* NYSDOH 0602.

```

1
ATCC10231 (AY939786) GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATATG AATTGCAGAT 50
NYSDOH 0602 GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATATG AATTGCAGAT

51
ATTCGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCT CTGGTATTCC 100
ATTCGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCT CTGGTATTCC

101
GGAGGGCATG CCTGTTTGGAG CGTCGTTTCT CCCTCAAACC GCTGGGTTTG 150
GGAGGGCATG CCTGTTTGGAG CGTCGTTTCT CCCTCAAACC GCTGGGTTTG

151
GTGTTGAGCA ATACGACTTG GGTTTGCTTG AAAGACGGTA GTGGTAAGGC 200
GTGTTGAGCA ATACGACTTG GGTTTGCTTG AAAGACGGTA GTGGTAAGGC

201
GGGATCGCTT TGACAATGGC TTAGGTCTAA CCAAAAAACAT TGCTTGCGGC 250
GGGATCGCTT TGACAATGGC TTAGGTCTAA CCAAAAAACAT TGCTTGCGGC

251
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GGTAACGTCC ACCACGTATA TCTTCAAAC TTTGACCTCAA ATCAGGTAGG

301
ACTACCCGCT GAACTTAAGC ATATCAATAA GCGGAGGA
ACTACCCGCT GAACTTAAGC ATATCAATAA GCGGAGGA

```

Alignment of primary sequences of the ITS2 regions of *C. albicans* ATCC10231 and PT specimen *C. albicans* NYSDOH 0602.

Further Reading:

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Figure 13. Four-day-old, white, glossy, and smooth colony of *Candida albicans* on Sabouraud's dextrose agar.

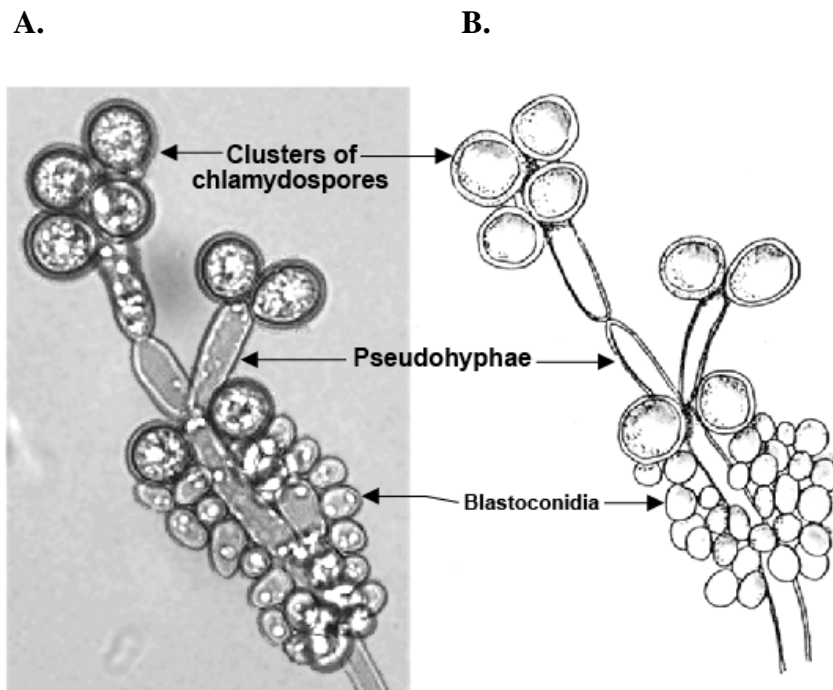


Figure 14. Microscopic morphology of *Candida albicans* on corn meal agar with Tween 80 shows terminal chlamydo spores on pseudohyphae with blastoconidia (left; 400 \times magnification, right; line diagram not to scale).

Y-2 *Candida dubliniensis*

Source: Sputum / Bronchial wash / Urine

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	9
Laboratories with correct ID:	44
Laboratories with incorrect ID:	8
(<i>Candida parapsilosis</i>)	(6)
(<i>Candida albicans</i>)	(2)

History: *Candida dubliniensis* is a chlamydospores-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 (9).

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. *C. dubliniensis* has also been isolated from other body sites including lungs, vagina, blood, and feces.

Ecology: *C. dubliniensis* is globally distributed, but may be restricted to humans as there is only one *C. dubliniensis* isolation from a nonhuman source - tick samples from an Irish seabird colony (7).

Laboratory Diagnosis:

1. Culture – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was white to cream, smooth, and soft (Figure 15). This isolate of *C. dubliniensis* did not grow at 42°C.
2. Microscopic morphology – Lactophenol cotton blue mount showed abundant branched pseudohyphae and true hyphae with blastoconidia. Many chlamydospores in single, pairs, chains, and clusters were observed on Corn meal agar (Figure 16).
3. Differentiation from other yeasts – Phenotypically, *C. dubliniensis* is practically indistinguishable from *C. albicans*. One physiologic feature that does appear to be fairly stable is that *C. dubliniensis* grows poorly at 42°C or not at all at 45°C while *C. albicans* grows well at these temperatures. In addition, *C. dubliniensis* is able to assimilate glycerol, but not xylose nor trehalose. However, *C. albicans* is the opposite. Some commercial yeast identification kits such as the API 20C AUX, VITEK II, or the ID 32C have the codes for *C. dubliniensis* included in the databases. These two closely related yeasts can also be distinguished by molecular tools.
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.

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA. 100% identity was found between this PT specimen and *C. dubliniensis* M

334a (Genbank accession number: AJ249484) for ITS1 and *C. dubliniensis* YN57-151205 (Genbank accession number: DQ355938) for ITS2 region.

Comments: This specimen was validated in the current test event. This is the second time a *C. dubliniensis* was validated in NYSDOH Mycology PT program. This specimen was sent out earlier as an educational specimen in the Mycology PTP October 1997 event, and as a test specimen in October 2000, June 2003, May 2005, January 2007, and September 2007 PT events. In the current test event, about 85% laboratories were able to identify *C. dubliniensis*, which is a notable improvement over last validated event. As summarized earlier in this section, a number of physiological differences could be used to distinguish these two closely related *Candida* species.

Sequences alignment:

```

Query      1      TCCGTAGGTGAACCTGCGGAAGGATCATTACTGATTTGCTTAATTGCACCACATGTGTTT 60
AJ249484   1      TCCGTAGGTGAACCTGCGGAAGGATCATTACTGATTTGCTTAATTGCACCACATGTGTTT 60

Query      61      TGTTTTGGACAAAACCTTGCTTTGGCGGTGGGCCTCTACCTGCCGCCAGAGGACATAAACTT 120
AJ249484   61      TGTTTTGGACAAAACCTTGCTTTGGCGGTGGGCCTCTACCTGCCGCCAGAGGACATAAACTT 120

Query      121     ACAACCAAATTTTTTATAAACTTGTCACGAGATTATTTTTAATAGTCAAAAACCTTCAACA 180
AJ249484   121     ACAACCAAATTTTTTATAAACTTGTCACGAGATTATTTTTAATAGTCAAAAACCTTCAACA 180

Query      181     ACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGC 218
AJ249484   181     ACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGC 218

```

Alignment of primary sequence of the ITS1 regions of *C. dubliniensis* M 334a and PT specimen *C. dubliniensis* NYSDOH 0907.

```

Query      1      CATCGATGAAGAACGCAGCGAAATGCGATACGTAATATGAATTGCAGATATTCGTGAATC 60
DQ355938   158     CATCGATGAAGAACGCAGCGAAATGCGATACGTAATATGAATTGCAGATATTCGTGAATC 217

Query      61      ATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGC 120
DQ355938   218     ATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGC 277

Query      121     GTCGTTTCTCCCTCAAACCCCTAGGGTTTGGTGTGAGCAATACGACTTGGGTTTGCTTG 180
DQ355938   278     GTCGTTTCTCCCTCAAACCCCTAGGGTTTGGTGTGAGCAATACGACTTGGGTTTGCTTG 337

Query      181     AAAGATGATAGTGGTAAGGCGGAGATGCTTGACAATGGCTTAGGTGTAACCAAAAAACATT 240
DQ355938   338     AAAGATGATAGTGGTAAGGCGGAGATGCTTGACAATGGCTTAGGTGTAACCAAAAAACATT 397

Query      241     GCTAAGGCGGTCTCTGGCGTCGCCATTTTATTCTTCAAACCTTGACCTCAAATCAGGTA 300
DQ355938   398     GCTAAGGCGGTCTCTGGCGTCGCCATTTTATTCTTCAAACCTTGACCTCAAATCAGGTA 457

Query      301     GGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA 340
DQ355938   458     GGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA 497

```

Alignment of primary sequence of the ITS2 regions of *C. dubliniensis* YN57-151205 and PT specimen *C. dubliniensis* NYSDOH 0907.

Further Reading:

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Figure 15. Four-day-old, white, glossy, and smooth colony of *Candida albicans* on Sabouraud's dextrose agar.

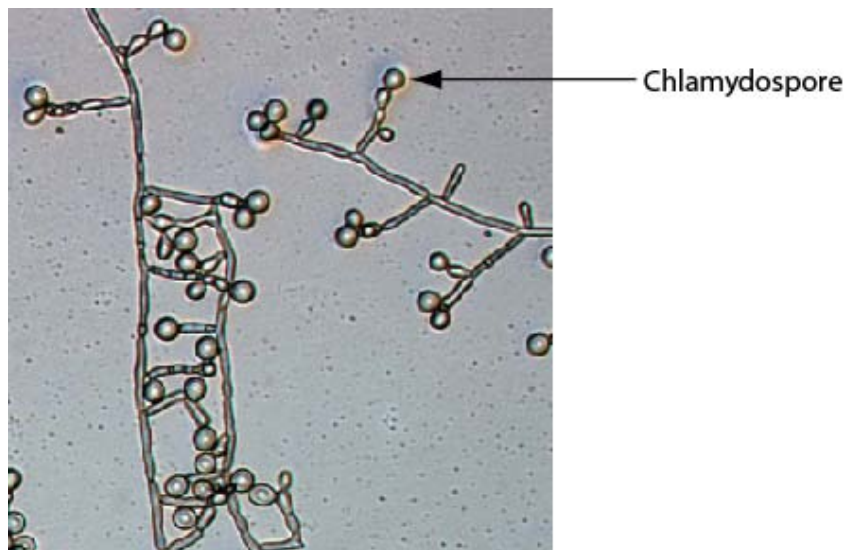


Figure 16. Microscopic morphology of *Candida dubliniensis* on corn meal agar with Tween 80, shows chlamydospores (A, 200× magnification).

Y-3 *Candida lipolytica*

Source: Tissue / Urine

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	48
Laboratories with incorrect ID:	4
(<i>Blastoschizomyces capitatus</i>)	(3)
(<i>Candida krusei</i>)	(1)

Clinical Significance: *Candida lipolytica* causes 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, after 7 days at 25°C, *C. lipolytica* colony was white to cream. The surface was wrinkled (Figure 17).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *C. lipolytica* showed abundant, multibranched true hyphae and infrequent blastoconidia along the hyphae (Figure 18). *Yarrowia lipolytica*, the teleomorph (sexual form) of *C. lipolytica*, formed ascospores on yeast malt agar in 3 to 7 days at 25°C.
3. **Differentiation from other yeasts** – *C. lipolytica* grows on media containing cycloheximide, grows well at 25°C, is urease positive, and negative on nitrate reactions. Sugars are not fermented by *C. lipolytica*. No growth at 42°C and positive growth on media containing cycloheximide differentiates it from *C. krusei*. Positive urease reaction and growth on media containing cycloheximide differentiates it from *C. lambia*. *C. lipolytica* 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. lipolytica* is less susceptible to amphotericin B, but more susceptible to caspofungin with amphotericin B (4). 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. lipolytica* is distinctly related to selected members of Genus *Candida*. Randomly amplified polymorphic DNA (RAPD) PCR has been used for the identification of *C. lipolytica* isolates from dairy products (1, 2).

Comments: *C. lipolytica* can be differentiated from *C. krusei/inconspicua* by its positive growth on the media containing cycloheximide. *C. lipolytica* has positive urease reaction, but *Blastoschizomyces capitatus* has negative reaction.

Further Reading:

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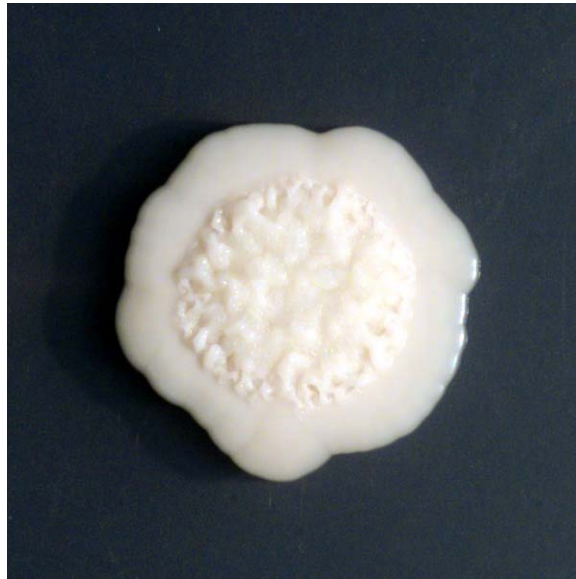
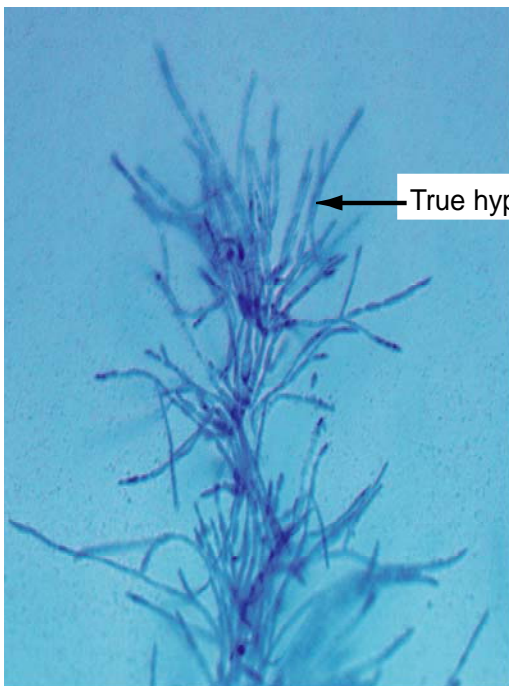


Figure 17. Seven-day-old, white to cream colony with wrinkled surface of *Candida lypholytica* on Sabouraud's dextrose agar.

A.



B.



Figure 18. Microscopic morphology of *Candida lypholytica* on corn meal agar with Tween 80 showing multibranched, true hyphae, and few blastoconidia (A, 200× magnification; B, line drawing not to scale).

Y-4 *Candida zeylanoides*

Source: Nail / Urine

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	51
Laboratories with incorrect ID:	1
(<i>Candida sake</i>)	(1)

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 cosmopolitan, 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 19).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *C. zeylanoides* forms long pseudohyphae, with verticillate, ovoid blastoconidia (Figure 20). Blastoconidia are produced in whorls around the pseudohyphae. Cells fusiform (spindle-shaped).
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.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 and ITS2 rDNA. The sequences are deposited in GenBank under the accession numbers AY382331 and AY382337, respectively.

```
ATCC 7351 (AF335930)      1                               50
NRRL 1774 (AY382331)      TCCGTAGGTG AACCTGCGGA AGGATCATTA CAGTATTCTT TTGCCAGCGC
                            TCCGTAGGTG AACCTGCGGA AGGATCATTA CAGTATTCTT TTGCCAGCGC

                            51                               100
                            TTAATTGCGC GCGGAAAAAC CTTACACACT ATGTTTTTTTT GATTTGAAAC
                            TTAATTGCGC GCGGAAAAAC CTTACACACT ATGTTTTTTTT GATTTGAAAC

                            101                              150
                            TTTTGCTTTG GTCTGACTTA GAAATGAGTT GGGCCAAAGG TTTTATACTA
                            TTTTGCTTTG GTCTGACTTA GAAATGAGTT GGGCCAAAGG TTTTATACTA

                            151                              200
                            AAAC TTCAAT TTTATTATTG AATTGTTAAT TAATTATATT GTCAATTTGT
                            AAAC TTCAAT TTTATTATTG AATTGTTAAT TAATTATATT GTCAATTTGT

                            201                              250
                            TGATTAAAT TCAAAAATCTT CAAAAC TTTC AACACGGAT CTCTTGGTTC
                            TGATTAAAT TCAAAAATCTT CAAAAC TTTC AACACGGAT CTCTTGGTTC
```

251
 TCGCATCGAT GAAGAACGCA GC
 TCGCATCGAT GAAGAACGCA GC

Alignment of primary sequences of the ITS1 regions of *C. zeylanoides* ATCC 7351 and PT specimen *C. zeylanoides* NRRL 1774. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

	1				50
ATCC 7351T (AF218976)	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATATG	AATTGCAGAT
NRRL 1774 (AY382337)	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATATG	AATTGCAGAT
	51				100
	TTTCGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCT	ATGGTATTCC
	TTTCGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCT	ATGGTATTCC
	101				150
	ATAGGGCATG	CCTGTTTGAG	CGTCATTTCT	CTCTCAAATC	TTCGGATTTG
	ATAGGGCATG	CCTGTTTGAG	CGTCATTTCT	CTCTCAAATC	TTCGGATTTG
	151				200
	GTTTTGAGTG	ATACTCTTAG	TCAGACTAAG	CGTTTGCTTG	AAATGTATTG
	GTTTTGAGTG	ATACTCTTAG	TCAGACTAAG	CGTTTGCTTG	AAATGTATTG
	201				250
	GCATGAGTGG	TACTAGATAG	TGCTGAACTG	TTTCAATGTA	TTAGGTTTAT
	GCATGAGTGG	TACTAGATAG	TGCTGAACTG	TTTCAATGTA	TTAGGTTTAT
	251				300
	CCAACTCGTT	GACCAGTATA	GTATTTGTTT	ATTACACAGG	CTCGGCCTTA
	CCAACTCGTT	GACCAGTATA	GTATTTGTTT	ATTACACAGG	CTCGGCCTTA
	301				350
	CAACAACAAA	CAAAGTTTGA	CCTCAAATCA	GGTAGGACTA	CCCCTGTAAC
	CAACAACAAA	CAAAGTTTGA	CCTCAAATCA	GGTAGGACTA	CCCCTGTAAC
	351				
	TTAAGCATAT	CAATAAGCGG	AGGA		
	TTAAGCATAT	CAATAAGCGG	AGGA		

Alignment of primary sequences of the ITS2 regions of *C. zeylanoides* ATCC 7351T and PT specimen *C. zeylanoides* NRRL 1774. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Comments: One laboratory reported this specimen as *Candida sake*, which does not grow on the media containing cycloheximide.

Further Reading:

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Figure 19. Seven-day-old, colony creamish white, butyrous, raised colony of *Candida zeylanoides* on Sabouraud's dextrose agar.

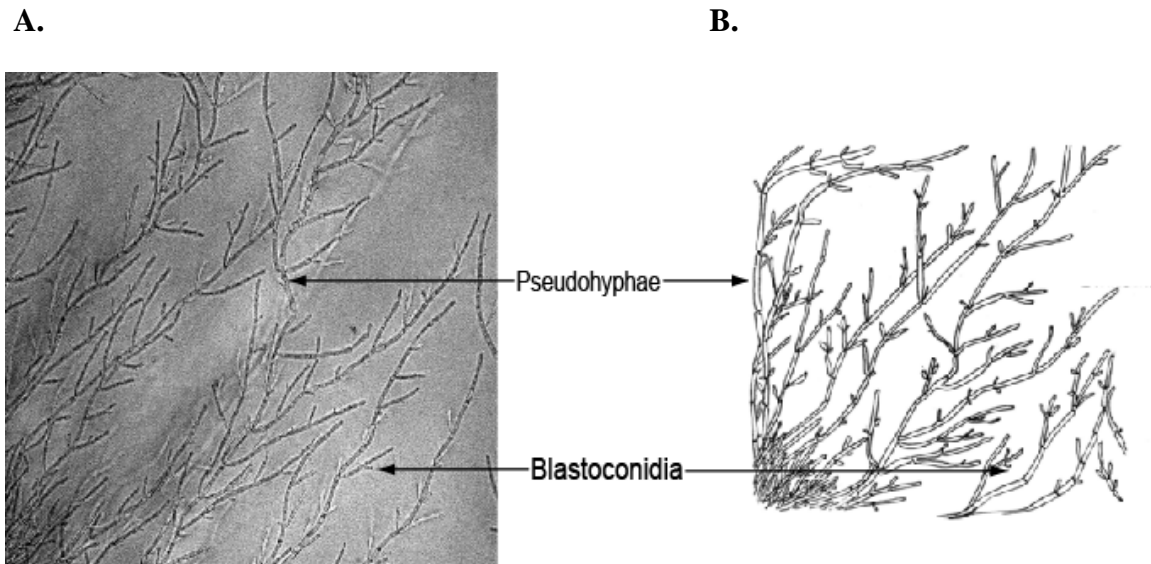


Figure 20. Microscopic morphology of *Candida zeylanoides* on corn meal agar with Tween 80, showing long pseudohyphae with verticillate, ovoid blastoconidia (left; 100x magnification, right; line drawing not to scale).

Y-5 *Cryptococcus albidus*

Source: CSF / Stool / Urine

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	51
Laboratories with incorrect ID:	1
(<i>Candida catenulata</i>)	(1)

Clinical Significance: *Cryptococcus albidus* is a rare causal agent of sepsis, wound infection, and pneumonia in immunocompromised patients.

Ecology: *C. albidus* is cosmopolitan, found on plants and in water. It is also found on skin of animals and humans.

Laboratory Diagnosis:

1. Culture – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was soft, mucoid, cream to pink (Figure 21).
2. Microscopic morphology – On corn meal agar with Tween 80, large, round budding yeast cells, no true hyphae or pseudohyphae are seen (Figure 22).
3. Differentiation from other yeasts – *C. albidus* does not grow on media containing cycloheximide, grows poorly at 37°C, produces urease enzyme, and assimilates nitrate. It is differentiated from *C. neoformans* by its inability to form brown colonies on niger seed agar. Although *C. terreus* is also nitrate-positive, it differs from *C. albidus* in assimilation of sorbitol and N-acetylglucosamine.
4. In vitro susceptibility testing – Almost all isolates are susceptible to amphotericin B, flucytosine, and azoles.
5. Molecular tests – Ribosomal DNA sequence analysis revealed diversity in *C. albidus*.

Comments: The absence of pseudohyphae or true hyphae in *C. albidus* distinguishes it from *C. catenulata*.

Sequences alignment:

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

```
CBS 5592 (AF444370)      1                               50
NRRL 2990                TCCGTAGGTG AACCTGCGGA AGGATCATTA ATGATTGACC GTCTGTGCGAG
                          TCCGTAGGTG AACCTGCGGA AGGATCATTA ATGATTGACC GTCTGTGCGAG

                          51                               100
                          CTTGCTCACA GGCACATCAT ATCCATAACA CCTGTGCACT TGTCGGATGG
                          CTTGCTCACA GGCACATCAT ATCCATAACA CCTGTGCACT TGTCGGATGG

                          101                              150
                          CTTAGTGAAG ACCGCAAGGT TGAATCTATC CATCTACTTT ACATAACAAT
                          CTTAGTGAAG ACCGCAAGGT TGAATCTATC CATCTACTTT ACATAACAAT

                          151                              200
                          TCTGTAAACAA ATGTAGTCTT ATTATAACAT AATAAAACTT TCAACAACGG
                          TCTGTAAACAA ATGTAGTCTT ATTATAACAT AATAAAACTT TCAACAACGG
```

```

201
ATCTCTTGGC TCTCGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA
ATCTCTTGGC TCTCGCATCG ATGAAGAACG CAGC
250

```

Alignment of primary sequences of the ITS1 regions of *C. albidus* CBS 5592 and PT specimen *C. albidus* NYSDOH 0508pt.

```

1
CBS 142 (AF145321) ATCTCTTGGC TCTCGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA
NYSDOH 0508pt      GCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA

51
TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAAC GCACCTTGCG
TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAAC GCACCTTGCG

101
CTCCTTGGTA TTCCGAGGAG CATGCCTGTT TGAGTGTCAT GAAAACCTC
CTCCTTGGTA TTCCGAGGAG CATGCCTGTT TGAGTGTCAT GAAAACCTC

151
AACCCTAGAT TGGTTAAAAC CTCTCTTTGG TTTGGATTTG GACGTTTGCC
AACCCTAGAT TGGTTAAAAC CTCTCTTTGG TTTGGATTTG GACGTTTGCC

201
GATGATAAGT CGGCTCGTCT TAAAAGTAAT AGCTGGATCT GTCTCGCGAC
GATGATAAGT CGGCTCGTCT TAAAAGTAAT AGCTGGATCT GTCTCGCGAC

251
ATGGTTTGAC TTGGCGTAAT AAGTATTTTCG CTAAGGACAT CTTCGGATGG
ATGGTTTGAC TTGGCGTAAT AAGTATTTTCG CTAAGGACAT CTTCGGATGG

301
CCGCGTTGCA GGACTIONAAGA CCGCTTTTCTA ATCCATTGAT CTTCGGATTA
CCGCGTTGCA GGACTIONAAGA CCGCTTTTCTA ATCCATTGAT CTTCGGATTA

351
ATACTCTTGA CATCTGGCCT CAAATCAGGT AGGACTACCC GCTGAACTTA
ATACTCTTGA CATCTGGCCT CAAATCAGGT AGGACTACCC GCTGAACTTA

401
AGCATATCAA TAAGCGGAGGA
AGCATATCAA TAAGCGGAGGA

```

Alignment of primary sequences of the ITS2 regions of *C. albidus* CBS 142 and PT specimen *C. albidus* NYSDOH 0509pt.

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Figure 21. Seven-day-old, mucoid, soft colony of *Cryptococcus albidus* on Sabouraud's dextrose agar.

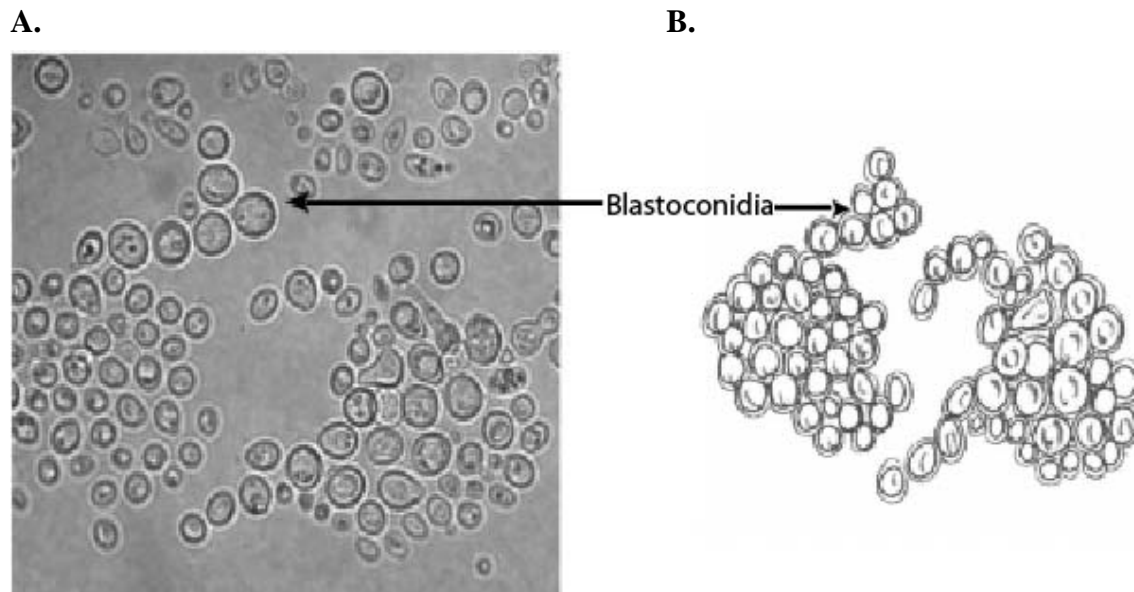


Figure 22. Microscopic morphology of *Cryptococcus albidus*. On corn meal agar culture, large, round blastoconidia are seen (A, 400× magnification; B, line drawing not to scale).

Y-Edu. *Kodamaea ohmeri*

Source: Blood / Mouth / Urine

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	7
Laboratories with correct ID:	27
Laboratories with incorrect ID:	25
(<i>Candida haemulonii</i>)	(19)
(<i>Candida magnoliae</i>)	(3)
(<i>Candida colliculosa</i>)	(1)
(<i>Candida inconspicua</i>)	(1)
(<i>Candida lusitaniae</i>)	(1)

Clinical Significance: *Kodamaea (Pichia) ohmeri* is a frequent causal agent of nosocomial fungemia in immunosuppressed patients. Also, it is an infrequent casual agent of urinary tract infections, brain abscess, and ocular infections.

Ecology: *K. ohmeri* is cosmopolitan in distribution.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was white, fluffy (Figure 23).
2. **Microscopic morphology** – On corn meal agar with Tween 80, few short pseudohyphae with blastoconidia and some ascospores were seen (Figure 24).
3. **Differentiation from other yeasts** – *K. ohmeri* ferments glucose, sucrose, and trehalose, grows at 37°C, and grows on media containing cycloheximide. It does not form pink pigment thereby differentiating from *Rhodotorula* species. It does not produce true hyphae, which differentiates it from *Candida ciferrii* and *Trichosporon beigelii*. Unlike *C. lusitaniae*, it is unable to grow at 45°C.
4. **In vitro susceptibility testing** – Most clinical isolates are susceptible to amphotericin B, 5FC, and azoles such as fluconazole, itraconazole, and ketoconazole; 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 *K. ohmeri* from *C. famata/Debaryomyces hansenii* complex. Isolates of *K. ohmeri* were identified using PCR to amplify ribosomal DNA, followed by restriction digestion of the PCR product.

Comments: *K. ohmeri* is the teleomorph of *Candida guilliermondii*. *K. ohmeri* is also named as *Pichia ohmeri*. Many participating labs reported this specimen as *Candida haemulonii*, which has no sexual state, none or simple pseudohyphae. *Candida magnoliae* is nitrate and nitrite positive, but *K. ohmeri* is nitrate and nitrite negative.

Further Reading:

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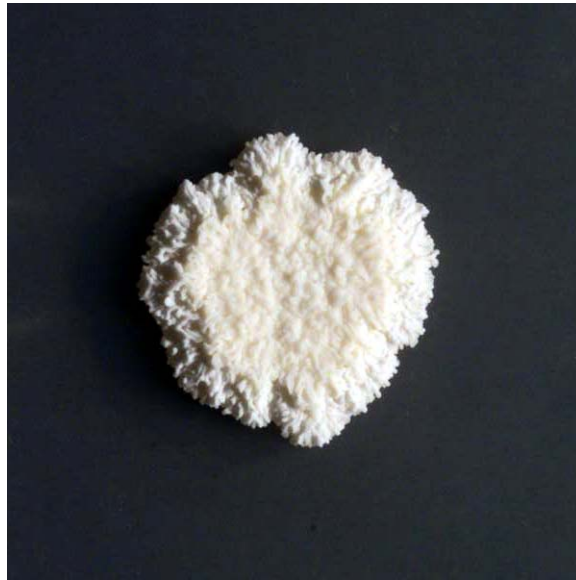


Figure 23. Seven-day-old, white and fluffy colony of *Pichia ohmeri* on Sabouraud's dextrose agar.

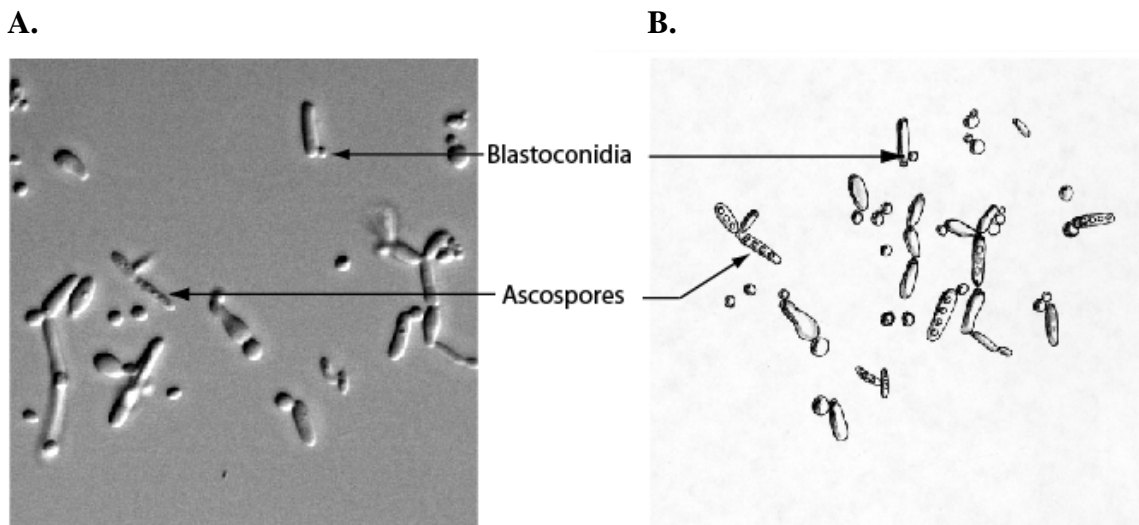


Figure 24. Microscopic morphology of *Pichia ohmeri* on corn meal agar with Tween 80 showing short pseudohyphae with blastoconidia and some ascospores (A, 200 \times magnification; B, line drawing not to scale).

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). Subcommittee on Antifungal Susceptibility Testing is the current standard reference guide for determining the 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 where the results could be read after 24 hr incubation rather than after 48 hr. There are 10 drugs in the antifungal susceptibility testing drug panel of NYSDOH Mycology Proficiency Test Program - amphotericin B, anidulafungin, caspofungin, flucytosine (5-FC), fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. All participating laboratories select any number of antifungal drug(s) from the test panel based upon usual test procedures in their facilities.

Materials & Results: Thirty-one laboratories participated in this event for yeast susceptibility test. *Candida parapsilosis* M957 (S-1) was included in the September 30, 2009 antifungal proficiency testing 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 2. 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 3. Summary of Laboratories Performance September 2009 PT Event

Acceptable Responses/Total # Laboratories (%)		
	S- 1: <i>Candida parapsilosis</i> M957	
Amphotericin B	26/26(100)	
Anidulafungin	16/16 (100)	
Caspofungin	21/21 (100)	
Flucytosine (5-FC)	25/25 (100)	
Fluconazole	31/31 (100)	
Itraconazole	28/28 (100)	
Ketoconazole	6/6 (100)	
Micafungin	15/15 (100)	
Posaconazole	17/17 (100)	
Voriconazole	23/23 (100)	

Table 4. Distribution of MIC ($\mu\text{g/ml}$) Reported by Participating Laboratories Using TREK Diagnostic System Sensititre YeastOne Panel, Etest, NCCLS Broth Microdilution Method

S-1: *Candida parapsilosis* M957

Drugs ($\mu\text{g/ml}$)	Total # of labs	0.008	0.015	0.016	0.023	0.03	0.047	0.06	0.1	0.12	0.25	0.5	0.75	1.0	2.0	4.0
Amphotericin B	26									2	7	14	1	2		
Anidulafungin	16													14	2	
Caspofungin	21											12		8	1	
Flucytosine (5-FC)	25					2	1	14	1	5	1	1				
Fluconazole	31										6	17		7	1	
Itraconazole	28	1				1	1	2		19	4					
Ketoconazole	6			2	1			1		2						
Micafungin	15										1			2	11	1
Posaconazole	17						1	11		4	1					
Voriconazole	23	7	11	3		1		1								

Table 5. Distribution of Interpretation Reported by Participating Laboratories

S-1: *Candida parapsilosis* M957

Antifungal Agent	Total # of labs	Susceptible	Susceptible-dose dependent	Non-susceptible	No interpretation
Amphotericin B	26	16			10
Anidulafungin	16	16			
Caspofungin	21	21			
Flucytosine (5-FC)	25	25			
Fluconazole	31	31			
Itraconazole	28	25	3		
Ketoconazole	6	2			4
Micafungin	15	14		1	
Posaconazole	17	18			5
Voriconazole	23	23			

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), subcommittee on Antifungal Susceptibility Testing, is the current standard reference guide for determining the antifungal susceptibility testing of pathogenic molds. The following 10 drugs were included in the antifungal susceptibility testing drug 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* M2036 was used. Laboratories were free to choose any number of drugs and preferred test method. Five out of eight laboratories used CLSI Microdilution method, three laboratories used YeastOne Colorimetric method, and one laboratory used Etest. The acceptable range of MIC_{80/100} (µg/ml) values were listed in the Table 6. The MIC values and interpretations upon yeast breakpoints reported by all the participating laboratories were summarized in the Table 7 and Table 8 respectively.

Table 6. Acceptable range of MIC values for Mold Antifungal Susceptibility Educational Sample: *Aspergillus fumigatus* M2036.

Acceptable Ranges of MIC (µg/ml) values		
Educational specimen: <i>Aspergillus fumigatus</i> M2036		
Amphotericin B	0.12 – 8.0	
Anidulafungin	Not Available	
Caspofungin	Not Available	
Flucytosine (5-FC)	8 – 256	
Fluconazole	64 – >256	
Itraconazole	0.06 – 1.0	
Ketoconazole	Not Available	
Micafungin	Not Available	
Posaconazole	0.015 – 1.0	
Voriconazole	0.06 – 1.0	

Discussion: This maiden event for antifungal susceptibility testing for molds was a moderate success. The isolate was selected from the study by Ramani *et al.* (2003) strain #44. The MIC values (µg/ml) of 2.0, 0.5, and 0.5 for amphotericin B, itraconazole, and voriconazole respectively were reported in this paper by using CLSI (NCCLS) broth microdilution (M38-A) testing method. Eight out of thirty-one laboratories, which hold antifungal susceptibility testing for yeasts permit, participated in this event. Acceptable results for antifungal susceptibility testing for molds were MICs +/-2 dilutions of the consensus results for any single drug. Basically all the participating laboratories reported the MIC values within the acceptable ranges for amphotericin B, 5-flucytosine, fluconazole, itraconazole, posaconazole, and voriconazole. The consensus values for anidulafungin, caspofungin, ketoconazole, and micafungin could not be generated since too few laboratories tested those drugs. There are no widely agreed breakpoints for antifungal

susceptibility testing 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 7. MIC ($\mu\text{g/ml}$) Values of Mold Antifungal Susceptibility Educational Sample: *Aspergillus fumigatus* M2036

Drugs ($\mu\text{g/ml}$)	Total # of labs	0.008	0.015	0.016	0.03	0.06	0.38	0.12	0.25	0.5	1.0	2.0	8	32	64	128	256	512
Amphotericin B	7							1		2	1	2	1					
Anidulafungin	3	1	1										1					
Caspofungin	4					1			1	1			1					
Flucytosine (5-FC)	7										1			1	1	2	1	
Fluconazole	7											1			1	1	4	1
Itraconazole	7					1	1		1	2	1	1						
Ketoconazole	1											1						
Micafungin	3	1		1									1					
Posaconazole	5				1	1		1	2									
Voriconazole	6								6									

Colors represent the testing method used:

- CLSI microdilution method
- YeastOne Colorimetric method
- Etest
- All three methods

Table 8. Distribution of Interpretation Reported by Participating Laboratories for Mold Antifungal Susceptibility Educational Sample: *Aspergillus fumigatus* M2036*

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

*Based upon yeast interpretation.

Further Reading:

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DIRECT DETECTION (*CRYPTOCOCCUS NEOFORMANS* ANTIGEN TEST)

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 (4, 6, 7 and 8), and showed it to be a valuable aid in establishing a diagnosis when culture was negative (5). Paired serum and CSF specimens allowed detection of antigen in confirmed cases (8). 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: Seventy laboratories participated in the September 30, 2009 direct detection antigen testing event. Two positive serum samples for cryptococcal antigen were included. The titers were 1:256 and 1:16 for Cn-Ag-2 and Cn-Ag-4 respectively. Titers within ± 2 dilutions of the reference and/or consensus results were the acceptable results for this event.

Results: The performance of 70 laboratories was satisfactory in this test event. One laboratory reported the specimen Cn-Ag-3 as positive with the titer of 1:16 instead of negative. One laboratory each reported the titer of specimen Cn-Ag-4 higher or lower than the acceptable titer range, respectively. The supplementary information on quantitative assays on *Cryptococcus neoformans* antigen test is summarized in Table 6.

Further Reading:

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Table 6. Summary of quantitative assay

The number of laboratories that reported titers is listed for positive test samples Cn-Ag-2 and Cn-Ag-4

Method	Sample	Cn-Ag-2 Titers								
		Total # of laboratories	64	128	200	256	400	512	1024	2048
EIA										
	(Meridien Diagnostic)	1						1		
Latex Agglutination										
	(Immuno-Mycologics)	3	1					2		
	(Meridien Diagnostic)	40		2	1	11	1	16	8	1
	(Remel)	4							4	
	(Wampole)	16	1			2		4	8	1

Method	Sample	Cn-Ag-4 Titers								
		Total # of laboratories	2	8	16	20	32	40	64	128
EIA										
	(Meridien Diagnostic)	1					1			
Latex Agglutination										
	(Immuno-Mycologics)	3	1		1		1			
	(Meridien Diagnostic)	40		3	20	1	9	1	6	
	(Remel)	4		1	1		1		1	
	(Wampole)	16	1		7		5		2	1

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