

Mycology Proficiency Testing Program Critique September 2007



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New York State Department of Health

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Schedule of 2007 Mycology PT Mailouts*[‡]

GENERAL

January 31, 2007
May 23, 2007
September 26, 2007

GENERAL POSTMARK DEADLINES

March 16, 2007
June 15, 2007
November 9, 2007

YEASTS ONLY

January 31, 2007
May 23, 2007
September 26, 2007

YEASTS ONLY POSTMARK DEADLINES

February 23, 2007
June 15, 2007
October 19, 2007

DIRECT DETECTION TESTING

January 31, 2007
September 26, 2007

DIRECT DETECTION TESTING POSTMARK DEADLINES

February 16, 2007
October 12, 2007

ANTIFUNGAL SUSCEPTIBILITY FOR YEASTS

January 31, 2007
May 23, 2007
September 26, 2007

ANTIFUNGAL SUSCEPTIBILITY FOR YEASTS POSTMARK DEADLINES

March 16, 2007
June 15, 2007
November 9, 2007

*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 September 2006. 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 or 80 percent of all participating laboratories. The referee laboratories are selected at random from among hospital laboratories participating in the program. They represent all geographical areas of New York State and must have a record of excellent performance during the preceding three years. The maximum score for each specimen is 20 based on the formula:

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

Acceptable results for antifungal susceptibility testing are MICs within +/-2 dilutions of the reference result for a particular organism against a single drug. If a result falls outside of this range, the laboratory gets a score of zero for that particular test component or set. One yeast is to be tested against following eight drugs: 5-fluorocytosine, amphotericin B, caspofungin, fluconazole, itraconazole, ketoconazole, posaconazole, and voriconazole. The participating laboratories are allowed to select any number of antifungal drug(s) from the test panel based upon customary practice in their facilities. A maximum score of 100 will be equally divided among the drugs selected by the individual laboratory.

For *Cryptococcus* antigen test, laboratories are evaluated on the basis of their responses and on overall performance for all the analytes tested in the Direct Detection category. Appropriate responses are determined by participant consensus requiring 80% agreement in the test. Qualitative/quantitative results are graded in relation to results given by participants. 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 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>Malbranchea</i> sp.	Not validated	
M-2	<i>Acremonium</i> sp.	<i>Acremonium</i> sp.	
M-3	<i>Chrysosporium</i> sp.	<i>Chrysosporium</i> sp.	
M-4	<i>Fusarium</i> sp.	<i>Fusarium</i> sp.	<i>Fusarium oxysporum</i> <i>Fusarium solani</i>
M-5	<i>Penicillium</i> sp.	<i>Penicillium</i> sp.	
M-Edu.	<i>Trichothecium</i> sp.		<i>Trichothecium roseum</i>

Mycology – Yeast Only

	Specimen Key	Validated Specimen	Other Acceptable Answers
Y-1	<i>Candida dubliniensis</i>	<i>Candida dubliniensis</i>	
Y-2	<i>Rhodotorula minuta</i>	<i>Rhodotorula minuta</i>	
Y-3	<i>Candida viswanathii</i>	Not validated	
Y-4	<i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>	
Y-5	<i>Trichosporon asahii</i>	<i>Trichosporon asahii</i>	

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

Drugs	Validated range (µg/ml)
5-fluorocytosine	0.125 – 2.0
Amphotericin B	0.06 – 2.0
Caspofungin	0.015 – 1.0
Fluconazole	0.06 – 4.0
Itraconazole	≤ 0.015 – 0.5
Ketoconazole	≤ 0.25
Posaconazole	≤ 0.125
Voriconazole	≤ 0.125

Mycology – Direct detection (*Cryptococcus* Antigen Test)

	Specimen Key	Validated Specimen	Other Acceptable Titer Range
Cn-Ag-1	Negative	Negative	
Cn-Ag-2	Positive (1:16)	Positive (1:16)	1:4 – 1:64
Cn-Ag-3	Negative	Negative	
Cn-Ag-4	Positive (1:128)	Positive (1:128)	1:32 – 1:512
Cn-Ag-5	Negative	Negative	
Cn-Ag-Edu	Positive (1:128)		1:32 – 1:512

LABORATORY PERFORMANCE SUMMARY

Mycology – General

	Correct Responses/ Total # Laboratories (%)	Referees (%)
M - 1 <i>Malbranchea</i> sp.	54/77 (70)	7/10 (70)
M - 2 <i>Acremonium</i> sp.	75/77 (97)	10/10 (100)
M - 3 <i>Chrysosporium</i> sp.	71/77 (92)	10/10 (100)
M - 4 <i>Fusarium</i> sp.	76/77 (99)	10/10 (100)
M - 5 <i>Penicillium</i> sp.	70/77 (91)	9/10 (90)

Mycology – Yeast Only

	Correct Responses/ Total # Laboratories (%)	Referees (%)
Y - 1 <i>Candida dubliniensis</i>	41/52 (79)	9/10 (90)
Y - 2 <i>Rhodotorula minuta</i>	51/52 (98)	10/10 (100)
Y - 3 <i>Candida viswanathii</i>	1/51 (2)	0/10 (0)
Y - 4 <i>Candida parapsilosis</i>	52/52 (100)	10/10 (100)
Y - 5 <i>Trichosporon asahii</i>	49/52 (94)	9/10 (90)

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

Correct Responses/Total # Laboratories (%)

5-fluorocytosine	22/22 (100)	Fluconazole	28/29 (97)	Posaconazole	11/12 (92)
Amphotericin B	23/23 (100)	Itraconazole	23/25 (92)	Voriconazole	20/20 (100)
Caspofungin	19/19 (100)	Ketoconazole	17/18 (94)		

Mycology – Direct detection (*Cryptococcus* Antigen Test)

	Correct Responses/Total # Laboratories (%)	
	Qualitative	Quantitative
Cn-Ag-1 Negative	75/75 (100)	NA
Cn-Ag-2 Positive (1:16)	75/75 (100)	66/69 (96)
Cn-Ag-3 Negative	74/75 (99)	NA
Cn-Ag-4 Positive (1:128)	75/75 (100)	67/69 (97)
Cn-Ag-5 Negative	75/75 (100)	NA

TEST STATISTICS

	General	Yeast Only	Antifungal Susceptibility Testing for Yeasts	Direct Detection
Number of participating laboratories	78	53	29	76
Number of referee laboratories	10	10	29	76
Number of laboratories responding by deadline	77	52	29	75
Number of laboratories responding after deadline	0	0	0	0
Number of laboratories not responding	1	1	0	1
Number of laboratories successfully completing this test	75	50	27	75
Number of laboratories unsuccessfully completing this test	3	3	2	1

Number of Laboratories Using Commercial Yeast Identification System*

API 20C AUX	39
AMS Vitek system	25
Remel Uni-Yeast-Tek	4
IDS Rapid System	3
Microscan	1

Number of Laboratories Using Commercial Antifungal Susceptibility Testing System/Method

YeastOne Colorimetric microdilution method	18
Etest	5
Disk diffusion method	1
Others [†]	6

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

EIA method	2
<i>Meridien Diagnostic</i>	2
Latex Agglutination method	73
<i>Immuno-Mycologics</i>	4
<i>Meridien Diagnostic</i>	44
<i>Remel</i>	5
<i>Wampole</i>	22

(*Include multiple systems used by some laboratories)

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

MOLD DESCRIPTIONS

M-1 *Malbranchea* sp.

Source: Lung

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	7
Laboratories with correct ID:	54
Laboratories with incorrect ID:	23
(<i>Arthrographis</i> sp.)	(21)
(<i>Emmonsia parva</i>)	(1)
(<i>Trichophyton terrestre</i>)	(1)

Clinical Significance: *Malbranchea* sp. is not a known human pathogenic fungus. It has been isolated from a variety of clinical specimens – skin lesions, toe nails, CSF, sinus, etc.

Ecology: *Malbranchea* sp. is found worldwide in soil, decaying vegetation and animal dung.

Laboratory Diagnosis:

1. **Culture** – Colonies on Sabouraud dextrose agar and potato dextrose agar were woolly to powdery in texture, grew rapidly, and color ranged from white, pink, buff to brown (Figure 1A). The reverse was white, yellow, to buff towards the center (Figure 1B). Like other saprobes, *Malbranchea* sp. was cycloheximide- sensitive.
2. **Microscopic morphology** – Lactophenol Cotton Blue or Calcofluor mount revealed hyaline, septate hyphae with no conidiophores. Fragmentation of hyphae caused formation of arthroconidia, which had the same diameter as that of the hyaline hyphae and alternate with disjuncter cells (Figure 2).
3. **Differentiation from other molds** – *Malbranchea* sp. produces alternate arthroconidia, which have the same width as that of the vegetative hyphae. Once this arthroconidia are liberated along with the ‘annular frills’ (the remnants of disjuncter cells), the fungus closely resembles *Coccidioides immitis*. *Arthrographis* sp. can also be confused with *Malbranchea* species. However *Arthrographis* sp. produces arthroconidia from conidiophores. There may be some resemblance to *Odiendron* sp., which is a dematiaceous, arthroconidia-forming fungi. These features are summarized in Table 1.
4. **In vitro susceptibility testing** – The limited susceptibility testing data available showed that this organism was susceptible to amphotericin B, and azoles like itraconazole, ketoconazole but resistant to fluconazole.
5. **Molecular tests** – Very limited information available. Internal transcribed spacer (ITS) regions can be used for *Malbranchea* sp. identification.

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 *Malbranchea filamentosa* UAMH9987 (Genebank accession number: AY177301) for both ITS1 and ITS2 regions.

Comments: This specimen was not validated in this test event although it was sent by our program previously (Oct. 2000 Test Event). The isolate sent in the present testing event resembled *C. immitis*. However, the isolate did not grow in the presence of cycloheximide, and also had a negative reaction for *C. immitis* DNA probe. Many labs reported this isolate as *Arthrographis* species, which could be easily

differentiated by presence of conidiophore. It is important to distinguish between *Malbranchea* species and *C. immitis* as the later is a highly virulent and increasingly being reported outside of its endemic zone in the Southwest. *Trichophyton terrestre* produces 2-6 cells long, cylindrical macroconidia, which are very different from arthroconidia of *Malbranchea* sp. *Emmonsia parva* can grow in the presence of cycloheximide but *Malbranchea* sp. does not.

Sequences alignment:

```

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AY177301  1      CATTAAAGTGTTAAGCCGGCGCCTCCGTGTGCCGGTGAAACTCCACCCCTTGACTACTATA  60
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AY177301  181     TAAAACCTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGC  232

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Alignment of primary sequence of the ITS1 regions of *Auxarthron filamentosum* (anamorph: *Malbranchea filamentosa*) UAMH 9987 and PT specimen *Malbranchea* sp. NYSDOH 0907.

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AY177301  274     ATCGAATCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGC  333
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AY177301  334     GTCATTGCAACCCTCAAGCGCGGCTTGTGTGTTGGGCCTCGTCCCCCGTGGACGTGCCCG  393
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AY177301  394     AAAGGCAGTGGCGGGCGTCCGTTTCGGTGCCCGAGCGTATGGGAACTCTTATACCGCTCGA  453
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|
|
Query      241     AGGGCCCGGCGGGCGCTGGTCAGAACCAAATCTTTTACCGGTTGACCTCGGATCAGG  296
|
|
|
AY177301  454     AGGGCCCGGCGGGCGCTGGTCAGAACCAAATCTTTTACCGGTTGACCTCGGATCAGG  509

```

Alignment of primary sequence of the ITS2 regions of *Auxarthron filamentosum* (anamorph: *Malbranchea filamentosa*) UAMH 9987 and PT specimen *Malbranchea* sp. NYSDOH 0907.

Further Reading:

1. Brenda, T.J. Jr. and Corey, J.P. 1994. *Malbranchea pulchella* fungal sinusitis. *Otolaryngology – Head and Neck Surgery*. 110: 501 – 504.
2. Currah, R.S. 1985. Taxonomy of the Onygenales: Arthrodermataceae, Gymnoascaceae, Myxotrichaceae, and Onygenaceae. *Mycotaxon*. 24: 1 –216.

3. Kaufman, L., Standard, P.G., Huppert, M., and Pappagianis, D. 1985. Comparison and diagnostic value of the coccidioidin heat-stable (HS and tube precipitin) antigens in immunodiffusion. *J Clin Microbiology*.
4. 22: 515 – 518.
5. Padhye, A.A., Smith, G., Standard, P.G., McLaughlin, D., and Kaufman, L. 1994. Comparative evaluation of chemiluminescent DNA probe assays and exotigen tests for rapid identification of *Blastomyces dermatitidis* and *Coccidioides immitis*. *J Clin Microbiology*. 32: 867 – 870.
6. Pan, S., Sigler, L., and Cole, G.T. 1994. Evidence for a phylogenetic connection between *Coccidioides immitis* and *Uncinocarpus reesii* (Onygenaceae). *Microbiology*. 140: 1481 – 1494.
7. Pounder, J.I., Hansen, D., and Woods, G.L. 2006. Identification of *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides* species by repetitive-sequence-based PCR. *J Clin Microbiol*. 44: 2977-2982.

TABLE 1: Summary of features that differentiate some arthroconidia- producing fungi.

Characteristic	<i>Malbranchea</i> sp.	<i>Coccidioides immitis/posadasii</i>	<i>Arthrographis</i> sp.	<i>Odiodendron</i> sp.
Growth on cycloheximide medium (25°C)	No growth	Growth	No growth	No growth
Conidiophores	None	Present	Present	Dark-colored
Growth and morphology (37°C)	No or poor growth	Thick walled spherules filled with endospores*	Very little growth	Very little growth
<i>C.immitis</i> GenProbe	Negative	Positive	Negative	Negative

***In vitro conversion to tissue form may not be easily obtained, may require prolonged incubation under 5% CO₂.**

A.



B.

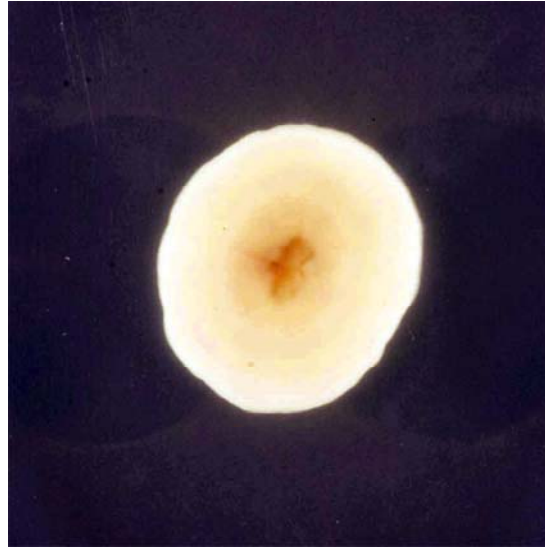
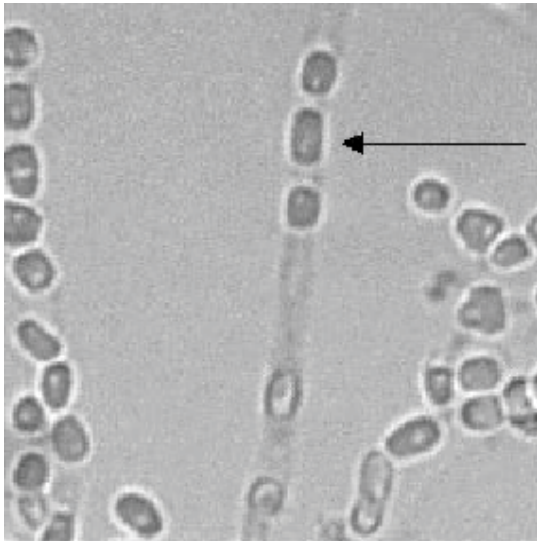


Figure 1. (A) One-week-old colony of *Malbranchea* sp. on Sabouraud's dextrose agar showing woolly texture. (B) The reverse of seven-day-old *Malbranchea* sp. colony on Sabouraud's dextrose agar

A.



B.



Figure 2. Microscopic morphology of *Malbranchea* species showing arthroconidia, which are similar in size to vegetative hyphae (A: 400× magnification; B: line drawing on right not to scale.)

M-2 *Acremonium* sp.

Source: Toe

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	75
Laboratories with incorrect ID:	2
(<i>Aureobasidium pullulans</i>)	(1)
(<i>Trichophyton terrestre</i>)	(1)

Clinical Significance: *Acremonium* sp. causes onychomycosis, keratitis, endophthalmitis, endocarditis, meningitis, peritonitis, and osteomyelitis, especially in immunocompromised patients.

Ecology: *Acremonium* sp. is cosmopolitan in distribution, commonly isolated from plant debris and soil.

Laboratory Diagnosis:

1. **Culture** – *Acremonium* sp. grew moderately rapidly. The colony was powdery to velvety, white to pale pink (Figure 3A). The reverse was pale to yellowish (Figure 3B).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed hyaline, fine and narrow septate hyphae, often in form of fascicle. Phialides were unbranched, solitary. Unicellular conidia accumulated in heads at the apices of the phialides, oblong to ovoid (Figure 4).
3. **Differentiation from other molds** – *Acremonium* species can be confused with certain non-macroconidia producing species of *Fusarium* and *Verticillium* strains, which produce solitary phialides. Both *Fusarium* and *Verticillium* spp. grow faster than *Acremonium* and produce deeply woolly colonies. *Acremonium* species can be distinguished from *Lecythophora* and *Phialemonium* spp. by the presence of septa between the base of phialides and hyphae. *Gliomastix* sp. is different from *Acrmonium* sp. by having olive-green to greenish-black colonies and chains or balls of dark conidia.
4. **In vitro susceptibility testing** – In general, *Acremonium* sp. is susceptible to amphotericin B, caspofungin, voriconazole, posaconazole, and itraconazole.
5. **Molecular tests** – Internal transcribed spacer (ITS) regions can be used for *Acremonium* spp. identification.

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 *Acremonium strictum* UW 580 (Genbank accession number: AY138848) for both ITS1 and ITS2 regions.

Comments: One participating laboratory reported this specimen as *Aureobasidium pullulans*, which has mucoid texture, becoming black with age; another laboratory reported it as *Trichophyton terrestre*, which forms cylindrical macroconidia.

Sequences alignment:

Query	1	TCCGTAGGTGAACCTGCGGAGGGATCATTACCAGAGTGCCCTAGGCTCTCCAACCCATTG	60
AY138848	1	TCCGTAGGTGAACCTGCGGAGGGATCATTACCAGAGTGCCCTAGGCTCTCCAACCCATTG	60
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[AY138848](#) 121 GTCCGCCGGGGAAAACCAAACCTGATTTAATCGTATTTCTCTGAGGGGCGAAAGCCCGA 180

Query 181 AAACAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATGAAGAAC 240
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[AY138848](#) 181 AAACAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATGAAGAAC 240

Query 241 GCAGC 245
 |||||
[AY138848](#) 241 GCAGC 245

Alignment of primary sequence of the ITS1 regions of *Acremonium strictum* UW 580 and PT specimen *Acremonium* sp. NYSDOH 0907.

Query 1 CATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATC 60
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[AY138848](#) 227 CATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATC 286

Query 61 ATCGAATCTTTGAACGCACATTGCGCCCGCCGGCACTCCGGCGGGCATGCCTGTCCGAGC 120
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[AY138848](#) 287 ATCGAATCTTTGAACGCACATTGCGCCCGCCGGCACTCCGGCGGGCATGCCTGTCCGAGC 346

Query 121 GTCATTTCAACCCTCAGGCCACCCTTCCGGGGGAGCGGGCCTGGTGCTGGGGATCGGCG 180
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[AY138848](#) 347 GTCATTTCAACCCTCAGGCCACCCTTCCGGGGGAGCGGGCCTGGTGCTGGGGATCGGCG 406

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[AY138848](#) 467 GTAGCACAACCTCGCACCGGAGAGCGGAACGACCACGCCGTGAAACCCCAATTTTTTAA 526

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[AY138848](#) 527 GGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA 586

Alignment of primary sequence of the ITS2 regions of *Acremonium strictum* UW 580 and PT specimen *Acremonium* sp. NYSDOH 0907.

Further Reading:

1. Chang, Y.H., Huang, L.M., Hsueh, P.R., Hsiao, C.H., Peng, S.F., Yang, R.S., and Lin, K.H. 2005. *Acremonium pyomyositis* in a pediatric patient with acute leukemia. *Pediatr Blood Cancer*. 44: 521-524.
2. Creti, A., Esposito, V., Bocchetti, M., Baldi, G., De Rosa, P., Parrella, R., and Chirianni, A. 2006. Voriconazole curative treatment for *Acremonium* species keratitis developed in a patient with concomitant *Staphylococcus aureus* corneal infection: a case report. *In Vivo*. 20: 169-171.
3. Doczi, I., Dosa, E., Varga, J., Antal, Z., Kredics, L., and Nagy, E. 2004. Etest for assessing the susceptibility of filamentous fungi. *Acta Microbiol Immunol Hung*. 51: 271-81.
4. Foell, J.L., Fischer, M., Seibold, M., Borneff-Lipp, M., Wawer, A., Horneff, G., and Burdach, S. 2007. Lethal double infection with *Acremonium strictum* and *Aspergillus fumigatus* during induction chemotherapy in a child with ALL. *Pediatr Blood Cancer*. 49: 858-861.

5. Garcia-Effron, G., Gomez-Lopez, A., Mellado, E., Monzon, A., Rodriguez-Tudela, J.L., and Cuenca-Estrella, M. 2004. *In vitro* activity of terbinafine against medically important non-dermatophyte species of filamentous fungi. *J Antimicrob Chemother.* 53: 1086-1089.
6. Pastorino, A.C., Menezes, U.P., Marques, H.H., Vallada, M.G., Cappellozi, V.L., Carnide, E.M., and Jacob, C.M. 2005. *Acremonium kiliense* infection in a child with chronic granulomatous disease. *Braz J Infect Dis.* 9: 529-534.

A.



B.



Figure 3. (A) Seven-day-old, powdery to velvet, white to pinkish colony of *Acremonium* sp. on Sabouraud's dextrose agar. (B) The reverse of seven-day-old *Acremonium* sp. colony on Sabouraud's dextrose agar

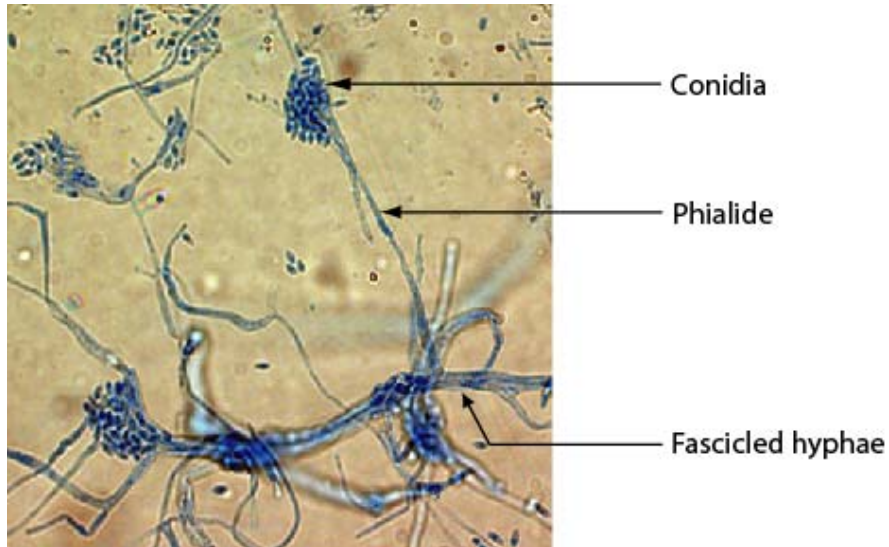


Figure 4. Microscopic morphology of *Acremonium* sp. showing septate, rope-like hyphae, unbranched phialides, and unicellular conidia accumulated in heads at the apices of the phialides (400× magnification).

M-3 *Chrysosporium* sp.

Source: Bronchial wash

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	71
Laboratories with incorrect ID:	6
(<i>Malbranchea</i> sp.)	(3)
(<i>Arthrographis</i> sp.)	(1)
(<i>Emmonsia parva</i>)	(1)
(<i>Scedosporium apiospermum</i>)	(1)

Clinical Significance: *Chrysosporium* sp. is occasionally reported from skin infection, or as an agent of onychomycosis. Invasive *Chrysosporium* infection of the nose and paranasal sinuses in an immunocompromised host has also been reported.

Ecology: *Chrysosporium* sp. is a common saprobe on plants and in soil distributed worldwide.

Laboratory Diagnosis:

1. **Culture** – *Chrysosporium* sp. grew moderately fast. On Sabouraud's dextrose agar, after 9 days at 25°C, the colony showed white to cream color on the surface and powdery to granular texture (Figure 5A). Reverse appeared yellow or buff (Figure 5B). The species of *Chrysosporium* sent in this testing was cycloheximide-resistant.
2. **Microscopic morphology** – Lactophenol cotton blue mount showed hyaline septate hyphae. Ovoid or club-shaped conidia with broad truncated bases were seen either singly or in short chains borne directly on hyphae, or in short conidiophores (Figure 6).
3. **Differentiation from other mold** – *Chrysosporium* sp. is distinct from *Emmonsia* sp. in not developing adiaspores at 37°C. It does not display thermal dimorphism and is negative with specific nucleic acid probe, which serves to differentiate it from *Blastomyces dermatitidis*. *Chrysosporium* sp. grows on the media with cycloheximide and is urease-positive, which distinguished it from *Sporotrichum* sp. Please refer to Table 2 for details.
4. **In vitro susceptibility testing** – Limited information is available. In general, *Chrysosporium* sp. is susceptible to amphotericin B, itraconazole, ketoconazole, and voriconazole. Fluconazole had higher MIC to *Chrysosporium* sp.
5. **Molecular tests** – Internal transcribed spacer (ITS) regions can be used for *Chrysosporium* sp. identification.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA. 96% and 97% identities were found between this PT specimen and *Chrysosporium articulatum* UAMH 4320 (Genbank accession number: AJ007841) for ITS1 and ITS2 regions, respectively.

Comments: Conidia of *Chrysosporium* sp. are pyriform with truncated bases, which are similar to those of *Scedosporium apiospermum*. However, *S. apiospermum* has large, dark, rounded cleistothecia with thick walls, while *Chrysosporium* sp. does not. *Arthrographis* sp. produces arthroconidia formed at the tips of conidiophores or intercalary in the hyphae, which are different from the arthroconidia of *Chrysosporium* sp., most often broader in diameter than the supporting hyphae. Arthroconidia of *Malbranchea* sp. are also

rectangular, of the same diameter as the hyphae from which they are formed, which is different from arthroconidia of *Chrysosporium* sp. *Emmonsia parva* develops adiaspores at 37°C, but *Chrysosporium* sp. does not.

Sequences alignment:

Query	1	TTCCGTAGGNG-NCCTGCGGAAGGATCATTAAAGTGTTTCGGAGCCTGGT-TCGGGCACC	58
AJ007841	24	TTCCGTAGGTGAACCTGCGGAAGGATCATTAAAGTGTTTCGGAGCCTGGTAT-GGGCATC	82
Query	59	TCAGCTCGAGGTGTCGGTGCCAGCGCCCCACACGTGTTTACTCAACTTGGTTGCCTTGG	118
AJ007841	83	TCAACTCGAGGTGTCGGTGCCAGCGCCCCACACGTGTTTACTCAACTTGGTTGCCTTGG	142
Query	119	CGAGCCTGCCCTGTGGCTGCTGGGGACGCCTCACGGTGTCCCGGGCTTGTGCTCGCCAG	178
AJ007841	143	TGAGCCTGCCCTTGTGGCTGCTGGGGATGCCTCACGGTGTCCCGGGCTCGTGCTCGCCAG	202
Query	179	TGGAACATTTGAACTCTTATGTGAAAATAGTCAGTCTGAGCATTATGCAAATTAATAAAA	238
AJ007841	203	TGGAACATTTGAACTCTTATGTGAAAATAGTCAGTCTGAGCATTATGCAAATTAATAAAA	262
Query	239	ACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAG	285
AJ007841	263	ACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAG	309

Alignment of primary sequence of the ITS1 regions of *Chrysosporium articulatum* UAMH 4320 and PT specimen *Chrysosporium* sp. NYSDOH 0907.

Query	1	GCATCGAT-NAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAAT	59
AJ007841	291	GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAAT	350
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AJ007841	351	CATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGGGGGCATGCCTGTTCGAG	410
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AJ007841	411	CGTCATTGCAACCCCTCAAGCACAGCTTGTGTGTTGGGCCATCGTCCCTC----TGGACG	466
Query	180	GGCCTGAAATGCAGTGGCAGCACCGAGTTCTGGTGTCTGAGTGTATGGGAATCTCTTATC	239
AJ007841	467	GGCCTGAAATGCAGTGGCAGCACCGAGTTCTGGTGTCTGAGTGTATGGGAATCTCTTATC	526
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AJ007841	527	GCTCAAAGACCCAATCGGCGCTGATGTGATGTTTATCCAGTTTACCTCGGATCAGGT	586
Query	300	AGGAGTACCCGCTGAACTTAAGCATATCAATAAGCGGA	337
AJ007841	587	AGGAGTACCCGCTGAACTTAAGCATATCAATAAGCGGA	624

Alignment of primary sequence of the ITS2 regions of *Chrysosporium articulatum* UAMH 4320 and PT specimen *Chrysosporium* sp. NYSDOH 0907.

Further Reading:

1. Bowman, M.R., Pare, J.A., Sigler, L., Naeser, J.P., Sladky, K.K., Hanley, C.S., Helmer, P., Phillips, L.A., Brower, A., and Porter, R. 2007. Deep fungal dermatitis in three inland bearded dragons (*Pogona vitticeps*) caused by the *Chrysosporium anamorph* of *Nannizziopsis vriesii*. *Med Mycol.* 45: 371-376.
2. Guerrero Palma, M.A., Avila Espin, L., Fernandez Perez, A., Moreno Leon, J.A. 2007. Invasive sinusal mycosis due to *Chrysosporium tropicum*. *Acta Otorrinolaringol Esp.* 58: 164-166.
3. Levy, F.E., Larson, J.T., George, E., and Maisel, R.H. 1991. Invasive *Chrysosporium* infection of the nose and paranasal sinuses in an immunocompromised host. *Otolaryngol. Head neck Surg.* 104: 384-388.
4. Roilides, E., Sigler, L., Bibashi, E., Katsifa, H., Flaris, N., and Panteliadis, C. 1999. Disseminated infection due to *Chrysosporium zonatum* in a patient with chronic granulomatous disease and review of non-*Aspergillus* fungal infections in patients with this disease. *J Clin Microbiol.* 37: 18-25.

TABLE 2: Differentiation of *Chrysosporium* species from some related fungi.

Characteristic	<i>Chrysosporium</i> sp.	<i>Emmonsia parva</i> var. <i>parva</i> and var. <i>crescens</i>	<i>Blastomyces dermatitidis</i>
Growth on cycloheximide medium (25°C)	No growth	Growth	Growth
Chlamydoconidia (25°C)	More than 20 µm in diameter	Absent	Absent
Chlamydoconidia / adiaspores (37°C)	Globose, pyriform, > 20 µm in diameter	Globose, thick walled chlamyconidia (10 – 20 µm); adiaspores (40 – 200 µm)	Yeast form with broad-base budding (8 –30 µm)
<i>B. dermatitidis</i> GenProbe	Negative	Negative	Positive

A.



B.

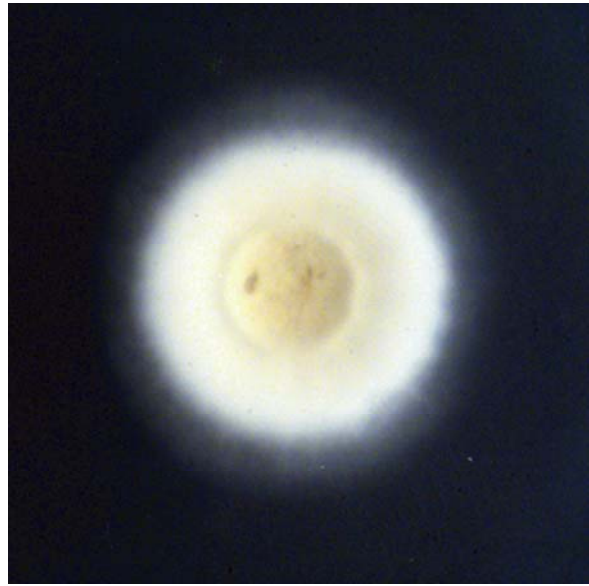
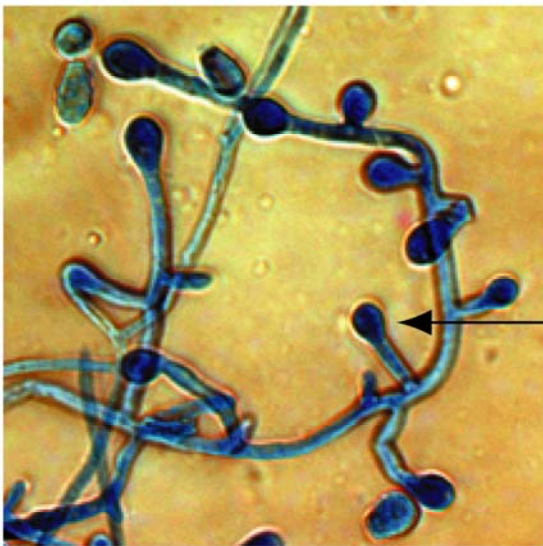


Figure 5. (A) Nine-day-old, white to cream colored powdery to granular colony of *Chrysosporium* sp. on Sabouraud's dextrose agar. (B) The reverse of nine-day-old *Chrysosporium* sp. colony on Sabouraud's dextrose agar

A.



B.

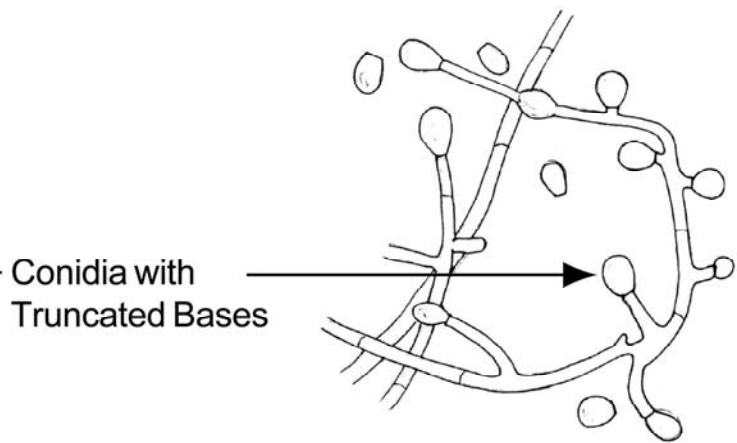


Figure 6. Microscopic morphology of *Chrysosporium* sp. showing hyaline septate hyphae. Ovoid or club-shaped conidia with broad truncated bases are seen either singly or in short chains borne directly on hyphae or in short conidiophores (A: 200× magnification; B: line diagram not to scale).

M-4 *Fusarium* sp.

Source: Nails

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	76
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.

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 7).
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 8). Chlamydospores may be present.
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 they did not observe macroconidia of *Fusarium*.

Further Reading:

1. Calado, N.B., Sousa, F. Jr, Gomes, N.O., Cardoso, F.R., Zaror, L.C., and Milan, E.P. 2006. *Fusarium* nail and skin infection: a report of eight cases from Natal, Brazil. *Mycopathologia*. 161: 27-31.
2. Klont, R.R., Eggink, C.A., Rijs, A.J., Wesseling, P., and Verweij, P.E. 2005. Successful treatment of *Fusarium* keratitis with cornea transplantation and topical and systemic voriconazole. *Clin Infect Dis*. 40: e110-112.
3. Hay, R.J. 2007. *Fusarium* infections of the skin. *Curr Opin Infect Dis*. 20: 115-117.
4. Ho, D.Y., Lee, J.D., Rosso, F., and Montoya, J.G. 2007. Treating disseminated fusariosis: amphotericin B, voriconazole or both? *Mycoses*. 50: 227-231.
5. Lin, H.C., Chu, P.H., Kuo, Y.H., and Shen, S.C. 2005. Clinical experience in managing *Fusarium solani* keratitis. *Int J Clin Pract*. 59: 549-554.
6. Qiu, W.Y., Yao, Y.F., Zhu, Y.F., Zhang, Y.M., Zhou, P., Jin, Y.Q., and Zhang, B. 2005. Fungal spectrum identified by a new slide culture and *in vitro* drug susceptibility using Etest in fungal keratitis. *Curr Eye Res*. 30: 1113-1120.

7. Sagnelli, C., Fumagalli, L., Prigitano, A., Baccari, P., Magnani, P., and Lazzarin, A. 2006. Successful voriconazole therapy of disseminated *Fusarium verticillioides* infection in an immunocompromised patient receiving chemotherapy. *J Antimicrob Chemother.* 57: 796-798.
8. Thomas, P.A., and Geraldine, P. 2007. Infectious keratitis. *Curr Opin Infect Dis.* 20: 129-141.
9. Weinstein, W.L., Moore, P.A., Sanchez, S., Dietrich, U.M., Wooley, R.E., Ritchie, B.W. 2006. *In vitro* efficacy of a buffered chelating solution as an antimicrobial potentiator for antifungal drugs against fungal pathogens obtained from horses with mycotic keratitis. *Am J Vet Res.* 67: 562-568.
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A.



B.

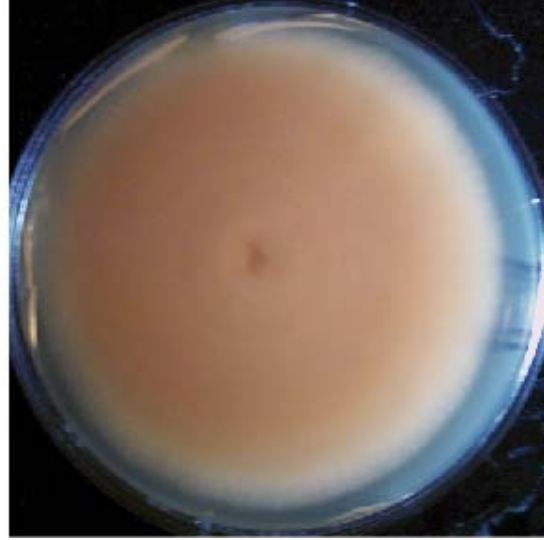
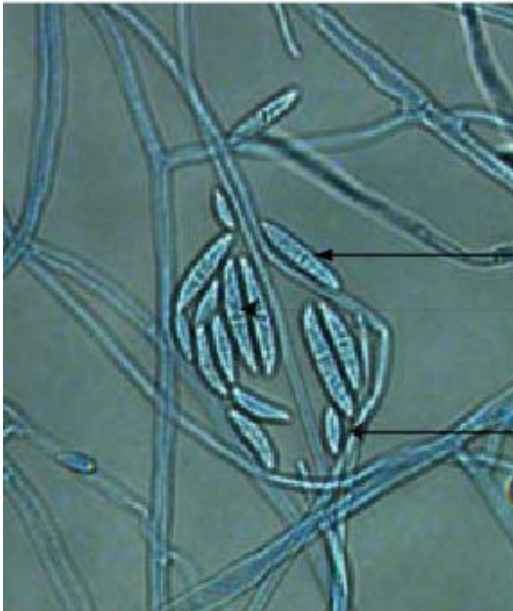


Figure 7. (L) Seven-day-old, woolly, orange to pinkish colony of *Fusarium* sp. on Sabouraud's dextrose agar. (R) The reverse side of seven-day-old *Fusarium* sp. colony on Sabouraud's dextrose agar.

A.



B.

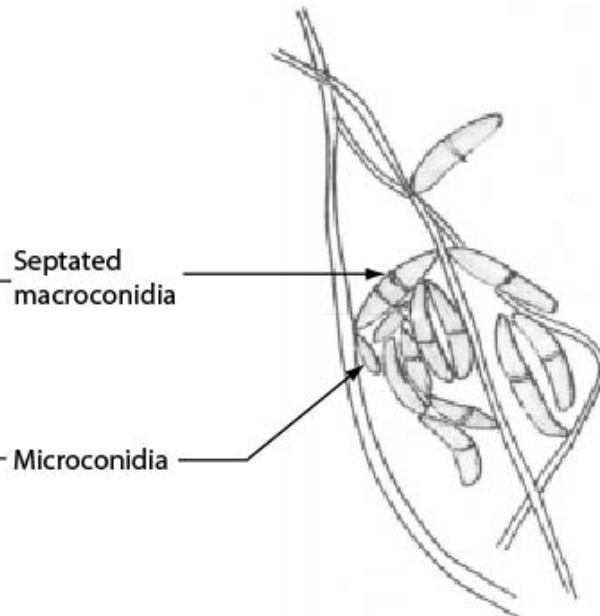


Figure 8. Microscopic morphology of *Fusarium* sp. showing curved, septate microconidia and elongated macroconidia (A, 200× magnification; B: line drawing not to scale)

M-5 *Penicillium* sp.

Source: Sinus

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	9
Laboratories with correct ID:	71
Laboratories with incorrect ID:	7
(<i>Paecilomyces</i> sp.)	(6)
(<i>Scedosporium apiospermum</i>)	(1)

Clinical Significance: *Penicillium* spp. other than *Penicillium marneffe* are commonly considered as laboratory contaminants. *Penicillium* spp. have been isolated from patients with keratitis, endophthalmitis, otomycosis, necrotizing esophagitis, pneumonia, endocarditis, peritonitis, and urinary tract infections. Some species are known to produce mycotoxins, which are nephrotoxic and carcinogenic.

Ecology: *Penicillium* spp. are widespread and are found in soil, decaying vegetables and fruits, and the air.

Laboratory Diagnosis:

1. **Culture** – *Penicillium* sp. grew rapidly, velvety to powdery in texture. The colony was initially white and then became blue green, gray green, olive gray in time (Figure 9A). The plate reverse was pale to yellowish (Figure 9B).
2. **Microscopic morphology** – Lactophenol cotton blue or Calcofluor mounts showed septate hyaline hyphae, simple or branched conidiophores, metulae, phialides. Metulae were secondary branches that form on conidiophores. The brush-like clusters of phialides, are referred to as "penicilli". The unicellular conidia were round, and formed chains at the tips of the phialides (Figure 10).
3. **Differentiation from other mold** – *Penicillium* sp. can be differentiated from *Paecilomyces* by having flask-shaped phialides and globose to subglobose conidia; from *Gliocladium* by having chains of conidia; and from *Scopulariopsis* by forming phialides.
4. **In vitro susceptibility testing** – In general, *Penicillium* sp. is susceptible to amphotericin B, ketoconazole, itraconazole, and voriconazole.
5. **Molecular tests** – Internal transcribed spacer (ITS) regions can be used for *Penicillium* spp. identification.

Comments: Five laboratories reported this specimen as *Paecilomyces* sp., which has thin phialides with elongated tips, but *Penicillium* sp. has phialides with thicker apices.

Further Reading:

1. Deshpande, S. D., and G. V. Koppikar. 1999. A study of mycotic keratitis in Mumbai. *Indian J Pathol Microbiol.* 42: 81-87.
2. Keceli, S., Yegenaga, I., Dagdelen, N., Mutlu, B., Uckardes, H., and Willke, A. 2005. Case report: peritonitis by *Penicillium* spp. in a patient undergoing continuous ambulatory peritoneal dialysis. *Int Urol Nephrol.* 37: 129-131.
3. Noritomi, D.T., Bub, G.L., Beer, I., da Silva, A.S., de Cleve, R., and Gama-Rodrigues, J.J. 2005. Multiple brain abscesses due to *Penicillium* spp infection. *Rev Inst Med Trop Sao Paulo.* 47: 167-170.
4. Zanatta, R., Miniscalco, B., Guarro, J., Gené, J., Capucchio, M.T., Gallo, M.G., Mikulicich, B., Peano, A. 2006. A case of disseminated mycosis in a German Shepherd dog due to *Penicillium purpurogenum*. *Med Mycol.* 44: 93-97.

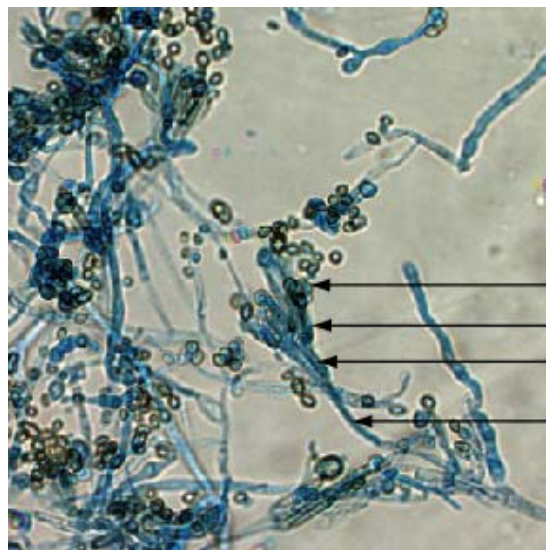
A.



B.



Figure 9. (A) Seven-day-old, white edge, blue green, to olive green colony of *Penicillium* sp. on Sabouraud's dextrose agar. (B) The reverse of seven-day-old *Penicillium* sp. colony on Sabouraud's dextrose agar



Conidia
Phialides
Metulae
Conidiophore

Figure 10. Microscopic morphology of *Penicillium* sp. showing the broom-shape of phialides and round conidia (200× magnification).

M-Edu. *Trichothecium* sp.

Source: Skin

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	76
Laboratories with incorrect ID:	1
(<i>Trichophyton terrestre</i>)	(1)

Clinical Significance: No human or animal diseases due to *Trichothecium* sp. have been reported. It is commonly considered as a laboratory contaminant.

Ecology: *Trichothecium* sp. is widely distributed on decaying vegetation and in the soil.

Laboratory Diagnosis:

1. **Culture** – *Trichothecium* sp. grew rapidly. On Sabouraud's dextrose agar at 25°C for 7 days, the colony was velvety to powdery, initially white and later becoming pale pink (Figure 11A). The reverse was pale (Figure 11B).
2. **Microscopic morphology** – Lactophenol cotton blue mounts showed septate hyphae, hyaline, unbranched conidiophore, and broadly club-shaped conidia. The conidia were two-celled, overlapping in an imbricate, zigzagging at the tip of the conidiophore (Figure 12).
3. **Differentiation from other mold** – *Trichothecium* sp. differs from *Microsporum nanum* by forming zigzag groups of conidia (compared to the solitary conidia of *Microsporum nanum*), by not perforating hair in vitro and by being inhibited by cycloheximide
4. **In vitro susceptibility testing** – No information available.
5. **Molecular tests** – Internal transcribed spacer (ITS) regions can be used for *Trichothecium* sp. identification.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA. 96% identities were found between this PT specimen and *Trichothecium roseum* (Genbank accession number: EF589898) for ITS1 and 98% identities were found between this PT specimen and *Trichothecium roseum* (Genbank accession number: U51982) for ITS2 region.

Comments: One laboratory reported this specimen as *Trichophyton terrestre*, which forms 2-6 cells long cylindrical macroconidia.

Sequences alignment:

Query	1	AACTCCCAACCCCTTTGTGAACCTTACCTACCGTTGCTTCGGCCGGACCGCCCCGGGCGCTG	60
EF589898	2	AACTCCC-ACCCTTTGTG-ACCTTACCCACCGTTGCTTCGGCCGGACCGCCCCGGG-GCAG	58
Query	61	CGTGCCCCGGACCCAAGGCGCCCGCCGGGGACCACACGAACCCTGTTTAA-CAAACATGT	119
EF589898	59	CGTGCCCCGGACCCAAGGCGCCCGCCGGGGACCACACGAACCCTGTTTAAACAA-CATGT	117
Query	120	GTATCCTCTGAGCGAGCCGAAAGGCAACAAAACAAATCAAAACTTTCAACAACGGATCTC	179
EF589898	118	GTATCCTCTGAGCGAGCCGAAAGGCAACAAAACAAATCAAAACTTTCAACAACGGATCTC	177

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Query      180  TTGGTTCTGGCATCGATGAAGAACGCAGC  208
          |||
EF589898  178  TTGGTTCTGGCATCGATGAAGAACGCAGC  206

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Alignment of primary sequence of the ITS1 regions of *Trichothecium roseum* and PT specimen *Trichothecium* sp. NYSDOH 0907.

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Query      1      GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT  60
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Query      61      CATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAG  120
          |||
U51982    235    CATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAG  294

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U51982    295     CGTCATTTCAACCCTCGG-CCCCCTTTTCCCGCTCGCGGGGAGGGGGCGGGCCCGG  353

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U51982    414     CGCTGCCTCCTCCGCGTAGTAGCACAAACCTCGCGGGCAGAAGGCGGCGCGGCCACGCCG  473

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U51982    474     TAAAACCCCAAACCTTTTACCAAGGT-GACCTCGGATCAGGTAGGAATACCCGCTGA  528

```

Alignment of primary sequence of the ITS2 regions of *Trichothecium roseum* and PT specimen *Trichothecium* sp. NYSDOH 0907.

Further Reading:

1. Aho R. 1983. Saprophytic fungi isolated from the hair of domestic and laboratory animals with suspected dermatophytosis. *Mycopathologia*. 83: 65-73.
2. Liou,G.Y. and Tzean,S.S. 1997. Phylogeny of the genus *Arthrotrrys* and allied nematode-trapping fungi based on rDNA sequences. *Mycologia* 89: 876-884.
3. Okhovvat, S.M. and Zakeri, Z. 2003. Identification of fungal diseases associated with imported wheat in Iranian silos. *Commun Agric Appl Biol Sci*. 68: 533-535.
4. Pandey, A., Agrawal, G.P., and Singh, S.M.1990. Pathogenic fungi in soils of Jabalpur, India. *Mycoses*. 33: 116-125.
5. Ulvund, M.J., Smith, J.D., and Grønstøl, H. 1984. Acute respiratory distress syndrome (ARDS) in lambs. Mycology and hypersensitivity. *Nord Vet Med*. 36: 98-102.

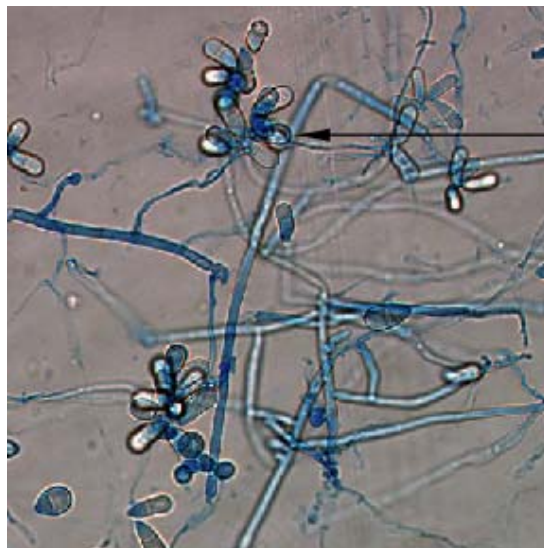
A.



B.



Figure 11. (A) Seven-day-old, cream colored velvety colony of *Trichothecium* sp. on Sabouraud's dextrose agar. (B) The reverse of seven-day-old *Trichothecium* sp. colony on Sabouraud's dextrose agar



Two-celled conidia
in zigzag arrangement

Figure 12. Microscopic morphology of *Trichothecium* sp. showing the two-celled conidia in zigzag arrangement (200× magnification).

YEAST DESCRIPTIONS

Y-1 *Candida dubliniensis*

Source: Blood / Throat / Stool / Urine

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	9
Laboratories with correct ID:	41
Laboratories with incorrect ID:	11
(<i>Candida albicans</i>)	(11)

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 13). 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 14).
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 or not at all at 42°C while *C. albicans* grows well at this temperature. 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 closed 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

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

Comments: This specimen was validated in the current test event. This is the first 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, and January 2007 PT events. In the current test event, about 79% laboratories were able to identify *C. dubliniensis*. As summarized earlier in this section, a number of physiological differences could be used to distinguish these two closely related *Candida* species. It has been reported that *C. dubliniensis* produces abundant chlamydospores, often in contiguous pairs or triplets but at least one study has not found this to be consistent, and therefore, the relative abundance of chlamydospores may not be a definite criterion.

Sequences alignment:

```

Query      1      TCCGTAGGTGAACCTGCGGAAGGATCATTACTGATTTGCTTAATTGCACCACATGTGTTT  60
          |||
AJ249484   1      TCCGTAGGTGAACCTGCGGAAGGATCATTACTGATTTGCTTAATTGCACCACATGTGTTT  60

Query      61      TGTTTTGGACAAACTTGCTTTGGCGGTGGGCCTCTACCTGCCGCCAGAGGACATAAACTT  120
          |||
AJ249484   61      TGTTTTGGACAAACTTGCTTTGGCGGTGGGCCTCTACCTGCCGCCAGAGGACATAAACTT  120

Query      121     ACAACCAAATTTTTTATAAACTTGTACGAGATTATTTTAAATAGTCAAACTTTCAACA  180
          |||
AJ249484   121     ACAACCAAATTTTTTATAAACTTGTACGAGATTATTTTAAATAGTCAAACTTTCAACA  180

Query      181     ACGGATCTCTTGTTCTCGCATCGATGAAGAACGCAGC  218
          |||
AJ249484   181     ACGGATCTCTTGTTCTCGCATCGATGAAGAACGCAGC  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     AAAGATGATAGTGGTAAGGCGGAGATGCTTGACAATGGCTTAGGTGTAACCAAAAACATT  240
          |||
DQ355938   338     AAAGATGATAGTGGTAAGGCGGAGATGCTTGACAATGGCTTAGGTGTAACCAAAAACATT  397

Query      241     GCTAAGGCGGTCTCTGGCGTCGCCATTTTATTCTTCAAACCTTTGACCTCAAATCAGGTA  300
          |||
DQ355938   398     GCTAAGGCGGTCTCTGGCGTCGCCATTTTATTCTTCAAACCTTTGACCTCAAATCAGGTA  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:

1. Alves, S.H., de Loreto, E.S., Linares, C.E., Silveira, C.P., Scheid, L.A., Pereira, D.I., Santuario, J.M. 2006. Comparison among tomato juice agar with other three media for differentiation of *Candida dubliniensis* from *Candida albicans*. *Rev Inst Med Trop Sao Paulo*. 48: 119-21.
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3. Cardenes-Perera, C.D., Torres- Lana, A., Alonso-Vargas, R., Moragues-Tosantas, M.D., Emeterio, J.P., Quindos-Andres, G., Arevalo-Morales, M.P. 2004. Evaluation of API ID 32C® and Vitek-2® to identify *Candida dubliniensis*. *Diagn Microbiol & Infect Dis*. 50: 219 – 221.
4. Graf, B., Trost, A., Eucker, J., Gobel, U.B., Adam, T. 2004. Rapid and simple differentiation of *C. dubliniensis* from *C. albicans*. *Diagn. Microbiol. & Infect Dis*. 48: 149 – 151.
5. Jabra-Rizk, M.A., Johnson, J.K., Forrest, G., Mankers, K., Meiller, T.F., Venezia, R.A. 2005. Prevalence of *Candida dubliniensis* fungemia at a large teaching hospital. *Clin Infect Dis*. 41: 1064 – 1067.
6. Mirhendi, H., makimura, K., Zomorodian, K., Maeda, N., Ohshima, T., Yamaguchi, H. 2005. Differentiation of *Candida albicans* and *Candida dubliniensis* using a single enzyme PCR-RFLP method. *Jpn J Infect Dis*. 58: 235 – 237.
7. Nunn, M.A., Schäfer, S.M., Petrou, M.A., and Brown, J.R.M. 2007. Environmental source of *Candida dubliniensis*. *Emerg Infect Dis* [serial on the Internet]. Available from <http://www.cdc.gov/EID/content/13/5/747.htm>
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9. Schabereiter-Gurtner, C., Selitsch, B., Rotter, M.L., Hirschl, A.M., Willinger, B. 2007. Development of novel real-time PCR assays for detection and differentiation of eleven medically important *Aspergillus* and *Candida* species in clinical specimens. *J Clin Microbiol*. [Epub ahead of print]
10. Sullivan, D.J., Moran, G.P., Pinjon, E., Al-Mosaid, A., Stokes, C., Vaughan, C., Coleman, D.C. 2004. Comparison of the epidemiology, drug resistance mechanisms and virulence of *Candida dubliniensis* and *Candida albicans*. *FEMS Yeast Research*. 4: 369 – 376.
11. Tsuruta, R., Oda, Y., Mizuno, H., Hamada, H., Nakahara, T., Kasaoka, S., and Maekawa, T. 2007. *Candida dubliniensis* isolated from the sputum of a patient with end-stage liver cirrhosis. *Intern Med*. 46: 597-600.
12. Us, E and Cengiz, S.A. 2007. Prevalence and phenotypic evaluation of *Candida dubliniensis* in pregnant women with vulvovaginal candidosis in a university hospital in Ankara. *Mycoses*. 50: 13-20.



Figure 13. Seven-day-old, white to cream, smooth, and soft colony of *Candida dublinensis* on Sabouraud's dextrose agar.

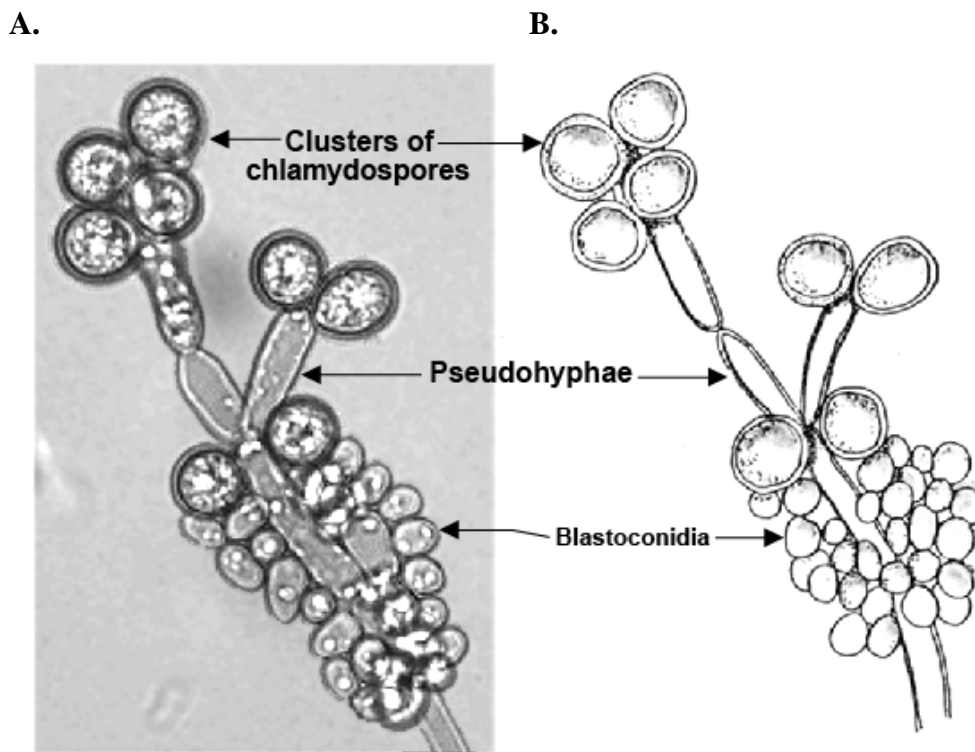


Figure 14. Microscopic morphology of *Candida dublinensis* on corn meal agar showing clusters of chlamydozoospores and blastoconidia (A, 400 \times magnification; B, line drawing not to scale).

Y-2 *Rhodotorula minuta*

Source: Catheter / Stool / Urine

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

Clinical Significance: *Rhodotorula minuta* is reported as a causative agent of infections in humans with AIDS and leukemia. It is isolated from blood, sputum, throat swabs, and feces.

Ecology: *R. minuta* is usually found in water and on oat leaves.

Laboratory Diagnosis:

1. Culture – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was pink, smooth, and soft (Figure 15).
2. Microscopic morphology – On corn meal agar with Tween 80, *R. minuta* had no pseudohyphae, round blastoconidia were seen (Figure 16).
3. Differentiation from other yeasts – *R. minuta* did not assimilate maltose, which differentiated it from *R. glutinis* and *R. mucilaginosa*.
4. In vitro susceptibility testing – *R. minuta* was susceptible to amphotericin B, but resistant to azoles.
5. Molecular tests – No information available.

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 regions of rDNA. 100% identity was found between this PT specimen and *R. minuta* (synonyms: *Rhodotorula slooffii*) CBS 5706 (Genebank accession number: AF444627) for ITS1 region.

Comments: One participating laboratories reported this specimen as *Sporobolomyces salmonicol*, which has true and pseudohyphae, but *R. minuta* does not.

Sequences alignment:

Query	1	CCGTAGGTGAACCTGCGGAAGGATCATTAATGAATTTTAGGACGTTCTTTTTAGAAAGTCC	60
AF444627	2	CCGTAGGTGAACCTGCGGAAGGATCATTAATGAATTTTAGGACGTTCTTTTTAGAAAGTCC	61
Query	61	GACCCTTTCATTTTCTTACACCGTGCACACACTTCTTTTTTACACACACTTTTAACACCT	120
AF444627	62	GACCCTTTCATTTTCTTACACTGTGCACACACTTCTTTTTTACACACACTTTTAACACCT	121
Query	121	TAGTATAAGAATGTAATAGTCTCTTAATTGAGCATAAAATAAAAACAAAACCTTTCAGCAAC	180
AF444627	122	TAGTATAAGAATGTAATAGTCTCTTAATTGAGCATAAAATAAAAACAAAACCTTTCAGCAAC	181
Query	181	GGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGC	216
AF444627	182	GGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGC	217

Alignment of primary sequence of the ITS1 regions of *R. minuta* (synonyms: *Rhodotorula slooffii*) CBS 5706 and PT specimen *R. minuta* NYSDOH 0907.

Further Reading:

1. Cutrona, A.F., Shah, M., Himes, M.S., and Miladore, M.A. 2002. *Rhodotorula minuta*: an unusual fungal infection in hip-joint prosthesis. *Am. J. Orthop.* 31: 137-140.
2. Garcia-Martos, P., Dominguez, I., Marin, P., Garcia-Agudo, R., Aoufi, S., and Mira, J. 2001. Antifungal susceptibility of emerging yeast pathogens. *Enferm. Infecc. Microbiol. Clin.* 19: 249-256.
3. Goldani, L.Z., Craven, D.E., and Sugar, A.M. 1995. Central venous catheter infection with *Rhodotorula minuta* in a patient with AIDS taking suppressive doses of fluconazole. *J. Med. Vet. Mycol.* 33: 267-270.
4. Pinna, A., Carta, F., Zanetti, S., Sanna, S., Sechi, L.A. 2001. Endogenous *Rhodotorula minuta* and *Candida albicans* endophthalmitis in an injecting drug user. *Br. J. Ophthalmol.* 85: 759.
5. Thanos, L., Mylona, S., Kokkinaki, A., Pomoni, M., Tsiouris, S., and Batakis, N. 2006. Multifocal skeletal tuberculosis with *Rhodotorula minuta* co-infection. *Scand J Infect Dis.* 38: 309-11.



Figure 15. Seven-day-old, soft, smooth, pink colony of *Rhodotorula minuta* on Sabouraud's dextrose agar.

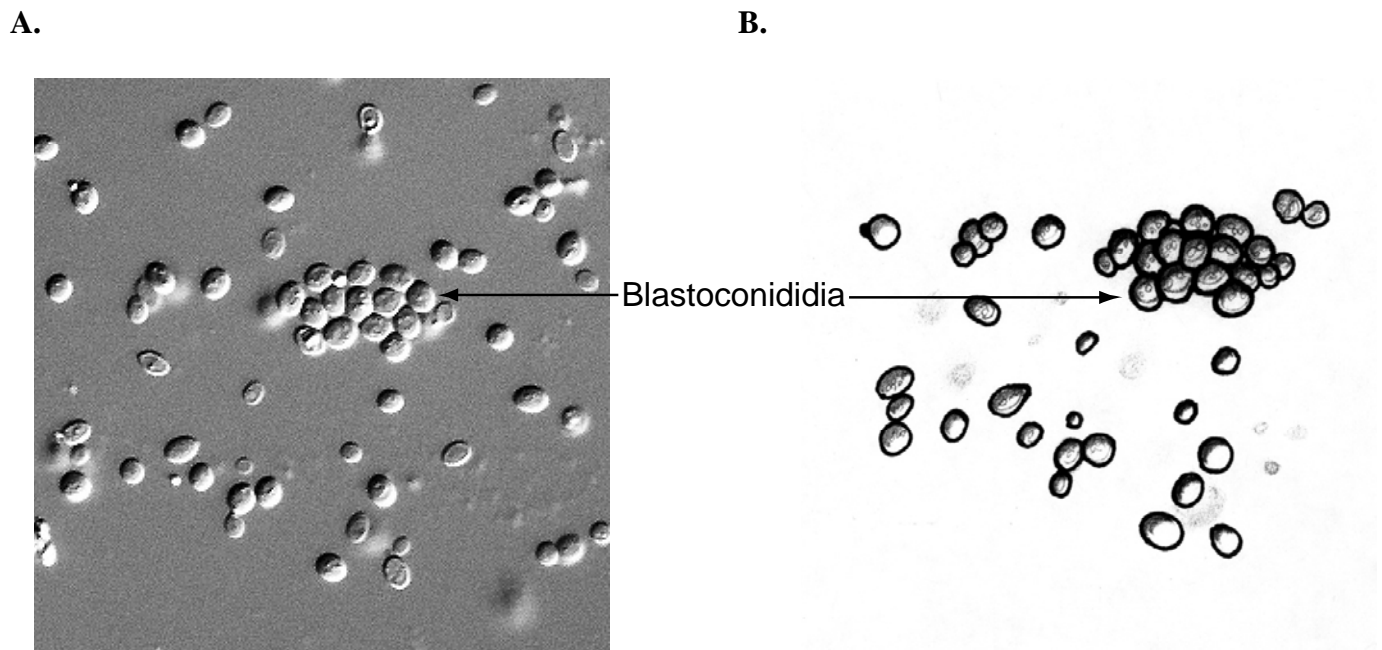


Figure 16. Microscopic morphology of *Rhodotorula minuta* on corn meal agar with Tween 80 showing round blastoconidia (A, 400× magnification; B, line drawing not to scale).

Y-3 *Candida viswanathii*

Source: CSF / Urine

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	0
Laboratories with correct ID:	1
Laboratories with incorrect ID:	50
(<i>Candida lusitaniae</i>)	(39)
(<i>Candida tropicalis</i>)	(5)
(<i>Candida haemulonii</i>)	(3)
(<i>Candida guilliermondii</i>)	(2)
(<i>Candida famata</i>)	(1)

Clinical Significance: *C. viswanathii* is a very rare yeast pathogen in clinical specimens. Only a few case reports exist on its recovery from the cerebrospinal fluid or sputum of patients with meningitis.

Ecology: *C. viswanathii* was first reported from the sputum and CSF from meningitis patient in India. A subsequent report described it from shrimp in Gulf of Mexico.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar at 25°C for 3 to 5 days, colony was white or cream, a little wrinkled, moist (Figure 17).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *C. viswanathii* formed long pseudohyphae and elongated, oval shaped blastoconidia. The truncated scar was observed at the attached site (Figure 18).
3. **Differentiation from other yeasts** – *C. viswanathii* grows on the media containing cycloheximide and grows well at 37°C. It ferments glucose and maltose, and many other physiological characteristics are very similar to *C. albicans* and *C. tropicalis*. However, on microscopic morphology, *C. viswanathii* has long pseudohyphae with elongated oval shaped conidia with truncate scars, which differentiates it from *C. tropicalis*. Absence of chlamydospore and germ tube differentiates it from *C. albicans*.
4. **In vitro susceptibility testing** – No information available.
5. **Molecular tests** – DNA hybridization and electrophoretic karyotyping or restriction enzyme analysis of PCR products obtained from the gene coding for the small ribosomal subunit 18S-rRNA were applied for differentiation of *C. viswanathii* from other medically relevant yeasts.

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. viswanathii* ATCC 22981 (Genebank accession number: AY139791) for ITS1 and *C. viswanathii* WM 239 (Genebank accession number: DQ249200) for ITS2 region.

Comments: There is no bicode for *C. viswanathii* in the API 20C AUX database. *C. viswanathii* grows on the media containing cycloheximide, differentiating it from *C. parapsilosis*, *C. lusitaniae*, *C. krusei*, and *C. zeylanoides*. *C. viswanathii* ferments glucose, but *C. lipolytica* does not. *C. viswanathii* has prominent truncated scars on the blastoconidia differentiate it from other physiologically close yeasts including *C. tropicalis*.

Sequences alignment:

```
Query      1      TCCGTAGGTGAACCTGCGGAAGGATCATTACTGATTTGCTTAATTGCACCACATGTGTTT 60
          |||
AY139791  1      TCCGTAGGTGAACCTGCGGAAGGATCATTACTGATTTGCTTAATTGCACCACATGTGTTT 60

Query      61      TTTACTGGACAGCTGCTTTGGCGGTGGGGACTCGTTTCCGCCGCCAGAGGTCACAACATA 120
          |||
AY139791  61      TTTACTGGACAGCTGCTTTGGCGGTGGGGACTCGTTTCCGCCGCCAGAGGTCACAACATA 120

Query      121     ACCAAACTTTTTATTACCAGTCAACCATACGTTTTTAATAGTCAAAACTTTCAACAACGGA 180
          |||
AY139791  121     ACCAAACTTTTTATTACCAGTCAACCATACGTTTTTAATAGTCAAAACTTTCAACAACGGA 180

Query      181     TCTCTTGGTTCTCGCATCGATGAAGAACGCAGC 213
          |||
AY139791  181     TCTCTTGGTTCTCGCATCGATGAAGAACGCAGC 213
```

Alignment of primary sequence of the ITS1 regions of *C. viswanathii* ATCC 22981 and PT specimen *C. viswanathii* NYSDOH 0907.

```
Query      1      GATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGC 60
          |||
DQ249200  1      GATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGC 60

Query      61      ATGCCTGTTTGGAGCGTCATTTCTCCCTCAAGCCC GCGGGTTTGGTGTGAGCAATACGCC 120
          |||
DQ249200  61      ATGCCTGTTTGGAGCGTCATTTCTCCCTCAAGCCC GCGGGTTTGGTGTGAGCAATACGCC 120

Query      121     AGGTTTGTGTTGAAAGACGTACGTGGAGACTATATTAGCGACTTAGGTTCTACCAAAACGC 180
          |||
DQ249200  121     AGGTTTGTGTTGAAAGACGTACGTGGAGACTATATTAGCGACTTAGGTTCTACCAAAACGC 180

Query      181     TTGTGCAGTCGGCCCACCACAGCTTTTCTAACTTTTGACCTCAAATCAGGTAGGACTACC 240
          |||
DQ249200  181     TTGTGCAGTCGGCCCACCACAGCTTTTCTAACTTTTGACCTCAAATCAGGTAGGACTACC 240

Query      241     CGCTGAACTTAAGCATATCAATAAGCGGAGGAAAA 275
          |||
DQ249200  241     CGCTGAACTTAAGCATATCAATAAGCGGAGGAAAA 275
```

Alignment of primary sequence of the ITS2 regions of *C. viswanathii* WM 239 and PT specimen *C. viswanathii* NYSDOH 0907.

Further Reading:

1. Lee, F.L., Fu, H.M., and Hsu, W.H. 1998. DNA hybridization and electrokaryotype study of some *Candida* species. *Int. J. Syst. Bacteriol.* 48: 1463-1466.
2. Maiwald, M., Kappe, R., and Sonntag, H.G. 1994. Rapid presumptive identification of medically relevant yeasts to the species level by polymerase chain reaction and restriction enzyme analysis. *J. Med. Vet. Mycol.* 32: 115-122.
3. McGinnis, M.R. 1983. Detection of fungi in cerebrospinal fluid. *Am. J. Med.* 75(1B):129-138.
4. Quindos, G., Lipperheide, V., and Ponton, J. 1993. Evaluation of two commercialized systems for the rapid identification of medically important yeasts. *Mycoses* 36: 299-303.
5. Sandhu, D.K., Sandhu, R.S., and Misra, V.C. 1976. Isolation of *Candida viswanathii* from cerebrospinal fluid. *Sabouraudia* 14: 251-254.



Figure 17. Four-day-old, white, a little wrinkled, and moist colony of *Candida viswanathii* on Sabouraud's dextrose agar.

A.

B.

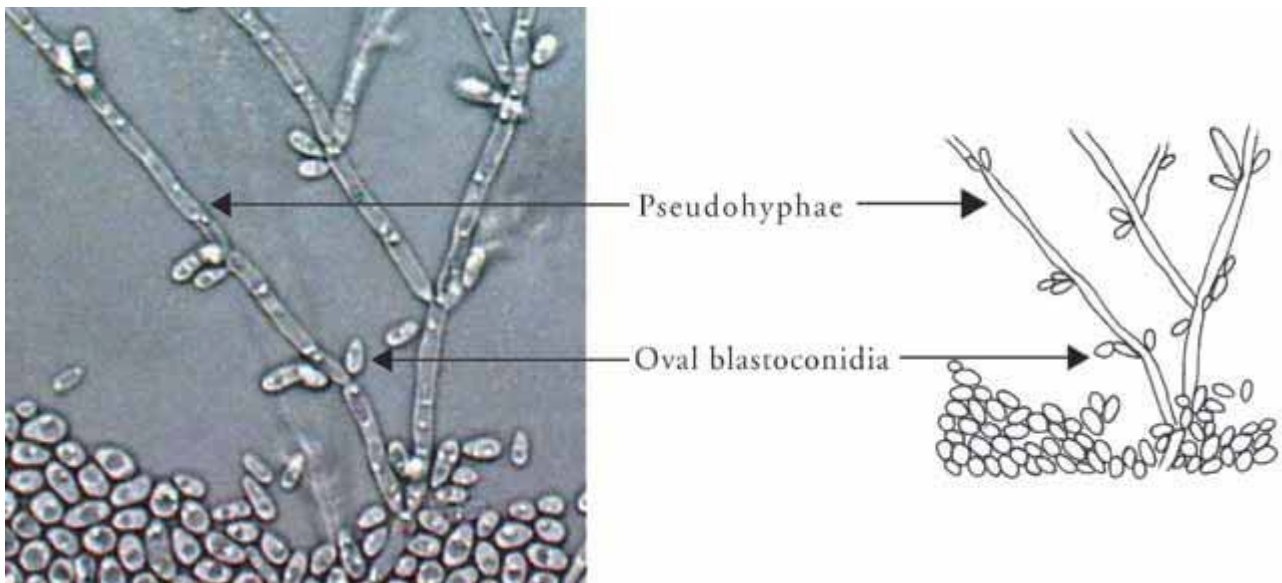


Figure 18. Microscopic morphology of *Candida viswanathii* on corn meal agar with Tween 80 showing long pseudohyphae and oval shaped blastoconidia (A, 400 X magnification; B, line diagram not to scale).

Y-4 *Candida parapsilosis*

Source: Stool / Urine

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	52

Clinical Significance: *Candida parapsilosis* is an increasingly important bloodstream pathogen. It is also increasingly prevalent in yeast-induced onychomycosis. It is implicated in candidal endocarditis, endophthalmitis, fungemia, and infection in burn patients. It is an important nosocomial pathogen in various hospital outbreaks, such as neonatal fungemia and endophthalmitis after cataract surgery.

Ecology: *C. parapsilosis* is found in fruit juices and water, and on the skin of humans and other mammals.

Laboratory Diagnosis:

1. Culture – On Sabouraud’s dextrose agar after 5 days at 25°C, colony was white to cream, dull with smooth surface (Figure 19).
2. Microscopic morphology – On corn meal agar with Tween 80, long, multibranched pseudohyphae, together with small elongated blastoconidia clustered on them, were seen (Figure 20).
3. Differentiation from other yeasts – *C. parapsilosis* ferments glucose, but not maltose, sucrose, lactose, or trehalose. It does not grow on media containing cycloheximide, but it grows at 37°C. It assimilates glucose, maltose, and sucrose, but it is urease- and nitrate-negative. Biochemically, it is very similar to *C. lusitaniae*, but microscopically, it forms long pseudohyphae that differentiates it from *C. lusitaniae*.
4. In vitro susceptibility testing – *C. parapsilosis* is susceptible to amphotericin B, 5-flucytosine, caspofungin, and azoles such as fluconazole, ketocoazole, itraconazole, and voriconazole. A few clinical isolates are reported as resistant to fluconazole.
5. Molecular tests – PCR assay of ITS regions of rDNA was used to identify *C. parapsilosis* in clinical specimens. Chromosome length polymorphism and RAPD procedures were used to characterize the genetic diversity of this organism.

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. parapsilosis* CBS 604 (Genebank accession number: AY391843) for ITS1 and ITS2 regions.

Comments: All laboratories correctly identified this specimen.

Sequence alignment:

```
Query      1      TTCCGTAGGTGAACCTGCGGAAGGATCATTACAGAATGAAAAGTGCTTAACTGCATTTTTT 60
           |||
AY391843   14      TTCCGTAGGTGAACCTGCGGAAGGATCATTACAGAATGAAAAGTGCTTAACTGCATTTTTT 73

Query      61      TCTTACACATGTGTTTTTCTTTTTTTTGAAAACCTTGCTTTGGTAGGCCTTCTATATGGGG 120
           |||
AY391843   74      TCTTACACATGTGTTTTTCTTTTTTTTGAAAACCTTGCTTTGGTAGGCCTTCTATATGGGG 133

Query      121     CCTGCCAGAGATTAAACTCAACCAAATTTTATTTAATGTCAACCGATTATTTAATAGTCA 180
           |||
AY391843   134     CCTGCCAGAGATTAAACTCAACCAAATTTTATTTAATGTCAACCGATTATTTAATAGTCA 193
```

```

Query      181  AAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAG   229
          |||
AY391843  194  AAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAG   242

```

Alignment of primary sequence of the ITS1 regions of *C. parapsilosis* CBS 604 and PT specimen *C. parapsilosis* NYSDOH 0907.

```

Query      1      GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATATTCGTGAAT   60
          |||
AY391843  224  GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATATTCGTGAAT   283

Query      61      CATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTTGGAG   120
          |||
AY391843  284  CATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTTGGAG   343

Query      121     CGTCATTTCTCCCTCAAACCCTCGGGTTTGGTGTGAGCGATACGCTGGGTTTGCTTGAA   180
          |||
AY391843  344  CGTCATTTCTCCCTCAAACCCTCGGGTTTGGTGTGAGCGATACGCTGGGTTTGCTTGAA   403

Query      181     AGAAAGGCGGAGTATAAACTAATGGATAGGTTTTTTTCCACTCATTGGTACAAACTCCAAA   240
          |||
AY391843  404  AGAAAGGCGGAGTATAAACTAATGGATAGGTTTTTTTCCACTCATTGGTACAAACTCCAAA   463

Query      241     ACTTCTTCCAAATTCGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAA   300
          |||
AY391843  464  ACTTCTTCCAAATTCGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAA   523

Query      301     TAAGCGGAG   309
          |||
AY391843  524  TAAGCGGAG   532

```

Alignment of primary sequence of the ITS2 regions of *C. parapsilosis* CBS 604 and PT specimen *C. parapsilosis* NYSDOH 0907.

Further Reading:

1. David, A., Risitano, D.C., Mazzeo, G., Sinardi, L., Venuti, F.S., and Sinardi, A.U. 2005. Central venous catheters and infections. *Minerva Anestesiol.* 71: 561-564.
2. Da Silva, C.L., dos Santos, R.M., and Colombo, A.L. 2001. Cluster of *Candida parapsilosis* primary bloodstream infection in a neonatal intensive care unit. *Braz. J. Infect. Dis.* 5: 32-36.
3. Deshpande, K. 2003. *Candida parapsilosis* fungaemia treated unsuccessfully with amphotericin B and fluconazole but eliminated with caspofungin: a case report. *Crit Care Resusc.* 5: 20-23.
4. Filioti, J., Spiroglou, K., and Roilides, E. 2007. Invasive candidiasis in pediatric intensive care patients: epidemiology, risk factors, management, and outcome. *Intensive Care Med.* 33: 1272-1283.
5. Garzoni, C., Nobre, V.A., and Garbino, J. 2007. *Candida parapsilosis* endocarditis: a comparative review of the literature. *Eur J Clin Microbiol Infect Dis.* Sep 6; [Epub ahead of print]
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7. Lopez-Ciudad, V., Castro-Orjales, M.J., Leon, C., Sanz-Rodriguez, C., de la Torre-Fernandez, M.J., Perez de Juan-Romero, M.A., Collell-Llach, M.D., Diaz-Lopez, M.D. 2006. Successful treatment of *Candida parapsilosis* mural endocarditis with combined caspofungin and voriconazole. *BMC Infect Dis.* 16: 73.

8. Yalaz, M., Akisu, M., Hilmioglu, S., Calkavur, S., Cakmak, B., Kultursay, N. 2006. Successful caspofungin treatment of multidrug resistant *Candida parapsilosis* septicaemia in an extremely low birth weight neonate. *Mycoses*. 49: 242-245.



Figure 19. Seven-day-old, white to cream, smooth colony of *Candida parapsilosis* on Sabouraud's dextrose agar.

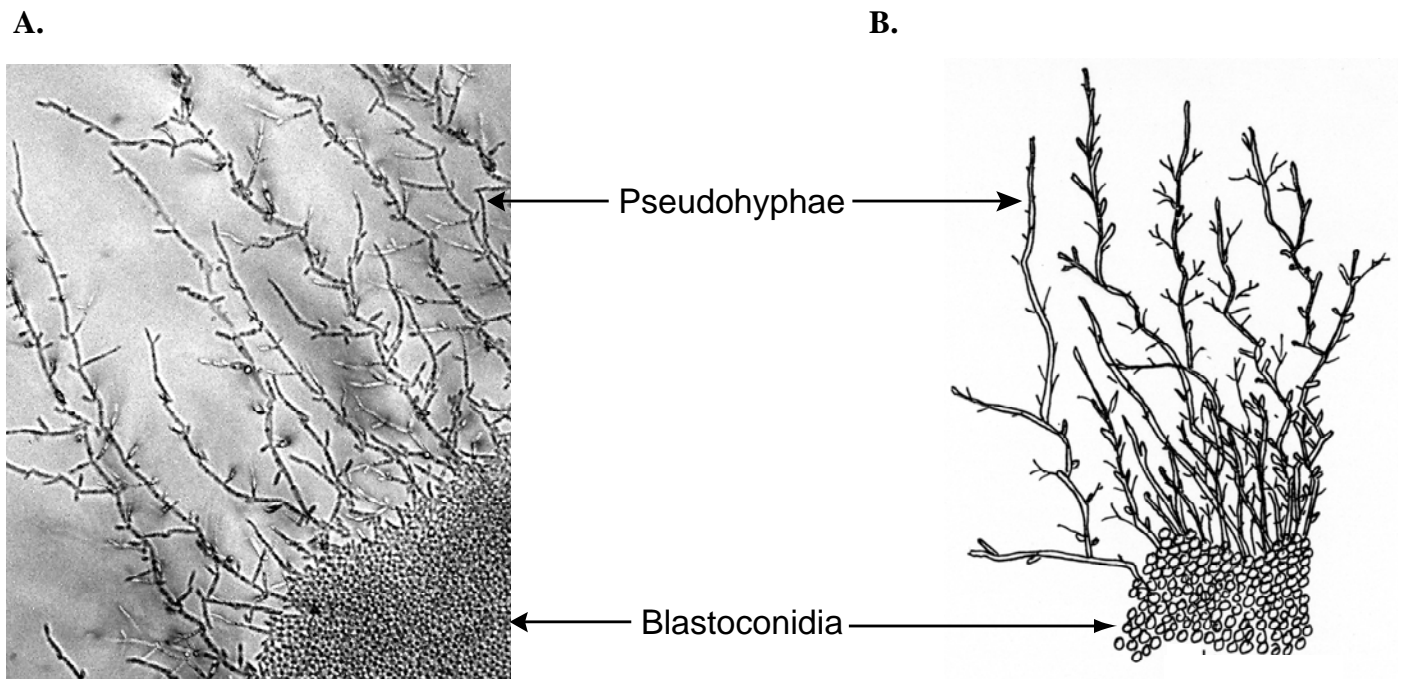


Figure 20. Microscopic morphology of *Candida parapsilosis* on corn meal agar with Tween 80 showing long, multibranched pseudohyphae together with small cluster of elongated blastoconidia (A, 400× magnification; B, line drawing not to scale).

Y-5 *Trichosporon asahii*

Source: Blood / Nail

Scoring:	No. Laboratories
Referee Laboratories with correct ID:	9
Laboratories with correct ID:	49
Laboratories with incorrect ID:	3
(<i>Trichosporon beigelii</i>)	(2)
(<i>Trichosporon</i> sp.)	(1)

Clinical Significance: *Trichosporon asahii* infections are not common but have been associated with a wide spectrum of clinical manifestations, ranging from superficial involvement in immunocompetent individuals to severe systemic disease in immunocompromised patients.

Ecology: *T. asahii* has been found from water, soil, and occasionally found on the human skin, in the mouth, and nails.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar, after 7 days at 25°C, *T. asahii* colony was white to yellowish. The surface was wrinkled, velvety (Figure 21).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *T. asahii* had true and pseudohyphae with blastoconidia singly or in short chains. Rectangular-to-oval arthroconidia were prominent and were fragmented from both the main and side branches of hyphae (Figure 22).
3. **Differentiation from other yeasts** – *T. asahii* is nonfermentative, urease-positive, nitrate-negative, cycloheximide resistant, and metabolically active for assimilation of a wide range of carbohydrates. It can be distinguished from *Geotrichum candidum* by its wooly colony and production of urease.
4. **In vitro susceptibility testing** – *T. asahii* is susceptible to amphotericin B, but reduced-susceptibility isolates are often recovered from patients who do not respond to therapy with this drug. The susceptibilities to flucytosine and azoles are variable.
5. **Molecular tests** – Sequence analysis of the ribosomal DNA intergenic spacer regions provided the powerful method to distinguish between phylogenetically closely related species and clinical isolates.

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 *T. asahii* CBS 7137 (Genbank accession number: AF444466) for ITS1 and ITS2 regions.

Comments: *T. asahii* is a new species split from *T. beigelii*, which is considered an invalidated name by Gueho and colleagues (1992). The new codebook for the API 20C listed three species in place of *T. beigelii*: *T. asahii*, *T. inkin*, and *T. mucoides*. In this system, separation of *T. asahii* and *T. mucoides* depends upon the assimilation of inositol and raffinose. *T. asahii* is negative for both at 48 hr, while *T. mucoides* is positive.

Sequence alignment:

```
Query      1      TCCGTAGGTGAACCTGCGGAAGGATCATTAGTGATTGCCTTTATAGGCTTATAACTATAT 60
          |||
AF444466  1      TCCGTAGGTGAACCTGCGGAAGGATCATTAGTGATTGCCTTTATAGGCTTATAACTATAT 60
```

```

Query      61      CCACTTACACCTGTGAACTGTTCTACTACTTTGACGCAAGTCGAGTATTTTTACAAACAAT  120
|          |          |          |          |          |          |          |          |          |          |          |          |          |
AF444466  61      CCACTTACACCTGTGAACTGTTCTACTACTTTGACGCAAGTCGAGTATTTTTACAAACAAT  120

Query      121     GTGTAATGAACGTCGTTTTATTATAACAAAATAAAACTTTCAACAACGGATCTCTTGGCT  180
|          |          |          |          |          |          |          |          |          |          |          |          |          |
AF444466  121     GTGTAATGAACGTCGTTTTATTATAACAAAATAAAACTTTCAACAACGGATCTCTTGGCT  180

Query      181     CTCGCATCGATGAAGAACGCAGC    203
|          |          |          |          |          |          |          |          |          |          |          |          |
AF444466  181     CTCGCATCGATGAAGAACGCAGC    203

```

Alignment of primary sequence of the ITS1 regions of *T. asahii* CBS 7137 and PT specimen *T. asahii* NYSDOH 0907.

```

Query      1       CATCGATGAAGAACGCAGCGAATTGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATC  60
|          |          |          |          |          |          |          |          |          |          |          |          |          |
AF444466  185     CATCGATGAAGAACGCAGCGAATTGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATC  244

Query      61      ATCGAATCTTTGAACGCAGCTTGCCTCTCTGGTATTCCGGAGAGCATGCCTGTTTCAGT  120
|          |          |          |          |          |          |          |          |          |          |          |          |          |
AF444466  245     ATCGAATCTTTGAACGCAGCTTGCCTCTCTGGTATTCCGGAGAGCATGCCTGTTTCAGT  304

Query      121     GTCATGAAATCTCAACCACTAGGGTTTCCTAATGGATTGGATTTGGGCGTCTGCGATTTC  180
|          |          |          |          |          |          |          |          |          |          |          |          |          |
AF444466  305     GTCATGAAATCTCAACCACTAGGGTTTCCTAATGGATTGGATTTGGGCGTCTGCGATTTC  364

Query      181     TGATCGCTCGCCTTAAAAGAGTTAGCAAGTTTGACATTAATGTCTGGTGTAAATAAGTTTC  240
|          |          |          |          |          |          |          |          |          |          |          |          |          |
AF444466  365     TGATCGCTCGCCTTAAAAGAGTTAGCAAGTTTGACATTAATGTCTGGTGTAAATAAGTTTC  424

Query      241     ACTGGGTCCATTGTGTTGAAGCGTGCTTCTAATCGTCCGCAAGGACAATTACTTTGACTC  300
|          |          |          |          |          |          |          |          |          |          |          |          |          |
AF444466  425     ACTGGGTCCATTGTGTTGAAGCGTGCTTCTAATCGTCCGCAAGGACAATTACTTTGACTC  484

Query      301     TGGCCTGAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA    357
|          |          |          |          |          |          |          |          |          |          |          |          |          |
AF444466  485     TGGCCTGAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA    541

```

Alignment of primary sequence of the ITS2 regions of *T. asahii* CBS 7137 and PT specimen *T. asahii* NYSDOH 0907.

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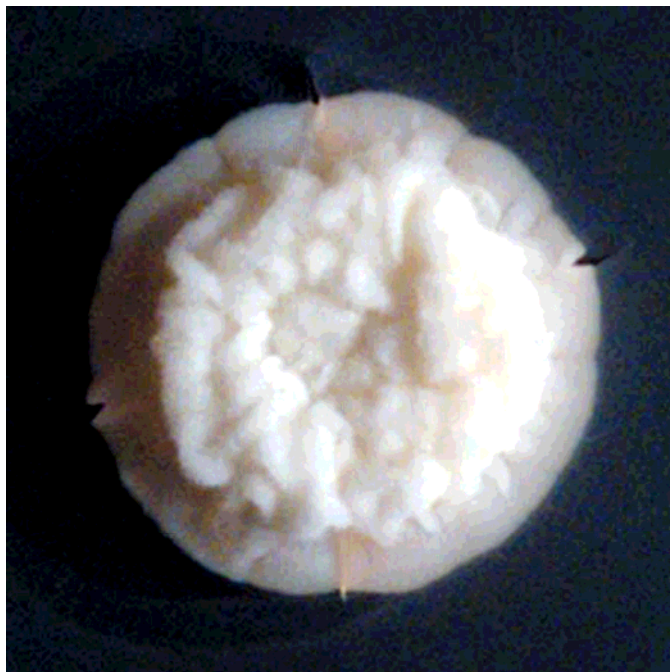
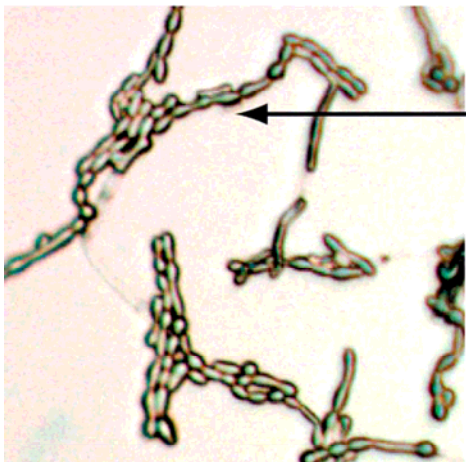
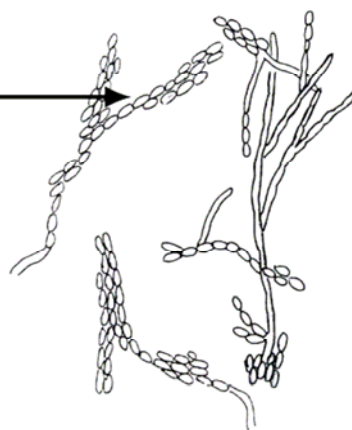


Figure 21. Seven-day-old, white to yellowish, wrinkled colony of *Trichosporon asahii* on Sabouraud's dextrose agar.

A.



B.



Arthroconidia

Figure 22. Microscopic morphology of *Trichosporon asahii* on corn meal agar with Tween 80 showing arthroconidia (A, 400× magnification; B, line drawing not to scale).

ANTIFUNGAL SUSCEPTIBILITY TESTING

Introduction: Document M27-A2 published by the National Committee for Clinical Laboratory Standards (NCCLS, now names as Clinical Laboratory Standards Institute, CLSI). Subcommittee on Antifungal Susceptibility Testing is the current standard reference guide for determining the antifungal susceptibility testing of pathogenic yeasts. It includes two methods, broth microdilution and broth macrodilution. 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.). The disk diffusion method approved by NCCLs (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. Starting from this test event, antifungal susceptibility testing drug panel was expanded from originally 2 drugs to 8 drugs right now. All participating laboratories could select any number of antifungal drug(s) from the test panel based upon customary practice in their facilities.

Materials & Results: Twenty-seven microbiology laboratories within the United States and one reference laboratory each from Canada and United Kingdom participated in this event. *Candida albicans* NYSDOH 0907 (S-1) was included in the September 26, 2007 antifungal proficiency testing event. This isolate was validated by all the participating laboratories. One laboratory each reported higher than validated MIC range for ketoconazole, fluconazole, and posaconazole. Two laboratories reported higher than validated MIC range for itraconazole.

Summary:

Drugs	Validated Range (µg/ml)	# of laboratories	% of laboratories reported MIC within validated range
5-fluorocytosine	0.125 – 2.0	22	100
Amphotericin B	0.06 – 2.0	23	100
Caspofungin	0.015 – 1.0	19	100
Fluconazole	0.06 – 4.0	29	97
Itraconazole	≤ 0.015 – 0.5	25	92
Ketoconazole	≤ 0.25	18	94
Posaconazole	≤ 0.125	12	92
Voriconazole	≤ 0.125	20	100

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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 (3, 5, 6 and 7), and showed it to be a valuable aid in establishing a diagnosis when culture was negative (4). Paired serum and CSF specimens allowed detection of antigen in confirmed cases (7). Parallel serologic studies for both antigen and antibody are recommended to ensure detection of extrameningeal cryptococcosis. Newly emerging disease states and therapies have been shown to increase the opportunity for nonspecific interference in some serum specimens. Pretreatment of serum specimens with pronase prior to utilization of the latex agglutination test reduces nonspecific interference, and enhances the detection of capsular polysaccharide antigens of *Cryptococcus neoformans*.

Materials & Methods: Seventy-six laboratories participated in this event. Two positive serum samples for cryptococcal antigen were included in the September 26, 2007 direct detection antigen testing event. One serum was of low to medium titers (1:16), another one was of high titer (1:128). Titers within ± 2 dilutions of the reference results were the acceptable results. One positive (titer 1:128) educational serum sample from another batch of *Cryptococcus* antigen preparation was included in this event too.

Results: The performance of 75 laboratories was satisfactory in this test event while 1 laboratory did not give the response before the deadline. The supplementary information on qualitative and quantitative assays on *Cryptococcus neoformans* antigen test is summarized in Table 3 and 4.

Comments: In last four test events, a number of laboratories obtained false positive results. These results were obtained with a particular test kit. In the current test event, none of the laboratories reported this problem possibly as a new batch of antigen was used among graded specimens. However, the previous problem was reproduced in the educational specimen, which used an old batch of antigen.

Further Reading:

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Table 3. Summary of qualitative assay

Method (Manufactures)	Sample	Cn-Ag-1		Cn-Ag-2		Cn-Ag-3		Cn-Ag-4		Cn-Ag-5	
	Total Number of laboratories	N*	P [†]	N	P	N	P	N	P	N	P
EIA (Total)	2	2	0	0	2	2	0	0	2	2	0
(Meridien Diagnostic)	2	2	0	0	2	2	0	0	2	2	0
Latex Agglutination (Total)	73	73	0	0	73	72	1	0	73	73	0
(Immuno-Mycologics)	4	4	0	0	4	4	0	0	4	4	0
(Meridien Diagnostic)	44	44	0	0	44	44	0	0	44	44	0
(Remel)	5	5	0	0	5	5	0	0	5	5	0
(Wampole)	22	22	0	0	22	21	1	0	22	22	0

* N: number of laboratories reporting Negative.

† P: number of laboratories reporting Positive.

Table 4. 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 (Manufactures)	Sample	Cn-Ag-2									Cn-Ag-4 Titers							
		2	4	8	10	16	32	64	128	256	8	16	32	64	80	128	256	512
EIA	Total # of laboratories																	
	(Meridien Diagnostic)	2		1			1						1				1	
Latex Agglutination	(Immuno-Mycologics)	3		2				1						1		1	1	
	(Meridien Diagnostic)	40	1	8	1	19	8	2	1			1	2	14	2	16	2	3
	(Remel)	4	1			2	1						3				1	
	(Wampole)	20	1	3		8	6	1		1		2	1	3		9	3	2

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