

Mycology Proficiency Testing Program

September 2010 Test Event

Critique



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New York State Department of Health

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Schedule of 2011 Mycology PT Mailouts*

Mycology Identification

January 26, 2011
May 25, 2011
September 27, 2011

Mycology Identification POSTMARK DEADLINES

March 11, 2011
June 17, 2011
November 14, 2011

Mycology Identification - Yeast Only

January 26, 2011
May 25, 2011
September 27, 2011

Mycology Identification - Yeast Only POSTMARK DEADLINES

February 18, 2011
June 17, 2011
October 24, 2011

Mycology Susceptibility

January 26, 2011
May 25, 2011
September 27, 2011

Mycology Susceptibility POSTMARK DEADLINES

February 18, 2011
June 17, 2011
October 24, 2011

Mycology Direct Detection

January 26, 2011
September 27, 2011

Mycology Direct Detection POSTMARK DEADLINES

February 11, 2011
October 17, 2011

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

TEST SPECIMENS AND GRADING POLICY

Test Specimens*

At least two strains of each mold specimen were examined for inclusion in the proficiency test event of September 2010. 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. Finally, ITS1 – ITS2 region of ribosomal genes was amplified, sequenced, and BLAST searched in two databases.

Grading Policy

A laboratory's response for each sample is compared with the response that reflects 80 percent agreement of 10 referee laboratories and/or 80 percent of all participating laboratories. The referee laboratories are selected at random from among hospital laboratories participating in the program. They represent all geographical areas of New York State and must have a record of excellent performance during the preceding three years. The 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 related, peer-reviewed publications. One yeast is to be tested against following drugs: amphotericin B, anidulafungin, caspofungin, flucytosine (5-FC), fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. The participating laboratories are allowed to select any number of antifungal drugs from the test panel based upon test practices in their facilities. A maximum score of 100 will be equally distributed to account for the drugs selected by an individual laboratory. If the result for any drug is incorrect then laboratory gets a score of zero for that particular test component or set.

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. The maximum score for this event is 100. 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 for an analyte, 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'.

*The use of brand and/or trade names in this report does not constitute an endorsement of the products on the part of the Wadsworth Center or the New York State Department of Health.

ANSWER KEY AND LABORATORY PERFORMANCE

Mycology – General

| | Specimen Key | Validated Specimen | Other Acceptable Answers | Correct Responses / Total # Laboratories (%) |
|---------------|----------------------------------|----------------------------------|--|---|
| M-1 | <i>Epidermophyton floccosum</i> | <i>Epidermophyton floccosum</i> | | 67/69 (97) |
| M-2 | <i>Cunninghamella</i> sp. | <i>Cunninghamella</i> sp. | <i>Cunninghamella elegans</i> <i>Cunninghamella bertholletiae</i> | 67/68 (98) |
| M-3 | <i>Syncephalastrum racemosum</i> | <i>Syncephalastrum racemosum</i> | <i>Syncephalastrum</i> sp. | 66/68 (97) |
| M-4 | <i>Nigrospora</i> sp. | <i>Nigrospora</i> sp. | <i>Nigrospora sphaerica</i> | 69/69 (100) |
| M-5 | <i>Aspergillus nidulans</i> | <i>Aspergillus nidulans</i> | <i>Emericella nidulans</i> | 65/68 (95) |
| M-Edu. | <i>Microsporum audouinii</i> | | | |

Mycology – Yeast Only

| | Specimen Key | Validated Specimen | Other Acceptable Answers | Correct Responses / Total # Laboratories (%) |
|--------------|-----------------------------|-----------------------------|---|---|
| Y-1 | <i>Candida parapsilosis</i> | <i>Candida parapsilosis</i> | | 53/53 (100) |
| Y-2 | <i>Candida tropicalis</i> | <i>Candida tropicalis</i> | | 53/53 (100) |
| Y-3 | <i>Candida dubliniensis</i> | <i>Candida dubliniensis</i> | | 44/53 (83) |
| Y-4 | <i>Candida krusei</i> | <i>Candida krusei</i> | <i>Candida krusei/Candida inconspicua</i> <i>Candida inconspicua</i> | 50/53 (94) |
| Y-5 | <i>Candida rugosa</i> | <i>Candida rugosa</i> | | 52/53 (98) |
| Y-Edu | <i>Candida famata</i> | | | |

Mycology – Antifungal Susceptibility Testing for Yeasts (S-1: *Candida albicans* M955)

| Drugs | Acceptable MIC (µg/ml) Range | Acceptable Interpretation | Acceptable Responses/Total # Laboratories (%) |
|--------------------|---|--|--|
| Amphotericin B | 0.06 – 1 | Susceptible / No interpretation | 24/24 (100) |
| Anidulafungin | 0.5 – 2 | Susceptible | 15/15 (100) |
| Caspofungin | 0.12 – 1 | Susceptible | 20/20 (100) |
| Flucytosine (5-FC) | 0.03 – 1 | Susceptible | 25/25 (100) |
| Fluconazole | 0.12 – 2 | Susceptible | 30/30 (100) |
| Itraconazole | 0.015 – 0.25 | Susceptible / Susceptible- dose dependent | 28/28 (100) |
| Ketoconazole | 0.008 – 0.06 | Susceptible / No interpretation | 6/6 (100) |
| Micafungin | 1 – 2 | Susceptible | 15/15 (100) |
| Posaconazole | 0.015 – 0.12 | Susceptible / No interpretation | 16/16 (100) |
| Voriconazole | 0.004 – 0.03 | Susceptible | 23/23 (100) |

Mycology – Direct detection (*Cryptococcus* Antigen Test)

| | Specimen Key | Validated Specimen | Correct Responses / Total # Laboratories (%) | Acceptable Titer Range | Correct Responses / Total # Laboratories (%) |
|----------------|---------------------|-------------------------------|---|-----------------------------------|---|
| Cn-Ag-1 | Negative | Negative | 70/70 (100) | NA | NA |
| Cn-Ag-2 | Negative | Negative | 70/70 (100) | NA | NA |
| Cn-Ag-3 | Negative | Negative | 69/70 (99) | NA | NA |
| Cn-Ag-4 | Positive (1:128) | Positive (1:128) | 69/70 (99) | 1:32 – 1:512 | 62/65 (95) |
| Cn-Ag-5 | Positive (1:512) | Positive (1:512) | 70/70 (100) | 1:64 – 1:2048 | 64/65 (98) |

TEST STATISTICS

| | General | Yeast Only | Antifungal Susceptibility Testing for Yeasts | Direct Detection |
|--|---------|---------------|--|---------------------|
| Number of participating laboratories | 69 | 53 | 30 | 70 |
| Number of referee laboratories | 10 | 10 | 30 | 10 |
| Number of laboratories responding by deadline | 69 | 53 | 30 | 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 | 68 | 52 | 30 | 70 |
| Number of laboratories unsuccessfully completing this test | 1 | 1 | 0 | 0 |

Number of Laboratories Using Commercial Yeast Identification System*

| | |
|---------------------|----|
| API 20C AUX | 32 |
| AMS Vitek | 7 |
| Vitek2 system | 23 |
| Remel Uni-Yeast-Tek | 1 |
| IDS Rapid System | 0 |
| Microscan | 2 |

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

| | |
|--|----|
| YeastOne Colorimetric microdilution method | 26 |
| Etest | 4 |
| Disk diffusion method | 0 |
| Others [†] | 3 |

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

| | |
|----------------------------|----|
| EIA method | 2 |
| <i>Meridien Diagnostic</i> | 2 |
| Latex Agglutination method | 68 |
| <i>Immuno-Mycologics</i> | 5 |
| <i>Meridien Diagnostic</i> | 44 |
| <i>Remel</i> | 8 |
| <i>Wampole</i> | 11 |

*Include multiple systems used by some laboratories

[†]Include laboratories using CLSI Microbroth dilution method

MOLD DESCRIPTIONS

M-1 *Epidermophyton floccosum*

Source: Foot

| | |
|---------------------------------------|------------------|
| Laboratory Performance: | No. Laboratories |
| Referee Laboratories with correct ID: | 10 |
| Laboratories with correct ID: | 67 |
| Laboratories with incorrect ID: | 2 |
| (<i>Microsporium audouinii</i>) | (1) |
| (<i>Trichophyton</i> sp.) | (1) |
| Outcome: | Validated |

Clinical Significance: A frequent casual agent of nail and skin infections in feet and groin. Unlike many other dermatophytes, *Epidermophyton floccosum* does not infect hair. There is one case report on invasive disease in immunocompromised patient with Behcet's syndrome.

Ecology: Anthropophilic (associated with humans), found worldwide.

Laboratory Diagnosis:

1. **Culture** – *E. floccosum* was a slow-growing fungus. Colonies measured up to 3 cm in 2 weeks. At 25°C, on Sabouraud's dextrose agar, colonies were initially white to yellow, later becoming greenish-yellow, folded in center and radically grooved, reverse tan (Figure 1).
2. **Microscopic morphology** – Lactophenol Cotton Blue mounts showed septate hyphae; macroconidia single or in clusters, smooth, thin-walled, clubshaped, with 2-6 septations (Figure 2). No microconidia were seen. Chlamydoconidia were present in old cultures.

3. **Differentiation from other dermatophytes** – *E. floccosum* is differentiated from other fungi by slow growth, absence of microconidia, and club-shaped macroconidia. The second species in this genus, *E. stockdaleae*, has been isolated from soil. It is differentiated from *E. floccosum* by its production of longer conidia with nine septations.
4. **In vitro susceptibility testing** – Most clinical isolates were susceptible to terbinafine and variably to griseofulvin, itraconazole, ketoconazole, and clotrimazole.
5. **Molecular tests** – ITS1 sequences of clinical isolates were species-specific. Specific DNA bands in arbitrarily primed polymerase chain reaction (AP – PCR) also provided rapid identification of dermatophytes including *E. floccosum*.

Comments: One laboratory reported this organism as *Microsporium audouinii* probably due to misidentification of the club-shaped macroconidia as terminal chlamydoconidia. *E. floccosum* does not produce any microconidia, which differentiates it from many *Trichophyton* species.

Sequences alignment:

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 *Epidermophyton floccosum* ATCC 52066 (Genebank accession number: EF631604) for ITS1 and ITS2 regions.

```

Query 1      TTAACGCGCAGGCCGCGAGTCGGCCCCGTCCCCCTTCTCTCTGAATGCTGGACGGTGTTCGCC 60
          |||
Sbjct 1701   TTAACGCGCAGGCCGCGAGTCGGCCCCGTCCCCCTTCTCTCTGAATGCTGGACGGTGTTCGCC 1760

Query 61     GGCCACACGCCCATTTCTTGTCTACACTACCCGGTTGCCTCGGCCGGGCCGCGCCCCCTAGG 120
          |||
Sbjct 1761   GGCCACACGCCCATTTCTTGTCTACACTACCCGGTTGCCTCGGCCGGGCCGCGCCCCCTAGG 1820

Query 121    CTGCAGTGTTCGCTGCAGCGTCTCg9999999CCGTTCGGGGGATGGAGAAGGATGCCCCGG 180
          |||
Sbjct 1821   CTGCAGTGTTCGCTGCAGCGTCTCgGGGGGGGCGGTTCGGGGGATGGAGAAGGATGCCCCGG 1880

Query 181    CGGGGTTGATCGCTCCCCACCCCTGGACAGCGCTCGCCGAAGGAGTGATTCTCAGAAAT 240
          |||
Sbjct 1881   CGGGGTTGATCGCTCCCCACCCCTGGACAGCGCTCGCCGAAGGAGTGATTCTCAGAAAT 1940

Query 241    TCTACGAAATCTCCATAGGTGGTTTCAGTCTGAGCGTTGGCAAGCAAAAACCAGTCAAAAC 300
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Sbjct 1941   TCTACGAAATCTCCATAGGTGGTTTCAGTCTGAGCGTTGGCAAGCAAAAACCAGTCAAAAC 2000

Query 301    TTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT 360
          |||
Sbjct 2001   TTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT 2060

Query 361    AATGTGAATTGCAGAATTCCTGTAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGG 420
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Sbjct 2061   AATGTGAATTGCAGAATTCCTGTAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGG 2120

Query 421    TATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTTCAACCCCTCAAGCCCGGCTTGTGTGA 480
          |||
Sbjct 2121   TATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTTCAACCCCTCAAGCCCGGCTTGTGTGA 2180

Query 481    TGGACGACCGTCCGACCGCCTTTGCATCCCCGTTCACCGGGAGAGGAGAAAGGTGGAG 540
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Sbjct 2181   TGGACGACCGTCCGACCGCCTTTGCATCCCCGTTCACCGGGAGAGGAGAAAGGTGGAG 2240

Query 541    GGGACGCGCCCGAAAAGCAGTGGCCAGGCCGCGATTCCGGGGCCCTGGGCGAATGGGCAA 600
          |||
Sbjct 2241   GGGACGCGCCCGAAAAGCAGTGGCCAGGCCGCGATTCCGGGGCCCTGGGCGAATGGGCAA 2300

Query 601    CAAAACCAGCGCTTCAGGACCGGCCGGCTCTCTGGCCCTAGTTTCCGTGGGAGGACGA 660
          |||
Sbjct 2301   CAAAACCAGCGCTTCAGGACCGGCCGGCTCTCTGGCCCTAGTTTCCGTGGGAGGACGA 2360

Query 661    AAGGGGGCGACCCCTCTCTCCCTCCGCATTCAGGTTGACCTCGG 705
          |||
Sbjct 2361   AAGGGGGCGACCCCTCTCTCCCTCCGCATTCAGGTTGACCTCGG 2405

```

Alignment of primary sequence of the ITS1 and ITS2 regions of *Epidermophyton floccosum* ATCC 52066 and PT specimen *Epidermophyton floccosum* M1640.

Further Reading:

1. Jessup, C. J., N. S. Ryder, and M. A. Ghannoum. 2000. An evaluation of the *in vitro* activity of terbinafine. *Med. Mycol.* 38: 155-159.
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- gene sequences. *Mycopathologia*. 146: 111-113.
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 6. Mochizuki, T., M. Kawasaki, H. Ishizaki, and K. Makimura. 1999. Identification of several clinical isolates of dermatophytes based on the nucleotide sequence of internal transcribed spacer 1 (ITS 1) in nuclear ribosomal DNA. *J Dermatol*. 26: 276-281.
 7. Mochizuki, T., N. Sugie, and M. Uehara. 1997. Random amplification of polymorphic DNA is useful for the differentiation of several anthropophilic dermatophytes. *Mycoses*. 40: 405-409.
 8. Pau M, Atzori L, Aste N, Tamponi R, Aste N. 2010. Epidemiology of Tinea pedis in Cagliari, Italy. *G Ital Dermatol Venereol*. 145: 1-5.
 9. Seddon, M. E., and M. G. Thomas. 1997. Invasive disease due to *Epidermophyton floccosum* in an immunocompromised patient with Behcet's syndrome. *Clin Infect Dis*. 25: 153-154.
 10. Weitzman, I., N. X. Chin, N. Kunjukunju, and P. Della-Latta. 1998. A survey of dermatophytes isolated from human patients in the United States from 1993 to 1995. *J Am Acad Dermatol*. 39(2 Pt 1): 255-261.

A.

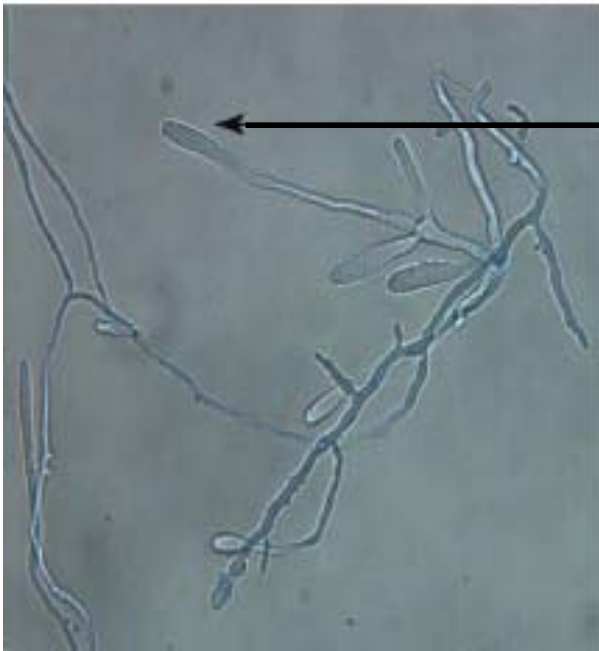


B.



Figure 1. (A) Ten-day-old, yellowish-white colony of *Epidermophyton floccosum* on Sabouraud's dextrose agar. (B) The reverse of ten-day-old colony of *Epidermophyton floccosum* on Sabouraud's dextrose agar.

A.



B.

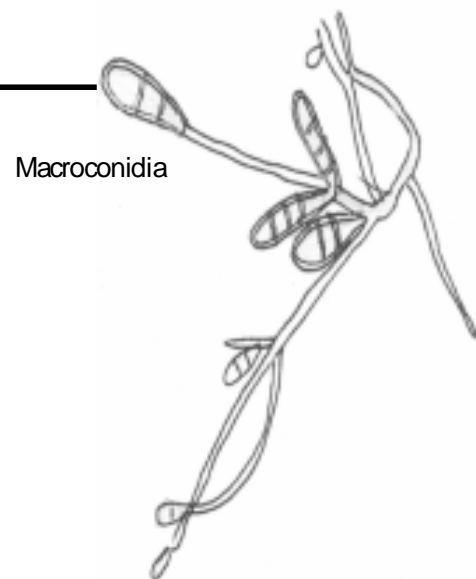


Figure 2. Microscopic morphology of *Epidermophyton floccosum*. Hyphae and smooth, thin walled, club-shaped macroconidia are seen (A, 400 \times magnification; B, line drawing not to scale).

M-2 *Cunninghamella* sp.

Source: Lung biopsy

| | |
|---------------------------------------|------------------|
| Laboratory Performance: | No. Laboratories |
| Referee Laboratories with correct ID: | 10 |
| Laboratories with correct ID: | 67 |
| Laboratories with incorrect ID: | 1 |
| (<i>Sepedonium</i> sp.) | (1) |
| Outcome: | Validated |

Clinical Significance: *Cunninghamella* sp. is occasionally reported as a causal agent of pulmonary zygomycosis or as an agent of nosocomial mycosis.

Ecology: *Cunninghamella* sp. is a common soil saprobe and widely found in decaying vegetables and animal matter.

Laboratory Diagnosis:

1. Culture – *Cunninghamella* sp. grew relatively rapidly. On Sabouraud's dextrose agar, after 5 days at 25°C, the colony showed white to gray color on the surface and cottony texture (Figure 3A). Reverse appeared pale or buff (Figure 3B).
2. Microscopic morphology – Lactophenol cotton blue mount showed broad, hyaline, and aseptate hyphae. Sporangiohores were branched with swollen vesicles at the end. Vesicles were covered with single-spored sporangioles supported by short denticles projecting from the vesicle (Figure 4).
3. Differentiation from other Zygomycetes – *Cunninghamella* sp. is distinct from other

common Zygomycetes or mucorales fungi by their single-spored sporangia formed on denticles on the vesicle surface.

4. In vitro susceptibility testing – *Cunninghamella* sp. is susceptible to amphotericin B, posaconazole, and itraconazole, but resistant to ketoconazole and miconazole.
5. Molecular tests – *Cunninghamella* spp. can be identified by means of panfungal polymerase chain reaction (PCR), direct DNA sequencing of the PCR products, and homology search with nucleotide basic local alignment search tool.

Comments: All the participating laboratories except one reported the acceptable answers for this specimen. This isolate we sent out in this PT event is *Cunninghamella elegans* which is a non-pathogen but closely related to *Cunninghamella bertholletiae*. These two species can be differentiated by the ability of growth at 45°C. *C. bertholletiae* grows at 45°C, but *C. elegans* does not.

Sequences alignment:

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA. 100% identity was found between this PT specimen and *Cunninghamella elegans* isolate 19-M-1 (Genebank accession number: EU076936) for ITS1 and ITS2 regions.

```

Query 1 TTCCGTAGGTGAACCTGCGGAAGGATCATTACTTATTCGGTCATTGGTTTTTTATTCAAAA 60
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Sbjct 14 TTCCGTAGGTGAACCTGCGGAAGGATCATTACTTATTCGGTCATTGGTTTTTTATTCAAAA 73

Query 61 ACCTTTGGCTTTAAATCATCCACAGTGTGGGAAATGTCTTCTAACGCTTGTGCCTGGTTC 120
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Sbjct 74 ACCTTTGGCTTTAAATCATCCACAGTGTGGGAAATGTCTTCTAACGCTTGTGCCTGGTTC 133

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Query 121 AGTCTAGTGCTGCCACTTGAGTTTACTCTTGGGTCAAGGGACCTTTGGGTAGTTTGTTC 180
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Sbjct 134 AGTCTAGTGCTGCCACTTGAGTTTACTCTTGGGTCAAGGGACCTTTGGGTAGTTTGTTC 193

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Sbjct 194 TTCGTGAGCAACCTCTTGTAACGGGGATAAGATTAATTTTATTATACTAAATTTTACTGA 253

Query 241 ACTGATAGACCATAAAATCTATGGTTGT'TTTTATTATAAAACaaaaaaaaCAACTTTCAGCA 300
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Sbjct 254 ACTGATAGACCATAAAATCTATGGTTGT'TTTTATTATAAAACAAAAAAAACAACTTTCAGCA 313

Query 301 ATGGATCTCTCGGCTTTCGCATCGATGAAGAACGC 335
          |||
Sbjct 314 ATGGATCTCTCGGCTTTCGTATCGATGAAGAACGC 348

```

Alignment of primary sequence of the ITS1 and ITS2 regions of *Cunninghamella elegans* isolate 19-M-1 and PT specimen *Cunninghamella elegans* M1221.

Further Reading:

1. Garbino J, Myers C, Ambrosioni J, Gumy-Pause F. 2010. Report of a successful treatment of pulmonary *Cunninghamella bertholletiae* infection with liposomal amphotericin and posaconazole in a child with GvHD and review of the literature. *J Pediatr Hematol Oncol.* 32: 85-87.
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8. Sato, M., Gemma, H., Sano, T., Ono, T., Atsumi, E., Ito, I., Chida, K., Nakamura, H. 2001. Pulmonary mucormycosis caused by *Cunninghamella bertholletiae* in a nonimmunocompromised woman. *Nihon Kokyuki Gakkai Zasshi* 39: 758-762.
9. Tomsikova, A. 2002. Causative agents of nosocomial mycoses. *Folia Microbiol.* 47: 105-112.

A.

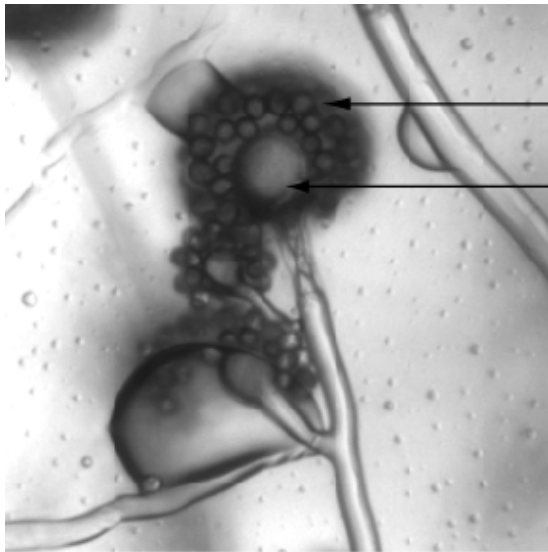


B.



Figure 3. (A) Five-day-old, white to gray cottony texture colony of *Cunninghamella* sp. on Sabouraud's dextrose agar. (B) The reverse of the colony shows pale or buff.

A.



B.

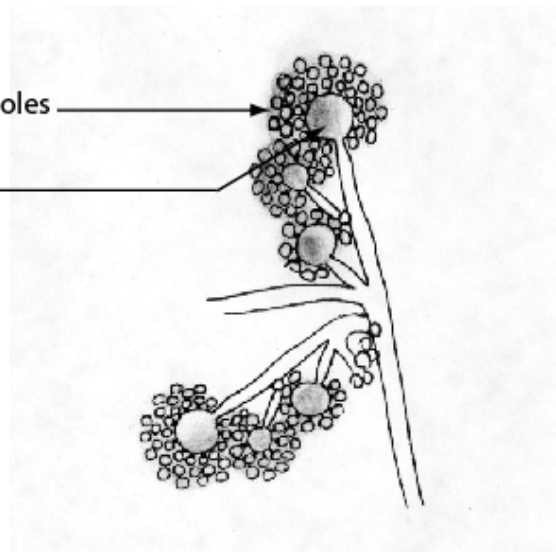


Figure 4. Microscopic morphology of *Cunninghamella* sp. showing broad, hyaline, and aseptate hyphae. Sporangiophores branched and end with a swollen vesicle covered with single-spored sporangioles (A, 200× magnification; B, line drawing not to scale).

M-3 *Syncephalastrum racemosum*

Source: Bronchial wash

| | |
|---|------------------|
| Laboratory Performance: | No. Laboratories |
| Referee Laboratories with correct ID: | 10 |
| Laboratories with correct ID: | 66 |
| Laboratories with incorrect ID: | 2 |
| (<i>Aspergillus niger</i>) | (1) |
| (<i>Cunninghamella bertholletiae</i>) | (1) |
| Outcome: | Validated |

Clinical Significance: *Syncephalastrum racemosum* is a rare pathogen in humans.

Ecology: *S. racemosum* is a saprobic fungus that has been isolated from environmental sources worldwide.

Laboratory Diagnosis:

1. **Culture** – *S. racemosum* grew rapidly. On Sabouraud's dextrose agar, after 5 days at 25°C, the colony produced low or tall, erect mycelia that covered the whole plate. The surface was white to gray and black (Figure 5A), and pale brown on reverse (Figure 5B).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed aseptate hyphae. Sporangioophores were branched and ended in round or oval terminal vesicles called ampullae. The vesicles were surrounded by rod- or club-shaped structures called merosporangia in which multiple, round to oval sporangiospores developed (Figure 6).
3. **Differentiation from other species** – It may be necessary to distinguish between the vesicle structures of *S. racemosum* and *Aspergillus* conidiophores. *Syncephalastrum* differs from *Aspergillus* by the presence of merosporangia and absence of phialides. In contrast to *Aspergillus*, the hyphae of

Syncephalastrum are nonseptate. *Syncephalastrum* is different from *Cunninghamella* by the terminal vesicle on the sporangiophore of *Syncephalastrum* bears finger-shaped sporangia called merosporangium on its surface. Each merosporangium carries linearly arranged sporangiospores inside.

4. **In vitro susceptibility testing** – *S. racemosum* is susceptible to amphotericin B, but resistant to 5-FC, fluconazole, itraconazole and ketocoazole.
5. **Molecular tests** – The nuclear small-subunit (18S) ribosomal DNA and domains D1 and D2 of the nuclear large-subunit (28S) ribosomal DNA was used to investigate phylogenetic relationships among representative species of Zygomycetes.

Comments: One laboratory reported this specimen as *Cunninghamella bertholletiae* possibly because it got mixed with the specimen M-2. *S. racemosum* can be distinguished from *Aspergillus niger* by the macro-morphology if the shape of the conidiophores causes confusion. *S. racemosum* has white, gray, to black very wooly, texture colony, but *A. niger* is granular with dark black conidia.

Sequences alignment:

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA. 94% identity was found between this PT specimen and strain FH9 (Genebank accession number: EU409811) for ITS1 and ITS2 regions.

A.



B.

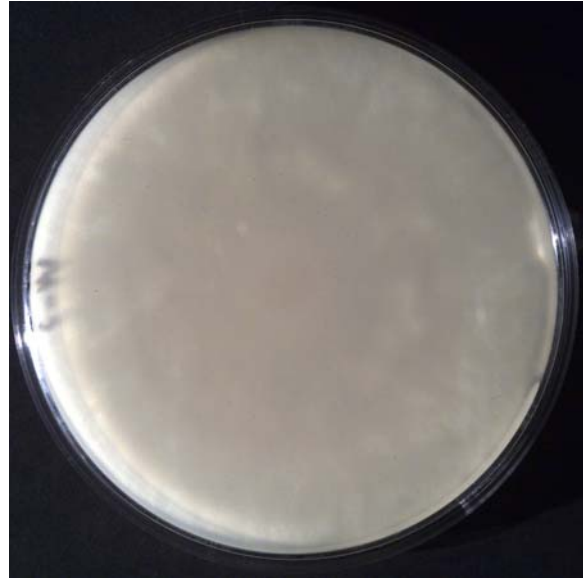
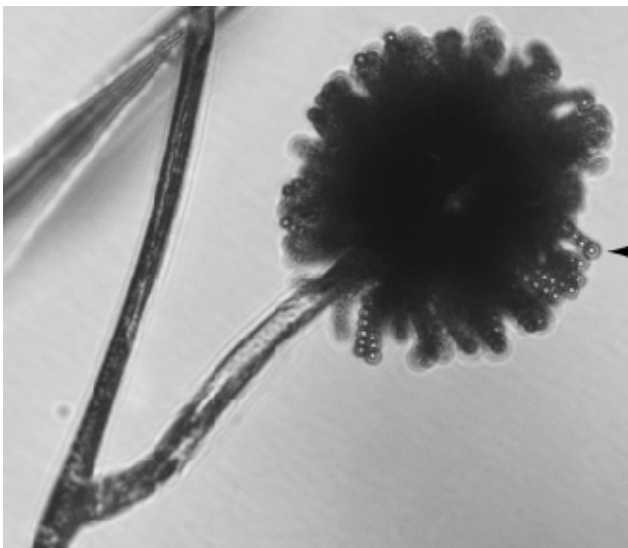


Figure 5. (A) Five-day-old, white to gray and black wooly texture colony of *Syncephalastrum racemosum* on Sabouraud's dextrose agar. (B) The reverse side of the colony appears pale brown.



← Merposporangia and sporangiospores

Figure 6. Microscopic morphology of *Syncephalastrum racemosum* showing merosporangia arranged around the vesicle at the apex of sporangiophore and round sporangiospores formed in a linear series in the interior of the merosporangia.

M-4 *Nigrospora* sp.

Source: Nail

| | |
|---------------------------------------|------------------|
| Laboratory Performance: | No. Laboratories |
| Referee Laboratories with correct ID: | 10 |
| Laboratories with correct ID: | 69 |
| Laboratories with incorrect ID: | 69 |
| Outcome: | Validated |

Clinical Significance: *Nigrospora* sp. is a rare pathogen and commonly encountered in the laboratory as contaminant.

Ecology: *Nigrospora* sp. is widespread and mainly found in the soil, decaying vegetable, leaves.

Laboratory Diagnosis:

1. Culture – *Nigrospora* sp. grew rapidly. On Sabouraud's dextrose agar, after 5 days at 25°C, the texture of the colony was velvet to woolly, and the color was white to cinnamon, became gray to black (Figure 7A). The reverse was brown (Figure 7B).
2. Microscopic morphology – Lactophenol cotton blue mount showed septate hyphae,

broad, flask-shaped conidiophores with black, unicellular, and oval to ellipsoidal conidia (Figure 8).

3. Differentiation from other molds – *Nigrospora* is differentiated from *Humicola* spp. by its very black, oblate conidia that originate from hyaline, inflated conidiophores.
4. In vitro susceptibility testing – No information is available.
5. Molecular tests – No information is available.

Comments: All the participating laboratories correctly identified this specimen.

Sequences alignment:

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 *Nigrospora sphaerica* strain 9032n3 (Genebank accession number: GQ919076) for ITS1 and ITS2 regions.

| | | | |
|-------|-----|---|-----|
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| Sbjct | 59 | GAGTTATCCAAC TCCCAAACCCATGTGAACTTATCTCTTTGTTGCCTCGGCGCAAGCTAC | 118 |
| Query | 61 | CCGGGACCTCGCGCCCCGGGCGGCCCGCGCGGACAAAACAAAAC TCTTGTTATCTTAG | 120 |
| Sbjct | 119 | CCGGGACCTCGCGCCCCGGGCGGCCCGCGCGGACAAAACAAAAC TCTTGTTATCTTAG | 178 |
| Query | 121 | TTGATTATCTGAGTGTCTTATTTAATAAGTCAAAACTTTCAACAACGGATCTCTTGGTTC | 180 |
| Sbjct | 179 | TTGATTATCTGAGTGTCTTATTTAATAAGTCAAAACTTTCAACAACGGATCTCTTGGTTC | 238 |
| Query | 181 | TGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA | 240 |
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| Query | 241 | ATCATCGAATCTTTGAACGCACATTTGCGCCATTAGTATTCTAGTGGGCATGCCTGTTCG | 300 |
| Sbjct | 299 | ATCATCGAATCTTTGAACGCACATTTGCGCCATTAGTATTCTAGTGGGCATGCCTGTTCG | 358 |

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Query 301 AGCGTCATTTCAACCCCTAAGCACAGCTTATTGTTGGGAACCTACGGCTTCGTAGTTCCCT 360
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Sbjct 359 AGCGTCATTTCAACCCCTAAGCACAGCTTATTGTTGGGAACCTACGGCTTCGTAGTTCCCT 418

Query 361 CAAAGACATTGGCGGAGTGGCAGTGGTCCTCTGAGCGTAGTAATCTTTTATCTCGCTTCT 420
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 419 CAAAGACATTGGCGGAGTGGCAGTGGTCCTCTGAGCGTAGTAATCTTTTATCTCGCTTCT 478

Query 421 GTTAGGTGCTGccccccGGCCGTAAAACCCCAAAA 456
          |||||||||||||||||||||||||||||||||||
Sbjct 479 GTTAGGTGCTGCCCCCGGCCGTAAAACCCCAAAA 514

```

Alignment of primary sequence of the ITS1 and ITS2 regions of *Nigrospora sphaerica* strain 9032n3 and PT specimen *Nigrospora sphaerica* M1963.

Further Reading:

1. Fan YM, Huang WM, Li W, Zhang GX. 2009. Onychomycosis caused by *Nigrospora sphaerica* in an immunocompetent man. *Arch Dermatol.* 145: 611-612.
2. Muralidhar S, Sulthana M. 1997. *Nigrospora* causing corneal ulcer--a case report. *Indian J Pathol Microbiol.* 40: 549-551.

A.



B.

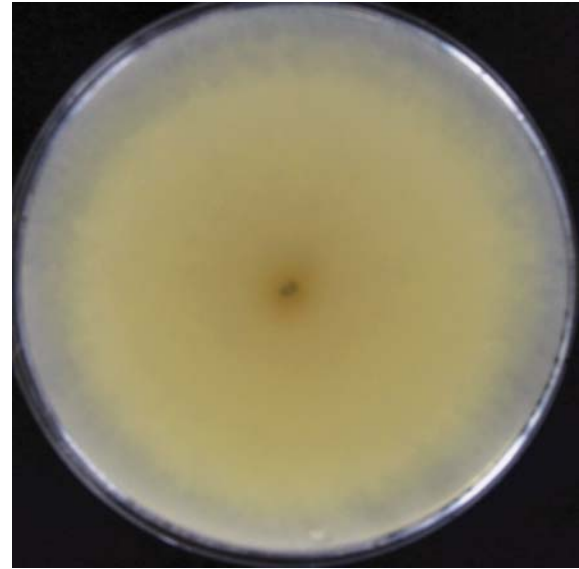
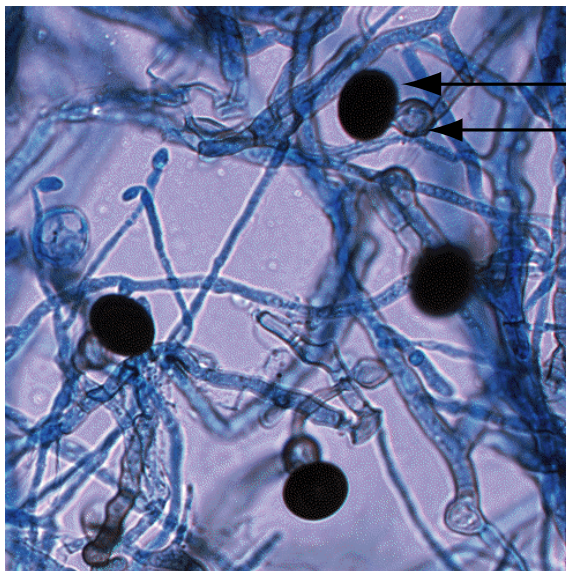


Figure 7. (A) Five-day old, white to gray woolly colony of *Nigrospora* sp. on Sabouraud's dextrose agar. (B) The reverse of the five-day-old colony of *Nigrospora* sp appeared brown.



Black, ovoid conidia
Inflated conidiophore

Figure 8. Microscopic morphology of *Nigrospora* sp. showing black, ovoid conidia with inflated conidiophore (400× magnification).

M-5 *Aspergillus nidulans*

Source: Sputum

| | |
|---------------------------------------|------------------|
| Laboratory Performance: | No. Laboratories |
| Referee Laboratories with correct ID: | 10 |
| Laboratories with correct ID: | 65 |
| Laboratories with incorrect ID: | 3 |
| (<i>Aspergillus versicolor</i>) | (2) |
| (<i>Aspergillus terreus</i>) | (1) |
| Outcome: | Validated |

Clinical Significance: Human infections of *Aspergillus nidulans* have been rarely reported. Most of these reports were from patients with chronic granulomatous disease involving skin, sinus, lungs etc.

Ecology: Cosmopolitan in soil.

Laboratory Diagnosis:

1. **Culture** – At 25°C, colony on Sabouraud’s dextrose agar was dark green with purplish peripheral pigment, powdery and rapid growing, and purple reverse (Figure 9).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed septate hyphae with brown, wavy conidiophores. Conidiophore ended in vesicle, which was subglobose with its upper half-covered by two series of sterigmata (biseriate). Conidia, measuring 5–7 µm in diameter, were round and smooth-rough walled. Round hülle cells and reddish color cleistothecia were also seen. Hülle cells are specialized structures made up of loose network of hyphae, having globose, vesiculose cells with thick walls that occur in certain groups of *Aspergilli*. Their characteristic shape provides a valuable diagnostic tool. Cleistothecia are sexual structures i.e. network of hyphae where mating between a and α strains occur. Ascospores (sexual spores) produced within these cleistothecia, are purple in color, lens shaped with equatorial crests (Figure 10).

3. **Differentiation from other *Aspergilli*** – *Aspergillus nidulans* can be distinguished by its dark green colony with purple reverse; microscopically, brown conidiophores, biseriate phialides, round hülle cells, cleistothecia with lens shaped ascospores with equatorial crests are characteristics.
4. ***In vitro* susceptibility testing** – Susceptibility testing results indicate that most of the isolates are susceptible to amphotericin B, voriconazole, and variably susceptible to itraconazole.
5. **Molecular tests** – *Aspergillus nidulans* has a well-defined genetic system, which allows it to be used as model organism in basic and applied research.

Comments: Three laboratories reported this organism as *Aspergillus versicolor*, and *Aspergillus terreus*. Microscopically, it is easy to differentiate *A. nidulans* from *A. versicolor* by the presence of cleistothecia and ridged ascospores. Similarly, colony morphology and microscopy of *A. nidulans* is quite unique from *A. terreus*. *A. nidulans* is green, buff to yellow on surface with purplish red to olive on reverse, while *A. terreus* is cinnamon to brown on surface with white to brown on the reverse. *A. nidulans* produce cleistothecia and hülle cells, while *A. terreus* has solitary, round aleuriconidia produced directly on hyphae.

Sequences alignment:

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 *Aspergillus nidulans* UOA/HCPF 8431B (Genbank accession number: FJ878644) for ITS1 and ITS2 regions.

```
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Query 61 TTGCTTCGGCGGGGAGCCCCCAGGGGGCGAGCCGCCGGGGACCCTGAACTTCATGCCT 120
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Sbjct 111 TTGCTTCGGCGGGGAGCCCCCAGGGGGCGAGCCGCCGGGGACCCTGAACTTCATGCCT 170

Query 121 GAGAGTGATGCAGTCTGAGCCTGAATACAAATCAGTCAAAACTTTCAACAATGGATCTCT 180
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Sbjct 171 GAGAGTGATGCAGTCTGAGCCTGAATACAAATCAGTCAAAACTTTCAACAATGGATCTCT 230

Query 181 TGGTTCGGCATCGATGAAGAACGCAGCGAACTGCGATAAGTAATGTGAATTGCAGAATT 240
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Query 361 CGGGGACGGGCCCCGAAAGGCAGCGGGCGGCACCGTGTCCGGTCCCTCGAGCGTATGGGGCT 420
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Query 421 TTGTCACCCGCTCGATTAGGGCCGGCCGGGCGCCAGCCGGCGTCTCCAACCTTA 474
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```

Alignment of primary sequence of the ITS1 and ITS2 regions of *Aspergillus nidulans* UOA/HCPF 8431B and PT specimen *Aspergillus nidulans* M1890.

Further reading:

1. Bukhari E, Alrabiaah A. 2009. First case of extensive spinal cord infection with *Aspergillus nidulans* in a child with chronic granulomatous disease. *J Infect Dev Ctries.* 3: 321 - 323.
2. Dellepiane RM, Tortorano AM, Liotto N, Laicini E, Di Landro G, Carnelli V, Pietrogrande MC. 2008. Invasive *Aspergillus nidulans* infection in a patient with chronic granulomatous disease. *Mycoses.* 51: 458 - 460.
3. de Souza CC, Pellizzon CH, Hiraishi M, Goldman MH, Goldman GH. 1998. Isolation and characterisation of cycloheximide – sensitive mutants of *Aspergillus nidulans*. *Current Genetics.* 33: 60 - 69.
4. Kim M, Shin JH, Suh SP, Ryang DW, Park CS, Kim C, Kook H, Kim J. 1997. *Aspergillus nidulans* infection in a patient with chronic granulomatous disease. *J Korean Medical Sci.* 12: 244 - 248.
5. Lucas GM, Tucker P, Merz WG. 1999. Primary cutaneous *Aspergillus nidulans* infection associated with a Hickman catheter

- in a patient with neutropenia. *Clin Infect Dis*. 29: 1594 - 1546.
6. Resen-Wolff A, Koch A, Friedrich W, Hahn G, Gahr M, Roesler J. 2004. Successful elimination of an invasive *Aspergillus nidulans* lung infection by voriconazole after failure of a combination of caspofungin and liposomal amphotericin b in a boy with chronic granulomatous disease. *The Pediatric Infect. Dis J*. 23: 584 - 586.
 7. Mizuki M, Chikuba K, Tanaka K. 1994. A case of chronic necrotizing pulmonary aspergillosis due to *Aspergillus nidulans*. *Mycopathologia*. 128: 75 - 79.
 8. Ng KP, Saw TL, Madasamy M, Soo Hoo T. 1999. Onychomycosis in Malaysia. *Mycopathologia*. 147: 29 - 32.
 9. Rösen-Wolff A, Koch A, Friedrich W, Hahn G, Gahr M, Roesler J. 2004. Successful elimination of an invasive *Aspergillus nidulans* lung infection by voriconazole after failure of a combination of caspofungin and liposomal amphotericin B in a boy with chronic granulomatous disease. *Pediatr Infect Dis J*. 23: 584 - 586.
 10. Yano S, Kobayashi K, Shishido S, Nakano H. 1999. Intrabronchial *Aspergillus nidulans* infection in an immunocompetent man. *Internal Medicine*. 38: 372 - 375.

A.

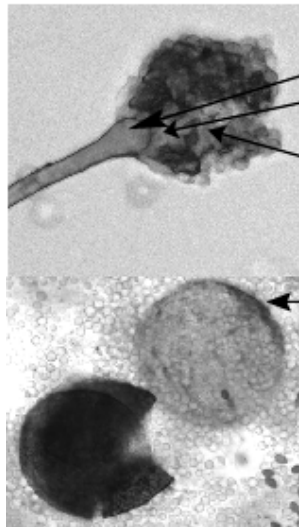


B.

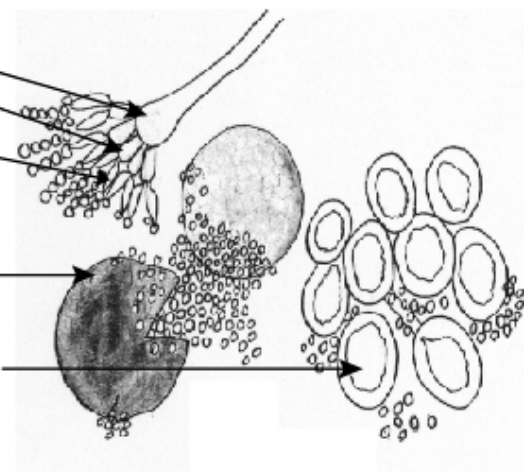


Figure 9. (A) Five-day-old, green to blue colony of *Aspergillus nidulans* with whitish edge on Sabouraud's dextrose agar. (B) The reverse of five-day old *Aspergillus nidulans* colony appeared purple.

A.



B.



Vesicle
Primary Sterigmata
Secondary Sterigmata

Cleistothecia
Hulle cells

Figure 10. Microscopic morphology of *Aspergillus nidulans* showing subglobose vesicle with biserial, columnar head, cleistothecia with ascus and ascospores, and hulle cells (A, 400X magnification; B, line drawing not to drawn to scale).

M-Edu *Microsporium audouinii*

Source: Skin

| Laboratory Performance: | No. Laboratories |
|---------------------------------------|------------------|
| Referee Laboratories with correct ID: | 2 |
| Laboratories with correct ID: | 16 |
| Laboratories with incorrect ID: | 53 |
| (<i>Trichophyton terrestre</i>) | (22) |
| (<i>Microsporium persicolor</i>) | (13) |
| (<i>Trichophyton interdigitale</i>) | (9) |
| (<i>Trichophyton</i> sp.) | (2) |
| (<i>Basidiobolus ranarum</i>) | (1) |
| (<i>Chrysosporium</i> sp.) | (1) |
| (<i>Emmonsia</i> sp.) | (1) |
| (<i>Trichophyton schoenleinii</i>) | (1) |
| (<i>Trichophyton tonsurans</i>) | (1) |

Clinical Significance: A causal agent of scalp and skin infections in young children. It rarely afflicts adults.

Ecology: This anthropophilic species is cosmopolitan in distribution. A decade ago, this fungus was the commonest cause of tinea capitis in North America but it is now seen mostly in Africa and South America.

Laboratory Diagnosis:

1. **Culture** – At 25°C, on Sabouraud’s dextrose agar, colonies grew moderately fast; downy, white to gray in color on the surface, with pale pink to salmon reverse (Figure 11).
2. **Microscopic morphology** – Lactophenol cotton blue mounts showed hyaline septate hyphae. Macroconidia and microconidia were rarely produced. Generally, sterile and pectinate hyphae were seen, with terminal or intercalary chlamydospores (Figure 12).
3. **Differentiation from other dermatophytes** – It is differentiated from other *Microsporium*

species by its production of brownish pigment on autoclaved rice grain. It does not perforate hair, and has no specific growth requirements.

4. **In vitro susceptibility testing** – Clinical isolates of *M. audouinii* were susceptible to various antifungal agents, but they have high MIC to fluconazole.
5. **Molecular tests** – Species identification of dermatophytes was done based on DNA sequences of nuclear ribosomal internal transcribed spacer regions and of the 5.8S ribosomal DNA region, and comparison with DNA sequence database.

Comments: *Trichophyton terrestre* does not grow at 37°C, which can be used to differentiate it from *M. audouinii*. *M. audouinii* is differentiated from *M. persicolor* by alkalization of BCP-milk solids-glucose medium. *M. audouinii* fails to perforate hair *in vitro*, but *T. terrestre*, *T. interdigitale*, and *M. persicolor* can perform this test.

Sequences alignment:

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA. 99% identity was found between this PT specimen and *Microsporium audouinii* strain IHEM 16245 (Genbank accession number: FJ479802) for ITS1 and ITS2 regions.

```

Query 1   ACGCGCAAGAGGTCGAAGTTGGCCCCGAAGCTCTTCCGTCTcccccccGGGCCTCCCGG 60
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Sbjct 69  GGAGGTTGCGGGCGGCAGGGGTGCTCCGGCCGCACGCCATTCTTGTCTACTGACCCG 128

Query 121  GTTGCTCGGCGGGCCGCGCTGCTGTGCTACAGCGGCCGTTcgggggggACGCCTGAGG 180
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Sbjct 608  TCTAGGACCGGCCGGTAGGCTGGCCTAAC 636

```

Alignment of primary sequence of the ITS1 and ITS2 regions of *Microsporium audouinii* strain IHEM 16245 and PT specimen *Microsporium* M1064.

Further reading:

1. Brasch J, Hugel R, Lipowsky F, Gräser Y. 2010. Tinea corporis caused by an unusual strain of *Microsporium audouinii* that perforates hair in vitro. *Mycoses*. 53: 360-362.
2. Brillowska-Dabrowska A, Swierkowska A, Lindhardt Saunte DM, Arendrup MC. 2010. Diagnostic PCR tests for *Microsporium audouinii*, *M. canis* and *Trichophyton* infections. *Med Mycol*. 48: 486-490.
3. Carrillo-Muñoz AJ, Fernandez-Torres B, Guarro J. 2003. In vitro antifungal activity of sertaconazole against 309 dermatophyte clinical isolates. *J Chemother*. 15: 555-557.

4. Donghi D, Hauser V, Bosshard PP. 2010. *Microsporum audouinii* tinea capitis in a Swiss school: assessment and management of patients and asymptomatic carriers. *Med Mycol.* [Epub ahead of print]
5. Fernandez-Torres, B., A. J. Carrillo, E. Martin, A. Del Palacio, M. K. Moore, A. Valverde, M. Serrano, and J. Guarro. 2001. In vitro activities of 10 antifungal drugs against 508 dermatophyte strains. *Antimicrob Agents Chemother.* 45: 2524-2528.
6. Ghannoum MA, Wraith LA, Cai B, Nyirady J, Isham N. 2008. Susceptibility of dermatophyte isolates obtained from a large worldwide terbinafine tinea capitis clinical trial. *Br J Dermatol.* 159: 711-713.
7. Graser, Y., A. F. Kuijpers, M. El Fari, W. Presber, and G. S. de Hoog. 2000. Molecular and conventional taxonomy of the *Microsporum canis* complex. *Med Mycol.* 38: 143-153.
8. Liu, D., S. Coloe, R. Baird, and J. Pedersen. 2000. Application of PCR to the identification of dermatophyte fungi. *J Med Microbiol.* 49: 493-497.
9. Rezusta A, Betrán A, Querol I, Palacián MP, Revillo MJ. 2009. Tinea capitis caused by *Trichophyton soudanense* and *Microsporum audouinii* in an adult: a case report. *Mycoses.* [Epub ahead of print]
10. Vella Zahra L, Vella Briffa D. 2003. Tinea capitis due to *Microsporum audouinii* in Malta. *Mycoses.* 46: 433-435.

A.



B.

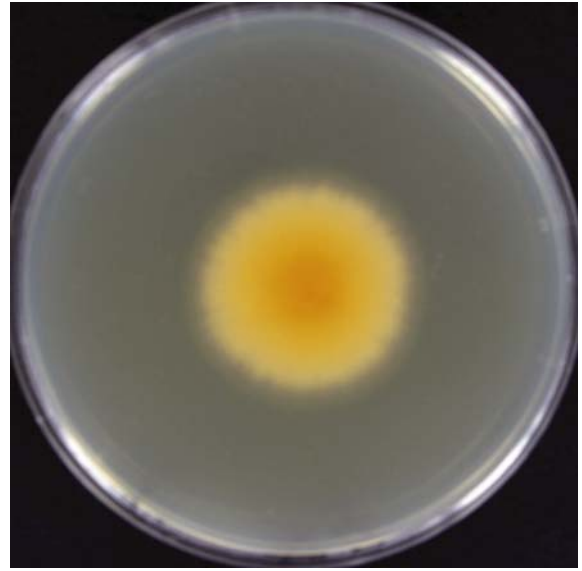
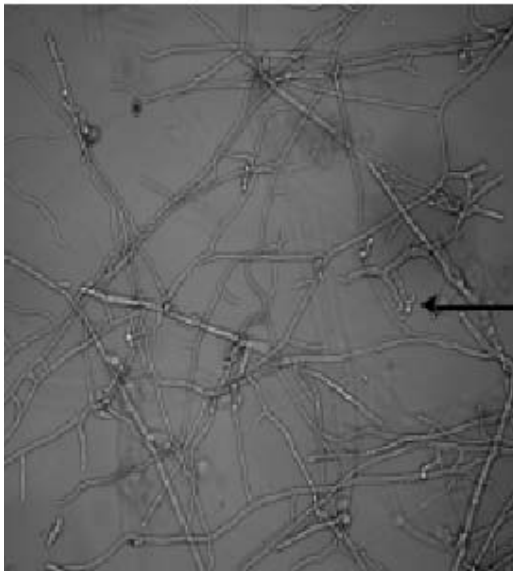


Figure 11. (A) Ten-day-old, white downy colony of *Microsporium audouinii* on Sabouraud's dextrose agar. (B) The reverse of ten-day-old *Microsporium audouinii* colony with yellow pigment.

A.



B.

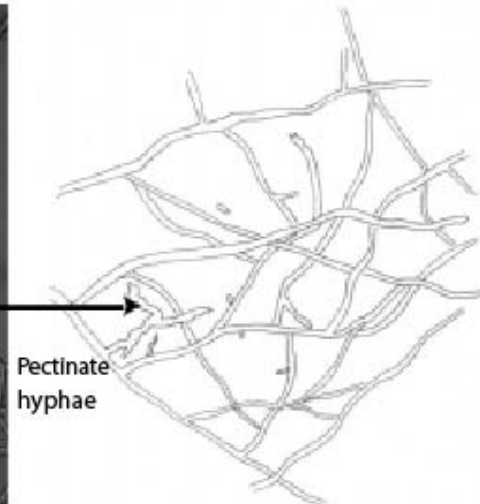


Figure 12. Microscopic morphology of *Microsporium audouinii*. Pectinate and sterile hyphae are seen (A, 200X magnification; B, line diagram not to scale).

Query 121 CCTGCCAGAGATTAAACTCAACCAAATTTTATTTAATGTCAACCGATTATTTAATAGTCA 180
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[AY391843](#) 194 AAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAG 242

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

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Query 181 AGAAAAGGCGGAGTATAAACTAATGGATAGGTTTTTTTCCACTCATTGGTACAAACTCCAAA 240
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[AY391843](#) 404 AGAAAAGGCGGAGTATAAACTAATGGATAGGTTTTTTTCCACTCATTGGTACAAACTCCAAA 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:

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5. 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.
6. 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.

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

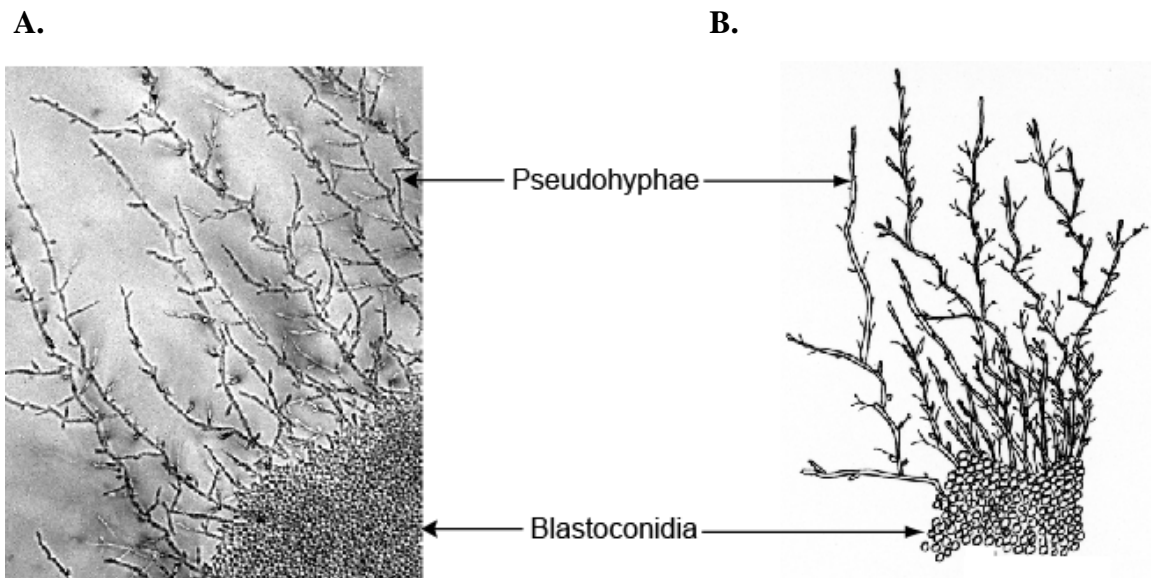


Figure 14. Microscopic morphology of *Candida parapsilosis* on corn meal agar with Tween 80 showing long, multibranched pseudohyphae together with small cluster of elongated blastoconidia (A, 400× magnification; B, line drawing not to scale).

Y-2 *Candida tropicalis*

Source: CSF / Sputum / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

Outcome:

No. Laboratories

10

53

0

Validated

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

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

Laboratory Diagnosis:

1. Culture – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was smooth to wrinkled, cream-colored and rapid-growing (Figure 15).
2. Microscopic morphology – On Corn meal agar with Tween 80, *C. tropicalis* showed long true hyphae and pseudohyphae, with either single or small clusters of blastoconidia (Figure 16).
3. Differentiation from other yeasts – *C. tropicalis* is differentiated from *C. albicans* and *C. dubliniensis* by variable growth on media containing cycloheximide, and by its

fermentation of glucose, maltose, sucrose, and trehalose. Occasionally, *C. tropicalis* can produce chlamydospores on corn meal agar.

4. In vitro susceptibility testing – Few strains of *C. tropicalis* has been reported with high amphotericin B MIC. *C. tropicalis* is generally susceptible to azoles and echinocandins, but variably susceptible to flucytosine.
5. Molecular tests – Reverse-hybridization line probe assay combined with PCR amplification of internal transcribed-spacer (ITS) regions was used for rapid identification of clinically significant fungal pathogens including *C. tropicalis*. The combination of pan-fungal PCR and multiplex liquid hybridization of ITS regions was developed for detection and identification of fungi in tissue specimens.

Comments: All the participating laboratories correctly identified this specimen.

Sequence alignment:

The identity of the test isolate was confirmed in Mycology PTP program by sequencing of its ITS1 region of rDNA. 100% identity was found between this PT specimen and *C. tropicalis* CBL Cd-3 (Genebank accession number: EU924133) for ITS1 region.

```

Query 1   TTTCCGTAGGTGAACCTGCGGAAGGATCATTACTGATTTGCTTAATTGCACCACATGTGT 60
          |||
Sbjct 23  TTTCCGTAGGTGAACCTGCGGAAGGATCATTACTGATTTGCTTAATTGCACCACATGTGT 82

Query 61  TTTTTATTGAACAAATTTCTTTGGTGGCGGGAGCAATCCTACCGCCAGAGGTTATAACTA 120
          |||
Sbjct 83  TTTTTATTGAACAAATTTCTTTGGTGGCGGGAGCAATCCTACCGCCAGAGGTTATAACTA 142

Query 121 AACCAAACTTTTTATTACAGTCAAACCTTGATTTATTATTACAATAGTCAAAACTTTCAA 180
          |||
Sbjct 143 AACCAAACTTTTTATTACAGTCAAACCTTGATTTATTATTACAATAGTCAAAACTTTCAA 202
    
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Query 181 CAACGGATCTCTTTGGTTCTCGCATCGATGAAGAACGCAG 219
          |||
Sbjct 203 CAACGGATCTCTTTGGTTCTCGCATCGATGAAGAACGCAG 241

```

Alignment of primary sequence of the ITS1 regions of *C. tropicalis* CBL Cd-3 and PT specimen *C. tropicalis* NYSDOH 0509.

Further Reading:

1. Dawson, N.L., Robles, H.A., and Alvarez, S. 2005. Recurrent *Candida tropicalis* meningitis. *Clinical Neurology and Neurosurgery*. 107: 243 – 245.
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3. Nucci, M. and Colombo, A.L. 2007. Candidemia due to *Candida tropicalis*: clinical, epidemiologic, and microbiologic characteristics of 188 episodes occurring in tertiary care hospitals. *Diagn Microbiol Infect Dis*. 58: 77-82.
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6. Pfaller MA, Castanheira M, Messer SA, Moet GJ, Jones RN. 2010. Variation in *Candida* spp. distribution and antifungal resistance rates among bloodstream infection isolates by patient age: report from the SENTRY Antimicrobial Surveillance Program (2008-2009). *Diagn Microbiol Infect Dis*. 68: 278-283.
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13. Yang, Y.L., Ho, Y.A., Cheng, H.H., Ho, M., and Lo, H.J. 2004. Susceptibilities of *Candida* species to amphotericin B and fluconazole: the emergence of fluconazole resistance in *Candida tropicalis*. *Infect Control Hosp Epidemiol*. 25: 60 – 64.



Figure 15. Seven-day-old, smooth-to-wrinkled, cream colored colony of *Candida tropicalis* on Sabouraud's dextrose agar.

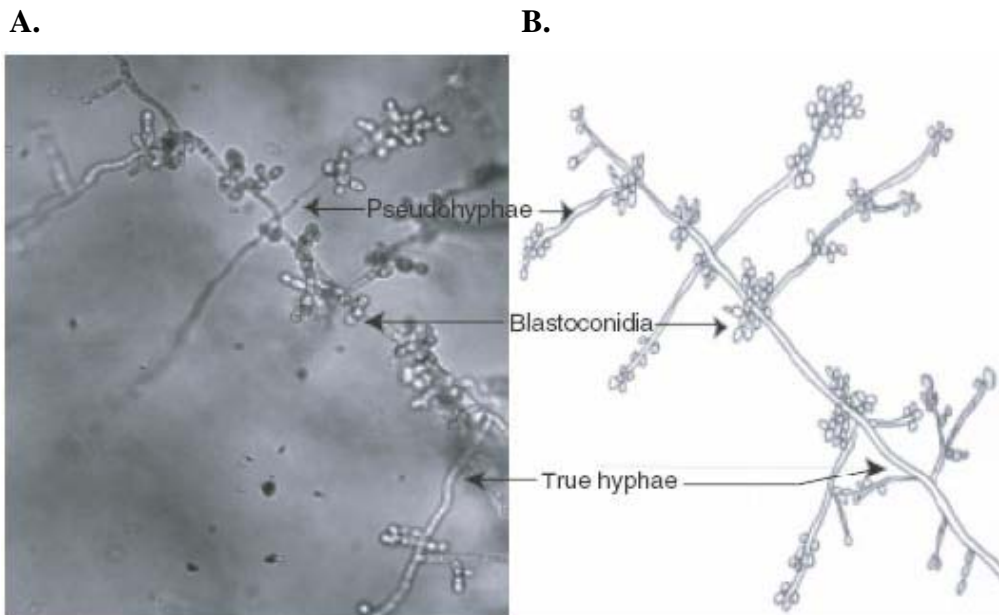


Figure 16. Microscopic morphology of *Candida tropicalis*, on corn meal agar with Tween 80, showing long true hyphae and pseudohyphae with clusters of blastoconidia (A, 400× magnification; B, line drawing not to scale).

Y-3 *Candida dubliniensis*

Source: Wound / Bronchial wash / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

(*Candida albicans*)

(*Candida viswanathii*)

(*Candida lusitaniae*)

(*Candida* sp.)

Outcome:

No. Laboratories

9

44

9

(5)

(2)

(1)

(1)

Validated

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.

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.

Laboratory Diagnosis:

1. Culture – On Sabouraud's dextrose agar after 7 days at 25°C, colony was white to cream, smooth, and soft (Figure 17). 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 18).
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. Mosaid et al. (2001) suggested that the ability of majority *C. dubliniensis* to produce rough colonies and chlamydospores on Staib agar and caffeic acid-ferric citrate agar, but *C. albicans* does not. 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.

Comments: This specimen was validated in the current test event. This is the third time that 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, September 2007, and

September 2009 PT events. In the current test event, about 83% laboratories were able to identify *C. dubliniensis*, which is similar as last validated event (September 2009). As summarized earlier in this section, a number of physiological differences could be used to distinguish these two closely related *Candida* species.

Sequences alignment:

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.

```

Query      1      TCCGTAGGTGAACCTGCGGAAGGATCATTACTGATTTGCTTAATTGCACCACATGTGTTT  60
AJ249484   1      TCCGTAGGTGAACCTGCGGAAGGATCATTACTGATTTGCTTAATTGCACCACATGTGTTT  60

Query      61      TGTTTTGGACAAACTTGCTTTGGCGGTGGGCCTCTACCTGCCGCCAGAGGACATAAACTT  120
AJ249484   61      TGTTTTGGACAAACTTGCTTTGGCGGTGGGCCTCTACCTGCCGCCAGAGGACATAAACTT  120

Query      121     ACAACCAAATTTTTTATAAACTTGTTCACGAGATTATTTTTAATAGTCAAAAACCTTCAACA  180
AJ249484   121     ACAACCAAATTTTTTATAAACTTGTTCACGAGATTATTTTTAATAGTCAAAAACCTTCAACA  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     AAAGATGATAGTGGTAAGGCGGAGATGCTTGACAATGGCTTAGGTGTAACCAAAAACATT  240
DQ355938   338     AAAGATGATAGTGGTAAGGCGGAGATGCTTGACAATGGCTTAGGTGTAACCAAAAACATT  397

Query      241     GCTAAGGCGGTCTCTGGCGTCGCCCATTTTATTCTTCAAACCTTTGACCTCAAATCAGGTA  300
DQ355938   398     GCTAAGGCGGTCTCTGGCGTCGCCCATTTTATTCTTCAAACCTTTGACCTCAAATCAGGTA  457

```




Figure 17. Four-day-old, white, glossy, and smooth colony of *Candida albicans* on Sabouraud's dextrose agar.

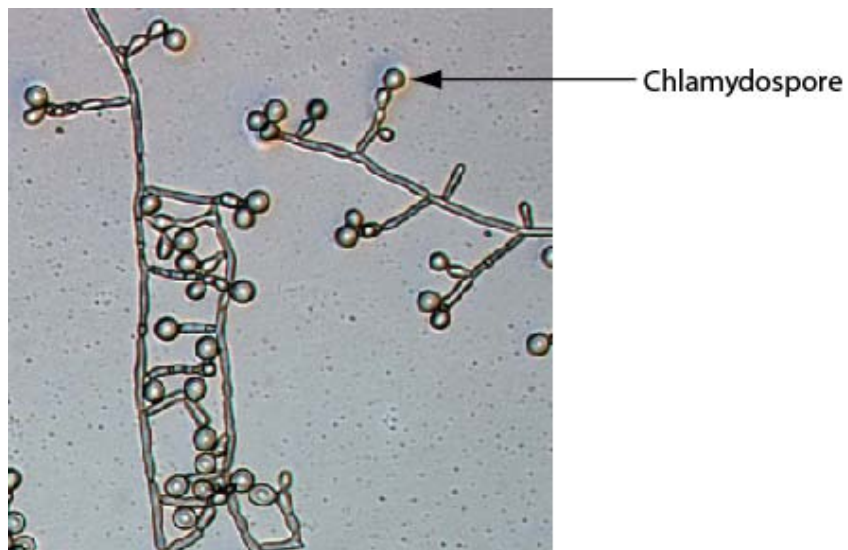


Figure 18. Microscopic morphology of *Candida dubliniensis* on corn meal agar with Tween 80, shows chlamydospores (A, 200× magnification).

Y-4 *Candida krusei*

Source: Vaginal Swab / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

(*Candida lambica*)

(*Candida lipolytica*)

(*Candida norvegenensis*)

Outcome:

No. Laboratories

10

50

3

(1)

(1)

(1)

Validated

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

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

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar, after 7 days at 25°C, *C. krusei* colony was soft, cream to buff, glassy and wrinkled (Figure 19).
2. **Microscopic morphology** – On Corn meal agar with Tween 80, *C. krusei* showed branched pseudohyphae with elongated blastoconidia (Figure 20).
3. **Differentiation from other yeasts** – *C. krusei* ferments glucose, but not sucrose or cellobiose, and does not grow on the media containing cycloheximide. It does not assimilate sucrose, which differentiates it from *C. parapsilosis* and *C. lusitanae*. It grows well at 42°C, differentiating it from *C. lambica*. It does not produce arthroconidia, thus differentiating it from *Blastoschizomyces capitatus*.

4. **In vitro susceptibility testing** – Clinical isolates are susceptible to amphotericin B and flucytosine. *C. krusei* is innately resistant to fluconazole and variably resistant to other azoles such as itraconazole and ketoconazole, but not voriconazole. *C. krusei* is also susceptible to anidulafungin, micafungin and caspofungin.
5. **Molecular tests** – DNA probes have been designed from the ITS regions and were incorporated into a reverse hybridization line probe assay for the detection of ITS PCR products for identification of fungal pathogens. Panfungal PCR and multiplex liquid hybridization were developed for the detection of clinically important yeasts in tissue specimens. PFGE, RFLP, and RAPD procedures were used for DNA fingerprinting and electrophoretic karyotyping of oral *C. krusei* isolates.

Comments: One laboratory each reported this specimen as *C. lambica*, *C. lipolytica*, and *C. norvegenensis*, respectively. *C. labmica* does not grow above 40°C, but *C. krusei* does. *C. lipolytica* can grow on the media containing cycloheximide, but *C. krusei* does not. Esculin hydrolysis test is recommended to differentiate *C. norvegenensis* from *C. krusei* when the same biocode occurs in API 20C AUX test.

Sequences alignment:

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

```

(AF411417)
NYSDOH 0108

1
TCCG TAGGTG AACCTGCGGA AGGATCATT A CTGTGATTTA GTACTACACT 50
CCG CAGGTT CACCTACGGA AGGATCATT A CTGTGATTTA GTACTACACT

51
GCGTGAGCGG AACGAAAAACA ACAACACCTA AAATGTGGAA TATAGCATAT 100
GCGTGAGCGG AACGAAAAACA ACAACACCTA AAATGTGGAA TATAGCATAT

101
AGTCGACAAG AGAAATCTAC GAAAAACAAA CAAAACCTTTC AACCAACGGAT 150
AGTCGACAAG AGAAATCTAC GAAAAACAAA CAAAACCTTTC AACCAACGGAT

151
CTCTTGGTTC TCGCATCGAT GAAGACGCA GC
CTCTTGGTTC TCGCATCGAT GAAGACGCA GC

```

Alignment of primary sequences of the ITS1 regions of *C. krusei* and PT specimen *C. krusei* NYSDOH 0108. Unmatched nucleotide bases are shaded.

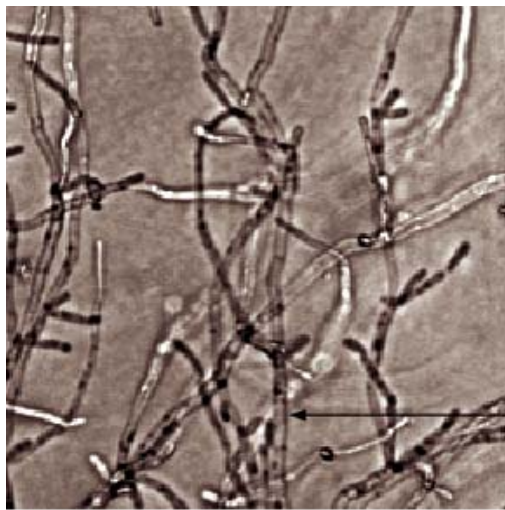
Further Reading:

- Munoz, P., Sanchez-Somolinos, M., Alcala, L., Rodriguez-Creixems, M., Pelaez, T., and Bonza, E. 2005. *Candida krusei* fungaemia: antifungal susceptibility and clinical presentation of an uncommon entity during 15 years in a single general hospital. *J Antimicrobial Chemotherapy*. 55: 188–193.
- Fanci, R., Guidi, S., Bonolis, M., and Bosi, A. 2005. *Candida krusei* fungemia in an unrelated allogeneic hematopoietic stem cell transplant patient successfully treated with caspofungin. *Bone Marrow Transplantation*. 35: 1215–1216.
- Dassanayake, R.S., Samaranayake, Y.H., and Samaranayake, L.P. 2000. Genomic diversity of oral *Candida krusei* isolates as revealed by DNA fingerprinting and electrophoretic karyotyping. *APMIS*. 108: 697-704.
- Hendolin, P.H., Paulin, L., Koukila-Kahkola, P., Anttila, V.J., Malmberg, H., Richardson, M., and Ylikoski, J. 2000. Panfungal PCR and multiplex liquid hybridization for detection of fungi in tissue specimens. *J. Clin. Microbiol.* 38: 4186-4192.
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- Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Nagy E, Dobiasova S, Rinaldi M, Barton R, Veselov A; the Global Antifungal Surveillance Group. 2008. *Candida krusei*, a Multidrug-Resistant Opportunistic Fungal Pathogen: Geographic and Temporal Trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001-2005. *J Clin Microbiol.* 46: 515-521.
- Sancak, B., Rex, JH., Chen, E., and Marr, K. 2004. Comparison of PCR- and *HinfI* Restriction endonuclease-based methods for typing of *Candida krusei* isolates. *J Clin Microbiol.* 42: 5889 – 5891.
- Schilling A, Seibold M, Mansmann V, and Gleissner B. 2007. Successfully treated *Candida krusei* infection of the lumbar spine with combined caspofungin/posaconazole therapy. *Med Mycol.* 46: 79-83.
- Sili, U., Yilmaz, M., Ferhanoglu, B., and Mert, A. 2007. *Candida krusei* arthritis in a patient with hematologic malignancy: successful treatment with voriconazole. *Clin Infect Dis.* 45: 897-898.
- Wiwanitkit V. 2010. Cardiac *Candida krusei* infection. *Ann Pediatr Cardiol.* 3: 93.



Figure 19. Seven-day-old soft wrinkled colony of *Candida krusei* on Sabouraud's dextrose agar.

A.



B.

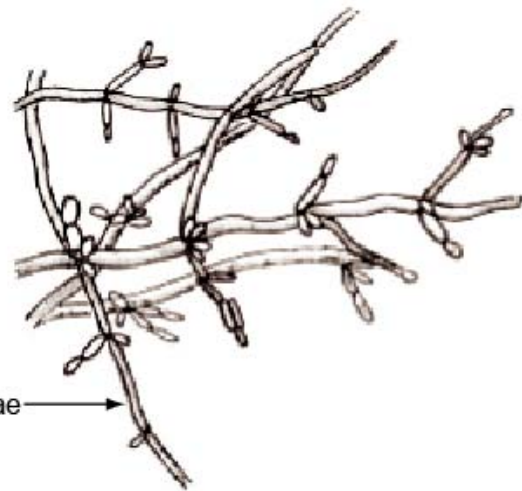


Figure 20. Microscopic morphology of *Candida krusei* on corn meal agar showing long, branched pseudohyphae with oval blastoconidia (A, 400× magnification; B, line drawing not to scale).

Y-5 *Candida rugosa*

Source: Blood / Catheter / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

(*Candida zeylanoides*)

Outcome:

No. Laboratories

10

52

1

(1)

Validated

Clinical Significance: *Candida rugosa* is an infrequent causal agent of fungemia in patients with indwelling catheters. Also, it is reported to cause infection in burn patients.

Ecology: *C. rugosa* is cosmopolitan, especially on dairy products.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar after 7 days at 25°C, colony was white to cream, wrinkled (Figure 21).
2. **Microscopic morphology** – On corn meal agar with Tween 80, branched pseudohyphae with chains of elongated blastoconidia were seen (Figure 22).
3. **Differentiation from other yeasts** – *C. rugosa* ferments only glucose, does not grow on media containing cycloheximide shows variable growth at 42°C, and is urea and nitrate negative. Microscopically, it forms branched pseudohyphae that differentiates it from *C. lusitanae* and *C. parapsilosis*. It does not form true hyphae, differentiating it from *Trichosporon beigeli*.
4. **In vitro susceptibility testing** – Clinical isolates are susceptible to caspofungin, 5-flucytosine, and various azoles such as fluconazole, ketocoanzole, and itraconazole. It is less susceptible to polyene antifungals like amphotericin B and nystatin.
5. **Molecular tests** – PCR assay of the ITS1 and ITS2 regions of ribosomal DNA was developed to identify *C. rugosa* in clinical specimens. A repetitive sequence-based PCR technique was developed to characterize the genotypic relatedness among *C. rugosa*

isolates (1). Karyotyping by PFGE was developed as a typing tool for discrimination among strains of *C. rugosa*.

Comments: All of the participating laboratories except one reported this specimen correctly in the current PT event. Generally, *C. rugosa* does not grow on the media containing cycloheximide but *C. zeylanoides* does.

Further Reading:

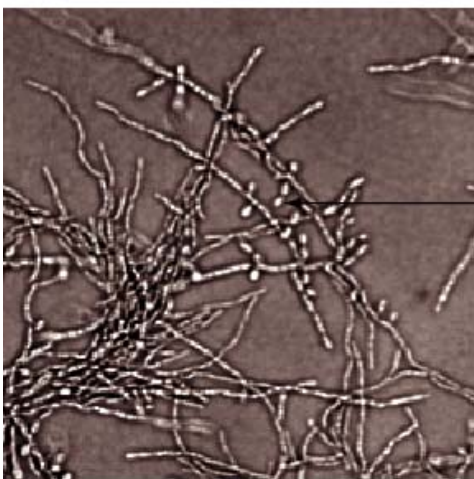
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Figure 21. Seven-day-old, cream colored, wrinkled colony of *Candida rugosa* on Sabouraud's dextrose agar.

A.



B.

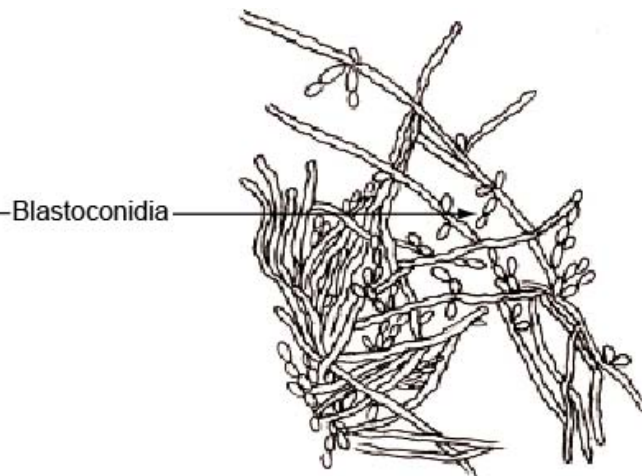


Figure 22. Microscopic morphology of *Candida rugosa* on corn meal agar with Tween 80 showing branched pseudohyphae with elongated blastoconidia (A, 200× magnification; B, line drawing not to scale).

Y-Edu *Candida famata*

Source: Skin / Catheter / Blood

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

(*Candida guilliermondii*)

(*Candida* sp.)

(*Candida zeylanoides*)

(*Candida lusitaniae*)

(*Candida sake*)

(*Cryptococcus albidus*)

(*Kluyveromyces* sp.)

No. Laboratories

7

36

17

(5)

(4)

(4)

(1)

(1)

(1)

(1)

Clinical Significance: *Candida famata* is an infrequent causal agent of nosocomial fungemia in immunosuppressed patients. Also, it is a rare causative agent of ocular infections, arthritis, and peritonitis.

Ecology: *C. famata* is cosmopolitan, found in plants, soil and dairy products.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar after 7 days at 25°C, colony was white to yellowish, soft, smooth to slightly wrinkled (Figure 23).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *C. famata* showed round to oval blastoconidia with no or rudimentary pseudohyphae, but with longer incubation (more than a week) primitive or well-developed pseudohyphae were seen (Figure 24).
3. **Differentiation from other yeasts** – *C. famata* ferments glucose, sucrose, and trehalose, grows at 37°C. It forms primitive to well developed pseudohyphae on corn meal agar or Dalmau plate when incubated longer, which differentiates it from *C. guilliermondii*. It does not produce true hyphae, which differentiates it from *C. ciferrii*. It does not grow at 45°C, differentiating it from *C. lusitaniae*.

4. ***In vitro* susceptibility testing** – Almost all clinical isolates are susceptible to amphotericin B, 5FC, and azoles such as fluconazole, itraconazole, ketoconazole, and voriconazole.
5. **Molecular tests** – Primers for large ribosomal subunit DNA sequences were used in PCR to differentiate *C. famata* from *C. guilliermondii*. The amplification of 340 bp of the large rDNA led to rapid and specific identification of *C. famata*. RAPD-PCR analysis was applied to identify *C. famata* in dairy product.

Comments: Five laboratories identified this isolate as *C. guilliermondii*. However, *C. famata* infrequently assimilates melezitose and raffinose (60%), while *C. guilliermondii* assimilates these two carbohydrates frequently (90%). Additionally, morphology on corn meal agar or Dalmau plate differentiates these two organisms. *C. famata*, when incubated for longer time on corn meal agar, produces rudimentary to well differentiated pseudohyphae, while *C. guilliermondii* produces few to moderate pseudohyphae. In the API 20C AUX yeast identification system, *C. famata* and *C. guilliermondii* are assigned the same biocode. Four laboratories reported this specimen as *Candida zeylanoides*, which does not ferment sucrose. *C. famata* does not grow at 45°C, differentiating it from *C. lusitaniae*.

Sequences alignment:

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 and ITS2 region of rDNA. The sequences are deposited in GenBank under the accession numbers AY282530 and AY282531, respectively.

```

1
CBS 789 (AF210326) TCCGTAGGTG AACCTGCGGA AGGATCATTA CAGTATTCTT TTTGCCAGCG
NYSDOH PT0103 (AY282530) TCCGTAGGTG AACCTGCGGA AGGATCATTA CAGTATTCTT TTTGCCAGCG

51
CTTAATTGCG CGGCGAAAAA ACCTTACACA CAGTGTTTTTT TGTATTATACA
CTTAATTGCG CGGCGAAAAA ACCTTACACA CAGTGTTTTTT TGTATTATACA

101
AGAACTTTTTG CTTTGGTCTG GACTAGAAAAT AGTTTGGGCC AGAGGTTTAC
AGAACTTTTTG CTTTGGTCTG GACTAGAAAAT AGTTTGGGCC AGAGGTTTAC

151
TGAACTAAAC TTCAATATTT ATATTGAATT GTTATTTATT TAATTGTCAA
TGAACTAAAC TTCAATATTT ATATTGAATT GTTATTTATT TAATTGTCAA

201
TTTGTTGATT AAATTCAAAA AATCTTCAAA ACTTTCAACA ACGGATCTCT
TTTGTTGATT AAATTCAAAA AATCTTCAAA ACTTTCAACA ACGGATCTCT

251
TGGTTCCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATATGAA
TGGTTCCTCGC ATCGATGAAG AACGCAGC

```

Alignment of primary sequences of the ITS1 regions of *C. famata* CBS 789 and PT specimen *C. famata* NYSDOH PT 0103. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1
CBS 789 (AF210326) ACGGATCTCT TGGTTCCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA
NYSDOH PT0103 (AY282531) GC ATCGATGAAG AACGCAGCGA AATGCGATAA

51
GTAATATGAA TTGCAGATTT TCGTGAATCA TCGAATCTTT GAACGCACAT
GTAATATGAA TTGCAGATTT TCGTGAATCA TCGAATCTTT GAACGCACAT

101
TGCGCCCTCT GGTATTCCAG AGGGCATGCC TGTTTGAGCG TCATTTCTCT
TGCGCCCTCT GGTATTCCAG AGGGCATGCC TGTTTGAGCG TCATTTCTCT

151
CTCAAACCTT CGGGTTTGGT ATTGAGTGAT ACTCTTAGTC GAACTAGGCC
CTCAAACCTT CGGGTTTGGT ATTGAGTGAT ACTCTTAGTC GAACTAGGCC

201
TTTGCTTGAA ATGTATTGGC ATGAGTGGTA CTGGATAGTG CTATATGACT
TTTGCTTGAA ATGTATTGGC ATGAGTGGTA CTGGATAGTG CTATATGACT

251
TTCAATGTAT TAGGTTTATC CAACTCGTTG AATAGTTTAA TGGTATATTT
TTCAATGTAT TAGGTTTATC CAACTCGTTG AATAGTTTAA TGGTATATTT

```

301

CTCGGTATTTC TAGGCTCGGC CTTACAATAT AACAAACAAG TTTGACCTCA
 CTCGGTATTTC TAGGCTCGGC CTTACAATAT AACAAACAAG TTTGACCTCA

350

351

AATCAGGTAG GATTACCCGC TGAACTTAAG CATATCAATA AGCGGAGGA
 AATCAGGTAG GATTACCCGC TGAACTTAAG CATATCAATA AGCGGAGGA

Alignment of primary sequences of the ITS2 regions of of *C. famata* CBS 789 and PT specimen *C. famata* NYSDOH PT0103. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

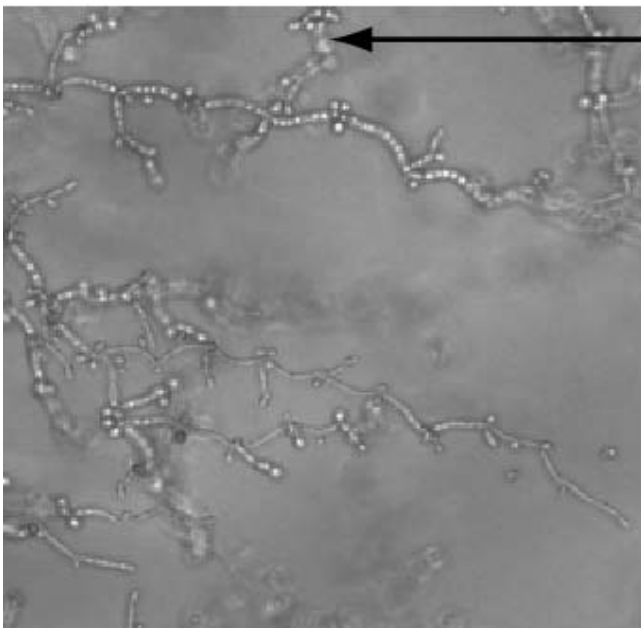
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Figure 23. Seven-day-old, white to yellowish, soft, smooth to slightly wrinkled colony of *Candida famata* on Sabouraud's dextrose agar.

A.



B.

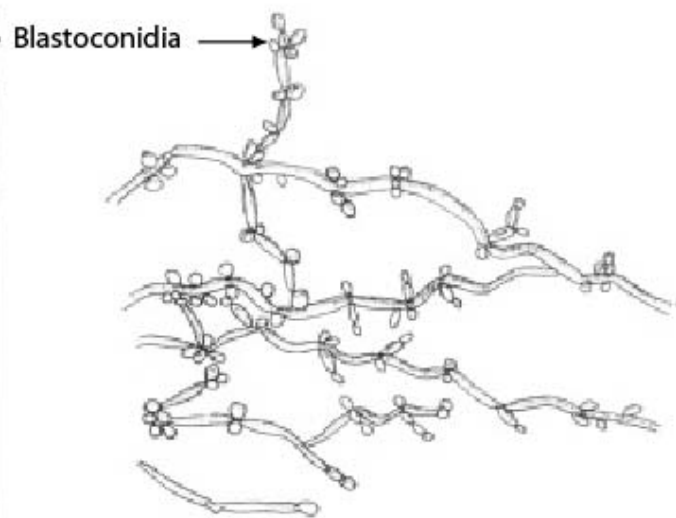


Figure 24. Microscopic morphology of *Candida famata* on corn meal agar with Tween 80 showing pseudohyphae with oval blastoconidia (A, 400 × magnification; B, line diagram not to scale).

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR YEASTS

Introduction: Documents of M27-A3 and M27-S3 published by Clinical Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards, NCCLS) is the current standard reference guide for antifungal susceptibility testing of pathogenic yeasts. FDA approved devices for antifungal susceptibility testing of yeasts includes Sensititre YeastOne Colorimetric Panel (Trek Diagnostic Systems Inc. Cleveland, OH) and Etest (AB BIODISK North America, Inc. Piscataway, NJ). The disk diffusion method approved by CLSI (M44-A) is another alternative for antifungal susceptibility testing of yeasts. There are 10 drugs in the antifungal susceptibility testing panel of NYSDOH Mycology Proficiency Test Program - amphotericin B, anidulafungin, caspofungin, flucytosine (5-FC), fluconazole,

itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. The participating laboratories are allowed to select any number of antifungal drug(s) from the test panel for testing based upon usual practices in their facilities.

Materials & Results: *Candida parapsilosis* (S-1) was the analyte in the September 29, 2010 antifungal proficiency testing event. Thirty laboratories participated in this event. The S-1 isolate was validated by all the participating laboratories. The acceptable results for antifungal susceptibility testings were based on consensus MIC values or interpretation per NCCLS/CLSI guidelines or other publications (Table 1).

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

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

* Adapted from CLSI draft document M27-S3 (December 2007)

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

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

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

⁴ **For Posaconazole, apply the voriconazole MIC interpretation as surrogate breakpoints**

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

Summary:

Table 2. Summary of Laboratory Performance, Antifungal Susceptibility Testing for Yeast Only, September 2010 PT Event

| Acceptable Responses/Total # Laboratories (%) | |
|--|-------------|
| S- 1: <i>Candida parapsilosis</i> | |
| Amphotericin B | 24/24 (100) |
| Anidulafungin | 15/15 (100) |
| Caspofungin | 20/20 (100) |
| Flucytosine (5-FC) | 25/25 (100) |
| Fluconazole | 30/30 (100) |
| Itraconazole | 28/28 (100) |
| Ketoconazole | 6/6 (100) |
| Micafungin | 15/15 (100) |
| Posaconazole | 16/16 (100) |
| Voriconazole | 23/23 (100) |

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

S-1: *Candida parapsilosis*

| Drugs (µg/ml) | Total # of labs | 0.004 | 0.008 | 0.015 | 0.03 | 0.047 | 0.06 | 0.094 | 0.12 | 0.19 | 0.25 | 0.38 | 0.5 | 0.75 | 1 | 2 |
|--------------------|-----------------|-------|-------|-------|------|-------|------|-------|------|------|------|------|-----|------|----|---|
| Amphotericin B | 24 | | | | | | 1 | | | | 8 | | 14 | 1 | | |
| Anidulafungin | 15 | | | | | | | | | | | | 4 | | 10 | 1 |
| Caspofungin | 20 | | | | | | | | 1 | | 4 | | 12 | | 3 | |
| Flucytosine (5-FC) | 25 | | | | 2 | | 7 | 1 | 14 | | | | | | 1 | |
| Fluconazole | 30 | | | | | | | | 1 | 1 | 1 | 1 | 20 | | 3 | 3 |
| Itraconazole | 28 | | | 1 | 1 | 1 | 5 | 1 | 13 | | 6 | | | | | |
| Ketoconazole | 6 | | 2 | 1 | 1 | | 2 | | | | | | | | | |
| Micafungin | 15 | | | | | | | | | | | | | | 9 | 6 |
| Posaconazole | 16 | | | 1 | 4 | | 10 | | 1 | | | | | | | |
| Voriconazole | 23 | 2 | 17 | 3 | 1 | | | | | | | | | | | |

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

S-1: *Candida parapsilosis*

| Antifungal Agent | Total # of labs | Susceptible | Susceptible -dose dependent | Intermediate | Resistant | Non-susceptible | No interpretation |
|--------------------|-----------------|-------------|-----------------------------|--------------|-----------|-----------------|-------------------|
| Amphotericin B | 24 | 13 | | | | | 11 |
| Anidulafungin | 15 | 15 | | | | | |
| Caspofungin | 20 | 20 | | | | | |
| Flucytosine (5-FC) | 25 | 25 | | | | | |
| Fluconazole | 30 | 30 | | | | | |
| Itraconazole | 28 | 23 | 5 | | | | |
| Ketoconazole | 6 | 2 | | | | | 4 |
| Micafungin | 15 | 15 | | | | | 4 |
| Posaconazole | 16 | 12 | | | | | 4 |
| Voriconazole | 23 | 23 | | | | | |

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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 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, 2010 direct detection antigen testing event. Two positive serum samples for cryptococcal antigen were included. The titers were 1:128 and 1:256 ~ 1:512 for Cn-Ag-3 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 positive result for specimen Cn-Ag-2. One laboratory reported negative and two laboratories reported the titer lower than the acceptable titer range for specimen Cn-Ag-4. One laboratory reported the titer lower than the acceptable titer range for specimen Cn-Ag-5. The supplementary information on quantitative assays on *Cryptococcus neoformans* antigen test is summarized in Table 5.

Further Reading:

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Table 5. Summary of quantitative assay

The number of laboratories that reported titers is listed for positive test samples Cn-Ag-4, and Cn-Ag-5.

| Method | Sample | Cn-Ag-4 Titers | | | | | | | | |
|-------------------------|--------------------------|----------------|----|----|----|----|-----|-----|-----|-----|
| | | 8 | 16 | 32 | 64 | 80 | 100 | 128 | 256 | 512 |
| Total # of laboratories | | 8 | 16 | 32 | 64 | 80 | 100 | 128 | 256 | 512 |
| EIA | | | | | | | | | | |
| | (Meridien Diagnostic) 2 | | | 1 | | | | 1 | | |
| Latex Agglutination | | | | | | | | | | |
| | (Immuno-Mycologics) 4 | | | | 1 | | | 2 | 1 | |
| | (Meridien Diagnostic) 41 | 1 | | 4 | 6 | 1 | 1 | 19 | 5 | 4 |
| | (Remel) 8 | | 1 | | 2 | | | | 4 | |
| | (Wampole) 10 | | | | 2 | | | 2 | 6 | |

| Method | Sample | Cn-Ag-5 Titers | | | | | | | |
|-------------------------|--------------------------|----------------|----|-----|-----|-----|-----|------|------|
| | | 16 | 64 | 128 | 200 | 256 | 512 | 1024 | 2048 |
| Total # of laboratories | | 16 | 64 | 128 | 200 | 256 | 512 | 1024 | 2048 |
| EIA | | | | | | | | | |
| | (Meridien Diagnostic) 2 | | 1 | | | 1 | | | |
| Latex Agglutination | | | | | | | | | |
| | (Immuno-Mycologics) 4 | | | | | 4 | | | |
| | (Meridien Diagnostic) 41 | 1 | 1 | 6 | 2 | 9 | 17 | 5 | |
| | (Remel) 8 | | | 2 | | 2 | 2 | 2 | |
| | (Wampole) 10 | | | 1 | | 3 | 1 | 4 | 1 |

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