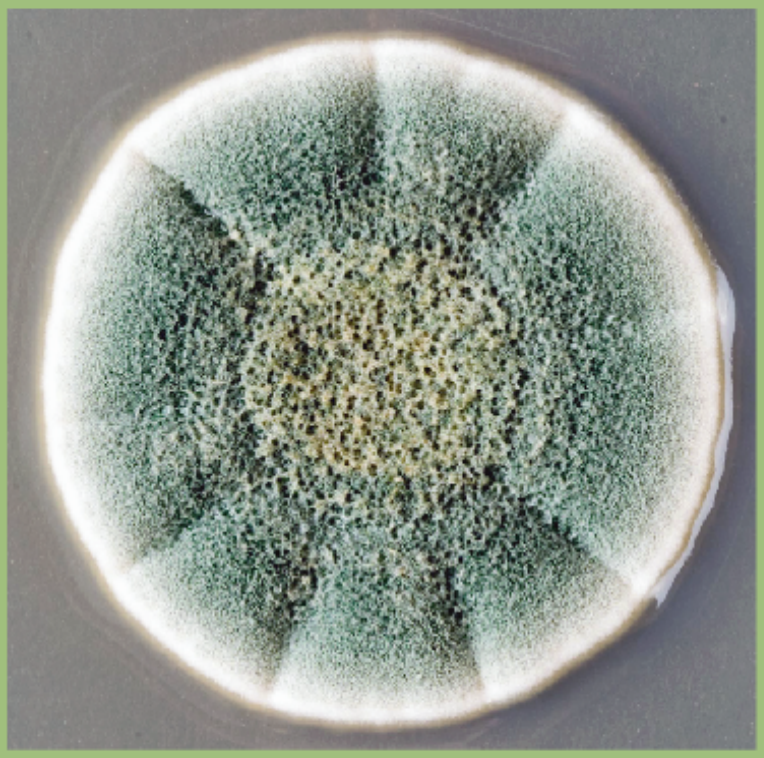
Mycology Proficiency Testing Program
January 2011 Test Event
Critique



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Schedule of 2011 Mycology PT Mailouts*

Mycology Identification	Mycology Identification POSTMARK DEADLINES
January 26, 2011	March 11, 2011
May 25, 2011	June 17, 2011
September 27, 2011	November 14, 2011

Mycology Identification - Yeast Only	Mycology Identification - Yeast Only POSTMARK DEADLINES
January 26, 2011	February 18, 2011
May 25, 2011	June 17, 2011
September 27, 2011	October 24, 2011

Mycology Susceptibility	Mycology Susceptibility POSTMARK DEADLINES
January 26, 2011	February 18, 2011
May 25, 2011	June 17, 2011
September 27, 2011	October 24, 2011

Mycology Direct Detection	Mycology Direct Detection POSTMARK DEALINES
January 26, 2011	February 11, 2011
September 27, 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	*Exophiala* spp.	*Exophiala* spp.	*Exophiala dermatitidis* *Wangiella dermatitidis*	67/69 (97)
M-2	*Aspergillus versicolor*	*Aspergillus versicolor*		66/69 (96)
M-3	*Chaetomium* spp.	Not validated	*Chaetomium globosum*	16/69 (23)
M-4	*Trichophyton rubrum*	*Trichophyton rubrum*		65/69 (94)
M-5	*Paecilomyces* spp.	*Paecilomyces* spp.	*Paecilomyces variotti*	68/69 (99)

Mycology – Yeast Only

	Specimen Key	Validated Specimen	Other Acceptable Answers	Correct Responses / Total # Laboratories (%)
Y-1	*Trichosporon* spp.	*Trichosporon* spp.	*Trichosporon asahii*	52/52 (100)
Y-2	*Cryptococcus albidus*	*Cryptococcus albidus*		52/52 (100)
Y-3	*Rhodotorula mucilaginosa*	*Rhodotorula mucilaginosa*		49/52 (94)
Y-4	*Rhodotorula minuta*	*Rhodotorula minuta*		52/52 (100)
Y-5	*Cryptococcus laurentii*	*Cryptococcus laurentii*		45/52 (87)

Mycology – Antifungal Susceptibility Testing for Yeasts (S-1: *Candida tropicalis* M2698)

Drugs	Acceptable MIC (μg/ml) Range	Acceptable Interpretation	Acceptable Responses/Total # Laboratories (%)
Amphotericin B	0.12 – 2	Susceptible / No interpretation	24/24 (100)
Anidulafungin	0.015 – 0.25	Susceptible	16/16 (100)
Caspofungin	0.015 – 0.25	Susceptible	21/21 (100)
Flucytosine (5-FC)	0.015 – 0.25	Susceptible	26/26 (100)
Fluconazole	0.25 – 4	Susceptible	30/30 (100)
Itraconazole	0.06 – 1	Susceptible / Susceptible-dose dependent	29/29 (100)
Ketoconzole	0.03 – 0.5	Susceptible / No interpretation	6/6 (100)
Micafungin	0.008 – 0.12	Susceptible	16/16 (100)
Posaconazole	0.03 – 0.5	Susceptible / No interpretation	17/17 (100)
Voriconazole	0.015 – 0.25	Susceptible	23/23 (100)

Mycology – Direct detection (*Cryptococcus* Antigen Test)

	Specimen Key (Titer)	Validated Specimen	Correct Responses / Total # Laboratories (%)	
			Qualitative	Quantitative
Cn-Ag-1	Positive (1:256)	Positive (1:256)	66/67 (99)	62/62 (100)
Cn-Ag-2	Negative	Negative	66/67 (99)	NA
Cn-Ag-3	Negative	Negative	66/67 (99)	NA
Cn-Ag-4	Negative	Negative	67/67 (100)	NA
Cn-Ag-5	Positive (1:128)	Positive (1:128)	67/67 (100)	60/62 (97)

TEST STATISTICS

	General	Yeast Only	Antifungal Susceptibility Testing for Yeasts	Direct Detection
Number of participating laboratories	69	53	30	67
Number of referee laboratories	10	10	30	10
Number of laboratories responding by deadline	69	53	30	66
Number of laboratories responding after deadline	0	0	0	1
Number of laboratories not responding	0	0	0	0
Number of laboratories successfully completing this test	66	52	30	65
Number of laboratories unsuccessfully completing this test	3	1	0	2

Number of Laboratories Using Commercial Yeast Identification System*

API 20C AUX	46
AMS Vitek	4
Vitek2 system	23
Remel Uni-Yeast-Tek	6
Microscan	1

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

Latex Agglutination method	67
Immuno-Mycologics	9
Inverness Medical	1
Meridien Diagnostic	48
Remel	9

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

MOLD DESCRIPTIONS

M-1 *Exophiala* spp.

Source: Blood / Skin

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	67
Other Acceptable Answers:	
Exophiala dermatitidis	17
Wangiella dermatitidis	13
Laboratories with incorrect ID:	2
(*Cladophialophora* sp.)	(1)
(Not identified)	(1)
Outcome:	Validated

Clinical Significance: *Exophiala dermatitidis* is an occasional causative agent of phaeohyphomycosis, which consists of a group of mycotic infections characterized by the presence of dematiaceous (dark-walled) septate hyphae and sometimes yeast or a combination of both in tissue. It is a neurotropic fungus, and central nervous system infections have been reported. It may also cause keratitis, otitis, pneumonia, and endocarditis. Disseminated infections may develop particularly in immunocompromised patients.

Ecology: *E. dermatitidis* is cosmopolitan fungus that inhabits the soil and plant material.

Laboratory Diagnosis:

1. Culture – *E. dermatitidis* grew slowly. On Sabouraud's dextrose agar after 5 days at 25°C, the colony was initially moist, yeast-like, and shiny. The color was black in the front and the reverse (Figure 1). Aerial hyphae developed after 3 to 4 weeks of incubation.
2. Microscopic morphology – Lactophenol cotton blue mount showed septate, brown hyphae, conidiophores, phialides, and yeast cells. When a young culture was examined microscopically, the predominant structure was phaeoid (brown), budding, yeast-like cells. As the colony grew older, hyphae and phialides were produced from these cells. Phialides were brown, and flask-shaped to cylindrical. They did not have collarettes. Conidia were brown, one-celled, and round to oval in shape. They were found in clusters at the apices of the phialides and down the sides of the conidiophores (Figure 2).
3. Differentiation from other *Exophiala* spp. – *E. dermatitidis* could grow at temperatures as high as 42°C, produce phialides but not annelides, and does not assimilate potassium nitrate.
4. *In vitro* susceptibility testing – *E. dermatitidis* is susceptible amphotericin B, itraconazole, terbinafine, and voriconazole.
5. Molecular tests – Nucleotide sequence of the large subunit (26S) ribosomal DNA D1/D2 domain of *E. dermatitidis* was used for differentiation of other pathogenic dematiaceous fungi and related taxa.

Comments: In this event, *Exophiala dermatitidis* was used. Speciation of this specimen was not required in this event since members of *Exophiala* genus are very similar to each other and is difficult to distinguish them based upon morphology. Their clinical

significance is similar as well. *E. dermatitidis* can be distinguished from *Cladophialophora* sp. by their conidiogenesis.

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 *Exophiala dermatitidis* AFTOL-ID 668 (Genebank accession number: DQ826738) for ITS1 and ITS2 regions.

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Alignment of primary sequence of the ITS1 and ITS2 regions of *Exophiala dermatitidis* AFTOL-ID 668 and PT specimen *Exophiala dermatitidis* M1998.

Further Reading:

1. Abliz, P., Fukushima, K., Takizawa, K., and Nishimura, K. 2004. Identification of pathogenic dematiaceous fungi and related taxa based on large subunit ribosomal DNA

D1/D2 domain sequence analysis. *FEMS Immunol Med Microbiol.* 40: 41-9.
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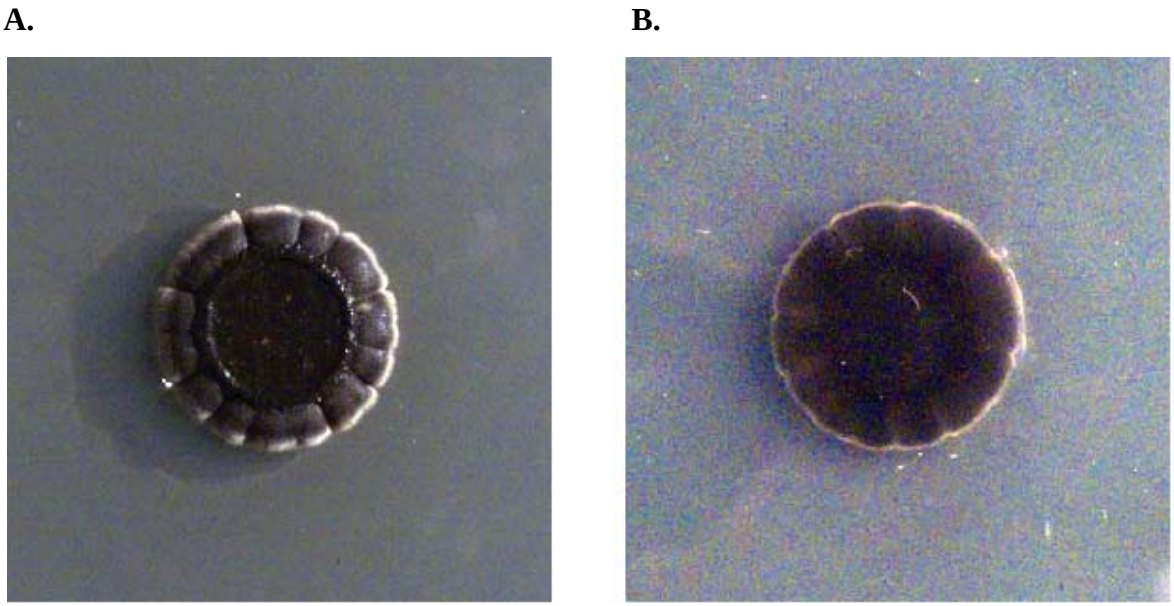

Figure 1. (A) Five-day-old, yeast like black colony of *Exophiala dermatitidis* on Sabouraud's dextrose agar. (B) Black reverse of five-day-old colony of *Exophiala dermatitidis* on Sabouraud's dextrose agar.

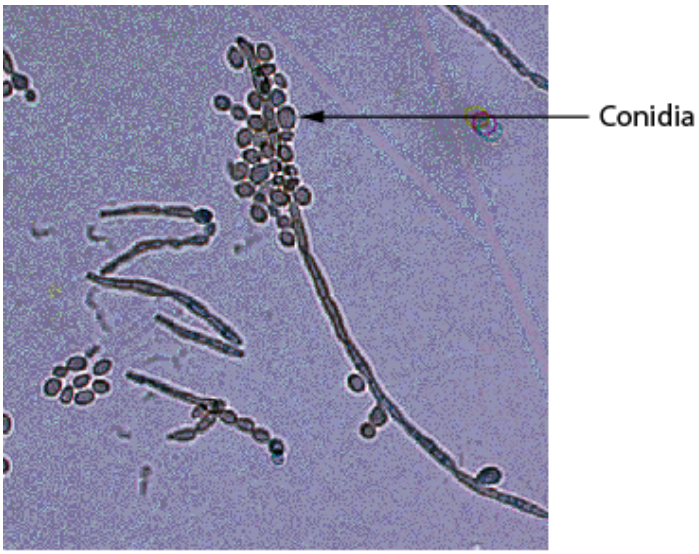

Figure 2. Microscopic morphology of *Exophiala dermatitidis* showing septated hyphae, conidiaphore and conidia (400× magnification).

M-2 *Aspergillus versicolor*

Source: Sputum / Finger

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	66
Laboratories with incorrect ID:	3
(*Aspergillus flavus*)	(1)
(*Penicillium* sp.)	(1)
(*Scopulariopsis* sp.)	(1)
Outcome:	Validated

Clinical Significance: *Aspergillus versicolor* is a major human allergen and a less common cause of aspergillosis. The fungus is also reported as etiologic agent of onychomycosis, otomycosis, osteomyelitis. Infection of the external auditory canal was also reported.

Ecology: *A. versicolor* is predominantly found in warmer climates. It is generally isolated from soil and plant materials. It is also found on food products especially cheese, and in air, and in house dust.

Laboratory Diagnosis:

1. Culture – *Aspergillus versicolor* grew moderately fast on Sabouraud's dextrose agar. After eight days at 25°C, *A. versicolor* colony was olive-green with clear to wine-red exudate on the surface (Figure 3A). The reverse was yellowish to brownish orange (Figure 3B).
2. Microscopic morphology – Lactophenol cotton blue mount showed radiate conidial head. Phialides were biseriate. Conidia were round, smooth, or rough (Figure 4A and 4B). Reduced conidiogenous structures were often seen (Figure 4C). Hülle cells were occasionally seen.
3. Differentiation from other *Aspergillus* species – *A. versicolor* usually produces round Hülle cells similarly to *A. nidulans,* but it has no cleistothecia, which distinguished it from *A. nidulans* and *A. glaucus*. *A. versicolor* has biseriate conidial heads, differentiating it from *A. flavus,* which has both uniseriate and biseriate conidial heads. The colony surface of *A. versicolor* is pale-green, compared to brown for *A. terreus* and black for *A. niger*. Please refer to Table 1 for more details.
4. *In vitro* susceptibility testing – *A. versicolor* is resistant to griseofulvin, fluconazole, and amphotericin B. MICs for itraconazole and ketoconazole are variable, but within a range of 0.50 - 4.0 μg/ml; in contrast, MICs for terbinafine are very low (<0.1 μg/ml).
5. Molecular tests – Nested PCR targeting of ribosomal DNA internal transcribed spacer regions was used for the identification of *Aspergillus versicolor* and related *Aspergillus* species. Reverse-hybridization line probe assay (LiPA) combined with PCR amplification was reported to detect and identify clinically significant fungal pathogens, including *A. versicolor* and related species.

Comments: One laboratory each reported this specimen as *A. flavus, Penicillium* sp., and *Scopulariopsis* sp. *A. flavus* is yellow-green colony. *A. versicolor* is olive-green colony and not only have biseriate phialides, but also produces reduced conidiogenous structures to be used to distinguish it from other species of *Aspergillus*. *A. versicolor* has reduced conidiogenous structures like *Penicillium* brush-like conidial head, but it also produces typical

radiate conidial head to distinguish it from *Penicillium* spp. and *Scopulariopsis* spp.

Sequence 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 versicolor* strain CBS 583.65 (Genebank accession number: EU076360) for ITS1 and ITS2 regions.

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Alignment of primary sequence of the ITS1 and ITS2 regions of *Aspergillus versicolor* strain CBS 583.65 and PT specimen *Aspergillus versicolor* M344 .

Further Reading:

1. Benndorf D, Müller A, Bock K, Manuwald O, Herbarth O, von Bergen M. 2008. Identification of spore allergens from the indoor mould *Aspergillus versicolor*. *Allergy.* 63: 454-460.
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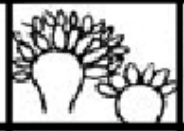
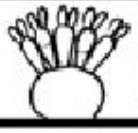
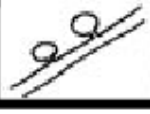
Table 1. Scheme for differentiation of *Aspergilli* most commonly involved in human diseases.

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

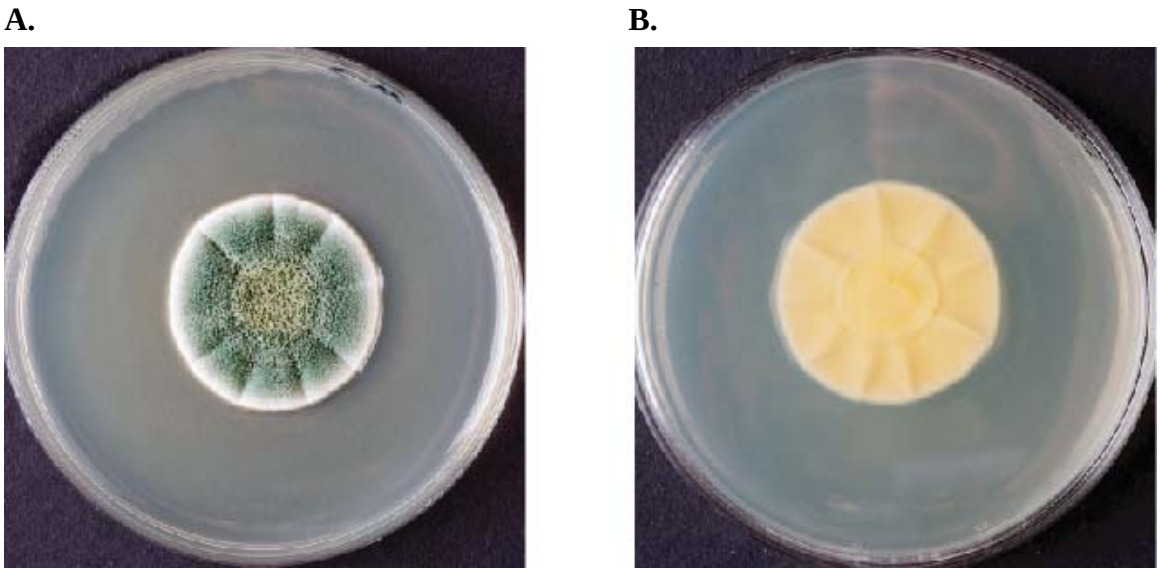


Figure 3. (A) Eight-day-old, olive-green colony of *Aspergillus versicolor* on Sabouraud's dextrose agar. (B) The reverse of eight-day-old *Aspergillus versicolor* colony on Sabouraud's dextrose agar.

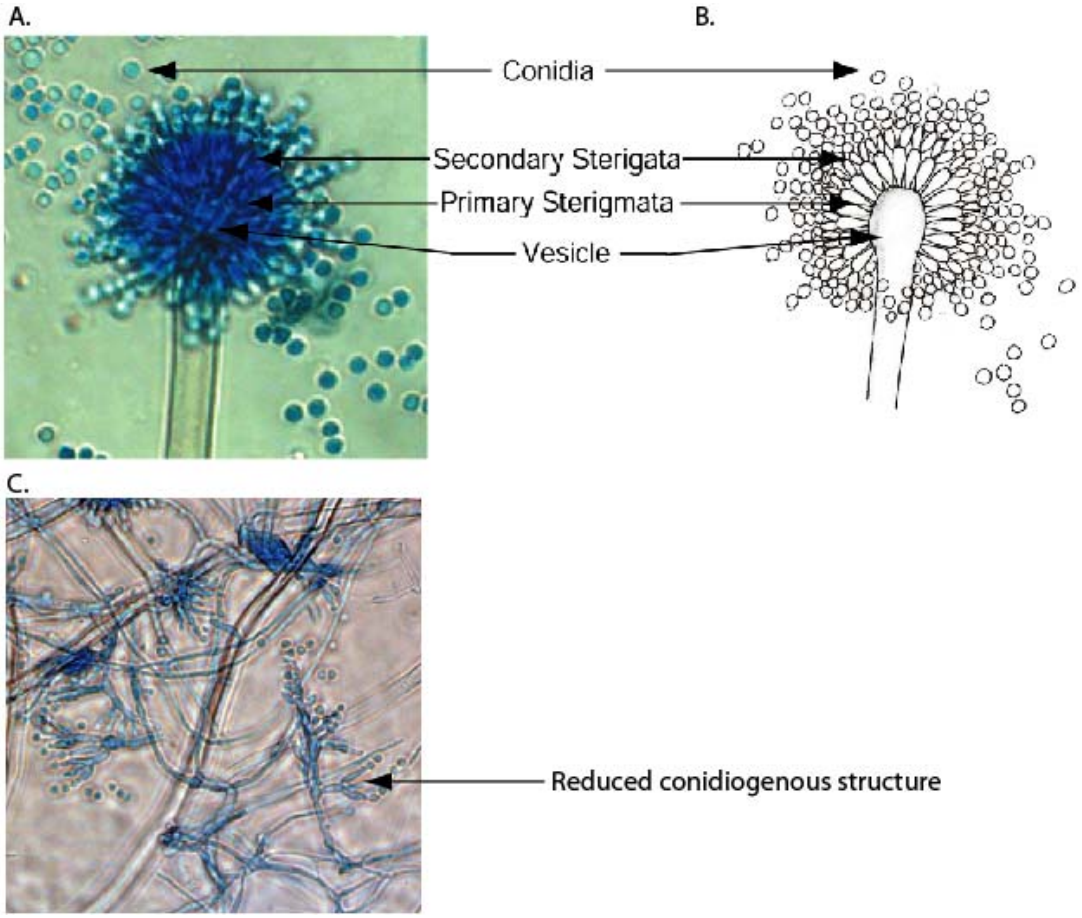


Figure 4. Microscopic morphology of *Aspergillus versicolor* showing typical radiate conidial heads with biseriate phialides and round, smooth, or rough conidia (A, 400× magnification; B, line drqwing not to scale; C, reduced conidiogenous structure).

M-3 *Chaetomium* spp.

Source: Foot

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	2
Laboratories with correct ID:	16
Other Acceptable Answers:	
Chaetomium globosum	1
Laboratories with incorrect ID:	53
(*Acremonium* sp.)	(19)
(*Scytalidium* sp.)	(16)
(*Trichoderma* sp.)	(8)
(*Chrysosporium* sp.)	(3)
(*Arthrographis* sp.)	(1)
(*Beauveria* sp.)	(1)
(*Malbranchea* sp.)	(1)
(*Phialemonium* sp.)	(1)
(*Scedosporium* sp.)	(1)
(*Scytalidium hyalinum*)	(1)
(*Trichophyton violaceum*)	(1)
Outcome:	Not validated

Clinical Significance: *Chaetomium* spp. is commonly encountered in clinical laboratories as a contaminant. It is occasionally reported as an agent in phaeohyphomycosis.

Ecology: *Chaetomium* sp. is mainly found in soil and on plant debris.

Laboratory Diagnosis:

1. Culture – *Chaetomium* sp. grew rapidly. On Sabouraud's dextrose agar, after 7 days at 25°C, the colony was white to gray, yellowish, wooly surface (Figure 5A), and pale yellow on reverse (Figure 5B).
2. Microscopic morphology – Lactophenol cotton blue mount showed round, oval, or flask-shaped perithecia (best seen on potato dextrose agar) with long, brown setae. Ascospores, oval to lemon shaped, emerged from the ostiole (opening) of the perithecium (Figure 6).
3. Differentiation from other molds – *Chaetomium* sp. is differentiated from other molds by its very typical perithecium, which is a large round or pear-shaped structure with a small rounded opening called ostiole (which differentiates it from cleistothecium) and containing asci and ascospores. The ascospores are unicellular and commonly lemon-shaped.
4. *In vitro* susceptibility testing – *Chaetomium* sp. is susceptible to amphotericin B, itraconazole, ketoconazole, ravuconazole, voriconazole, and albaconazole, but resistant to micafungin, fluconazole and flucytosine in general.
5. Molecular tests – Oligonucleotide fingerprinting of rRNA genes (OFRG) was reported to identify *Alternaria, Ascobolus, Chaetomium, Cryptococcus,* and *Rhizoctonia* clades.

Comments: This sample was not validated in this test event mainly because it did not produce perithecia, which is an important characteristics for the identification of *Chaetomium* spp.

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 *Chaetomium globosum* isolate wxm152 (Genebank accession number: HM061327) for ITS1 and ITS2 regions.

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Alignment of primary sequence of the ITS1 and ITS2 regions of *Chaetomium globosum* isolate wxm152 and PT specimen *Chaetomium* sp. M2464.

Further Reading:

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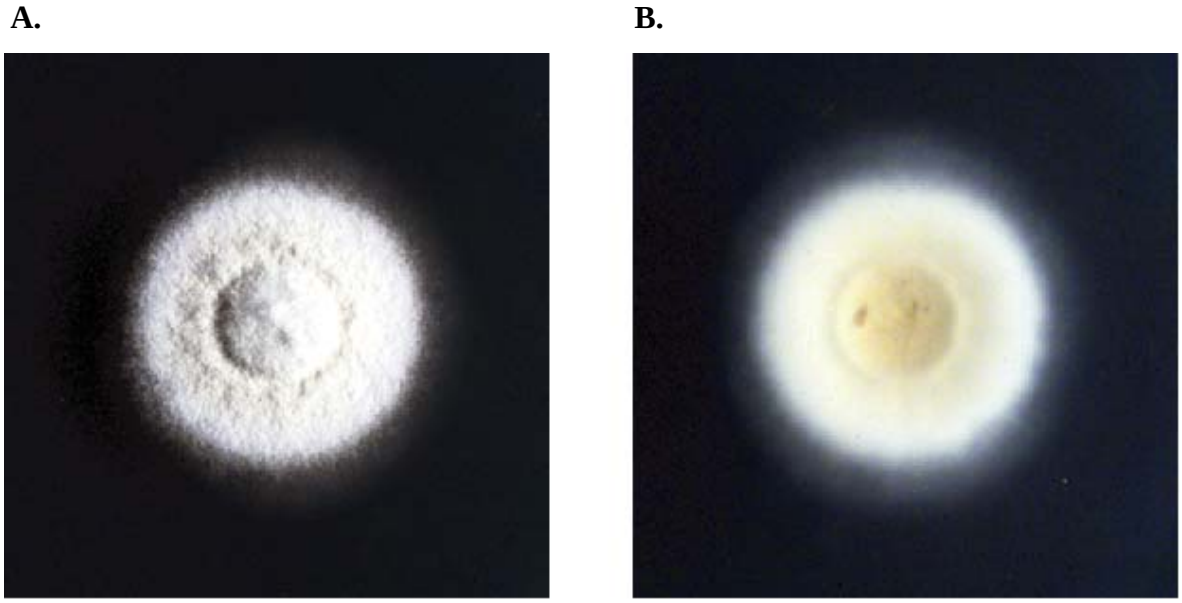

Figure 5. (A) Five-day-old, white to gray, yellowish, wooly colony of *Chaetomium* sp. on Sabouraud's dextrose agar. (B) The reverse of five-day-old *Chaetomium* sp. colony on Sabouraud's dextrose agar appeared pale yellow.

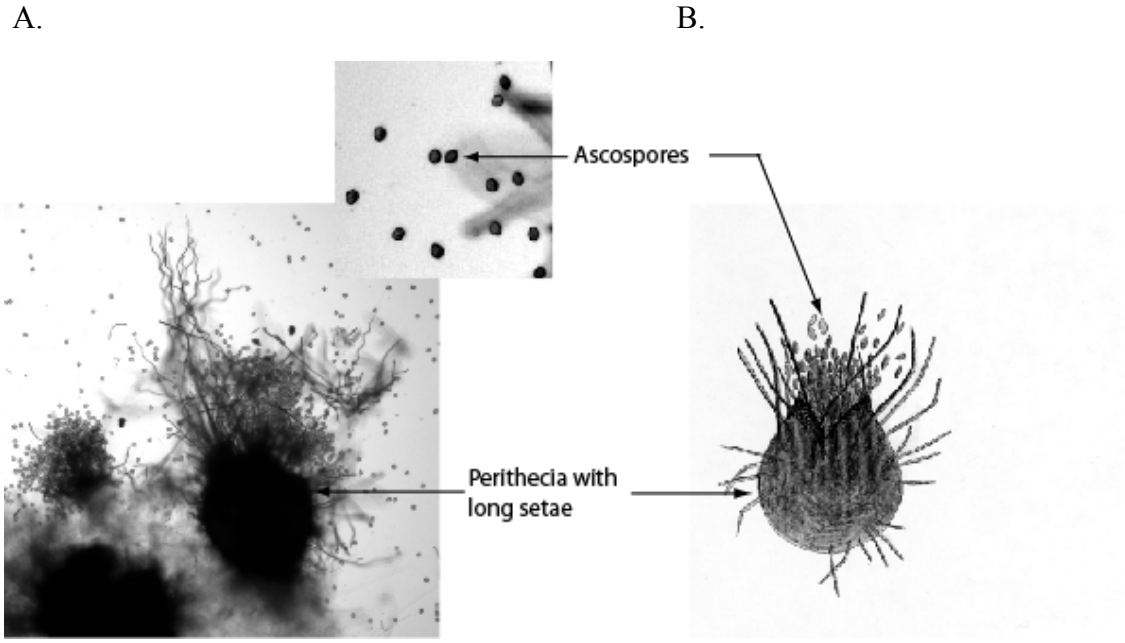

Figure 6. Microscopic morphology of *Chaetomium* sp. showing round, oval, or flask-shaped perithecia, oval to lemon shaped ascospores (A. 400× magnification; B. line drawing not to scale).

M-4 *Trichophton rubrum*

Source: Nail / Wound

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	65
Laboratories with incorrect ID:	4
(*Trichophyton tonsurans*)	(3)
(*Trichophyton* sp.)	(1)
Outcome:	Validated

Clinical Significance: A frequent causal agent of infections of the feet, toes, groin, finger nails, and skin. It rarely causes infection of scalp or hair.

Ecology: Cosmopolitan, anthropophilic.

Laboratory Diagnosis:

1. Culture – *T. rubrum* grew slowly on Sabouraud's dextrose agar. After 10 days, colonies were fluffy to powdery, white to buff, with wine-red to brown in color on reverse (Figure 7).
2. Microscopic morphology – Lactophenol cotton blue mounts showed hyaline septate hyphae, microconidia were solitary, tear-dropped and macroconidia were rare, multi-septate and pencil-shaped (Figure 8). Arthroconidia were rarely reported in the literature.
3. Differentiation from other dermatophytes – *T. rubrum* can be differentiated from *T. mentagrophytes* by tear drop-shaped solitary microconidia, no urease activity, no hair perforation, and no specific growth requirements. It can be differentiated from *T. terrestre* by good growth at 37°C, and production of red reverse pigment on potato-dextrose agar or cornmeal glucose agar, *T. rubrum* produces red pigment on reverse. Three new species, *T. fischeri, T. raubitschekii, and T. kanei* were described to be closely related to *T. rubrum*. Two of these species, *T. raubitschekii* and *T. kanei,* have been isolated from skin lesions. *T. raubitschekii* and *T. kanei* produce urease; *T. kanei* lacks microconidia, while *T. raubitschekii* produces variably shaped microconidia. *T. fischeri* resembles *T. rubrum* closely, but is non-pathogenic for humans.
4. *In vitro* susceptibility testing – *T. rubrum* are highly susceptible to terbinafine and variably to azoles.
5. Molecular tests – A species–specific DNA probe using highly variable internal transcribed spacer 2 region of the ribosomal DNA (ITS2) was developed to detect *T. rubrum* in culture and from clinical samples. Species identification of dermatophytes was done based on DNA sequences of nuclear ribosomal internal transcribed spacer regions (ITS), and of the 5.8S ribosomal DNA region, and comparison with DNA sequence database. Identification and differentiation of *T. rubrum* clinical isolates were reported using PCR-RFLP and RAPD.

Comments: Three laboratories reported this specimen as *T. tonsurans*. *T. rubrum* can be differentiated from *T. tonsurans* by tear drop-shaped solitary microconidia, and absence of urease activity, and specific growth requirements.

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 *Trichophyton rubrum* strain ATCC MYA-4607 (Genebank accession number: GU291266) for ITS1 and ITS2 regions.

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Alignment of primary sequence of the ITS1 and ITS2 regions of *Trichophyton rubrum* strain ATCC MYA-4607 and PT specimen *Trichophyton rubrum* M1704.

Further Reading:

1. Baeza, L.C., Matsumoto, M.T., Almeida, A.M., and Mendes-Giannini, M.J. 2006. Strain differentiation of *Trichophyton rubrum* by randomly amplified polymorphic DNA and analysis of rDNA nontranscribed spacer. *J Med Microbiol.* 55: 429-436.
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3. Cetinkaya, Z., Kiraz, N., Karaca, S., Kulac, M., Ciftci, I.H., Aktepe, O.C., Altindis, M., Kiyildi, N., and Piyade, M. 2005. Antifungal susceptibilities of dermatophytic agents isolated from clinical specimens. *Eur J Dermatol*. 15: 258-261.
4. Hryncewicz-Gwóźdź A, Jagielski T, Dobrowolska A, Szepietowski JC, Baran E. 2011. Identification and differentiation of *Trichophyton rubrum* clinical isolates using PCR-RFLP and RAPD methods. *Eur J Clin Microbiol Infect Dis*. [Epub ahead of print]
5. Khan MS, Ahmad I. 2011. Antifungal activity of essential oils and their synergy with fluconazole against drug-resistant strains of *Aspergillus fumigatus* and *Trichophyton rubrum*. *Appl Microbiol Biotechnol*. [Epub ahead of print]
6. Kobayashi, M., Ishida, E., Yasuda, H., Yamamoto, O., and Tokura, Y. 2006. Tinea profunda cysticum caused by *Trichophyton rubrum*. *J Am Acad Dermatol.* 54(2 Suppl): S11-3.
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10. Sachdeva S, Gupta S, Prasher P, Aggarwal K, Jain VK, Gupta S. 2010. *Trichophyton rubrum* onychomycosis in a 10-week-old infant. *Int J Dermatol*. 49: 108-109.
11. Smijs TG, Pavel S. 2011. The susceptibility of dermatophytes to photodynamic treatment with special focus on *Trichophyton rubrum*. *Photochem Photobiol*. 87: 2-13.

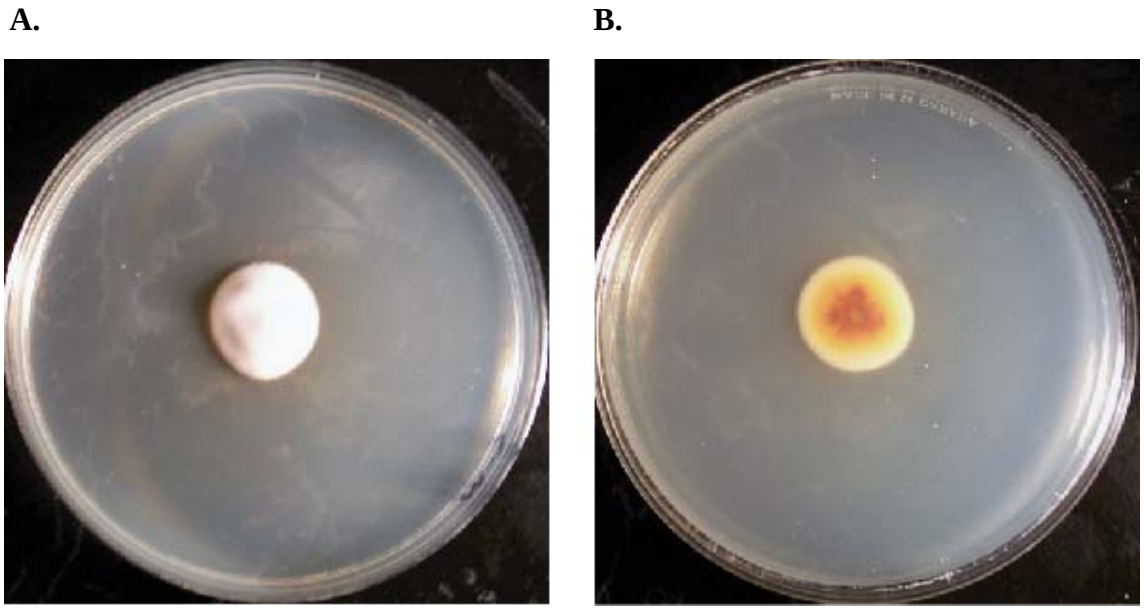

Figure 7. (A) Ten-day-old, buff, fluffy colony of *Trichophyton rubrum* on Sabouraud's dextrose agar. (B) The reverse side of ten-day-old *T. rubrum* colony on Sabouraud's dextrose agar.

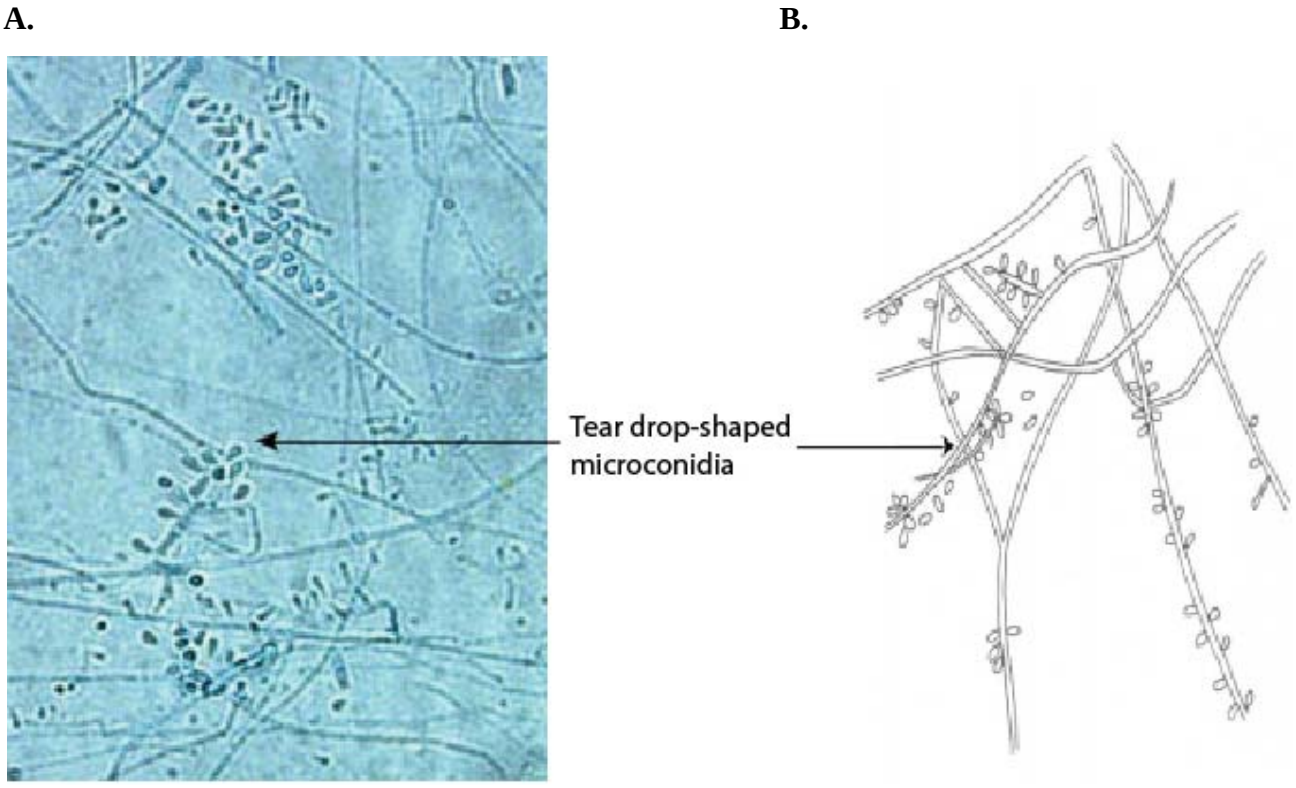

Figure 8. Microscopic morphology of *Trichophyton rubrum* showing tear drop-shaped microconidia, solitary along the hyphae (A. 200× magnification; B. line drawing not to scale).

M-5 *Paecilomyces* spp.

Source: Scalp / Bronchial wash

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	68
Other Acceptable Answers:	
Paecilomyces variotii	8
Laboratories with incorrect ID:	1
(*Cladophialophora* sp.)	(1)
Outcome:	Validated

Clinical Significance: *Paecilomyces variotii* is not a common pathogen in humans. Ocular infections including keratitis, endophthalmitis, and corneal ulcers have been reported.

Ecology: *Paecilomyces variotii* is found in soil and decaying plant materials worldwide.

Laboratory Diagnosis:

1. Culture – *Paecilomyces variotii* grew moderately fast. On Sabouraud's dextrose agar, after 7 days at 25°C, the colony showed yellowish brown to tan color and powdery surface (Figure 9A). Reverse appeared yellowish to tan (Figure 9B).
2. Microscopic morphology – Lactophenol cotton blue mount showed hyaline septate hyphae with simple, or irregular or verticillately branched conidiophores. Oval conidia form in divergent chains (Figure 10).
3. Differentiation from other molds – *Paecilomyces* sp. is distinguished from *Penicillium* and *Scopulariopsis* spp. by its thin phialides with elongation at the tips and its colony color, initially buff, becoming tan and eventually yellowish-brown. *Paecilomyces* sp. is distinguished from *Arthrographis* sp. by not forming arthroconidia.
4. *In vitro* susceptibility testing – In general, *Paecilomyces variotii* is susceptible to amphotericin B, miconazole, and 5FC but resistant to fluconazole, voriconazole, and ravuconazole.
5. Molecular tests – Molecular identification by sequencing of internal transcribed spacer (ITS) region is available.

Comments: One participating laboratory reported this specimen as *Cladophialophora* species. Phialides of *Paecilomyces* are swollen at the base, taper towards their apices and are organized slighly apart from each other. Conidiophores are absent or inconspicuous in *Cladophialophora* spp.

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 *Paecilomyces variotii* strain NRRL 1115 (Genebank accession number: AF033395) for ITS1 and ITS2 regions.

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Alignment of primary sequence of the ITS1 and ITS2 regions of *Paecilomyces variotii* strain NRRL 1115 and PT specimen *Paecilomyces variotii* M2723.

Further Reading:

1. Anita KB, Fernandez V, Rao R. 2010. Fungal endophthalmitis caused by *Paecilomyces variotii* in an immunocompetent patient following intraocular lens implantation. *Indian J Med Microbiol*. 28: 253-254.
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A. **B.**

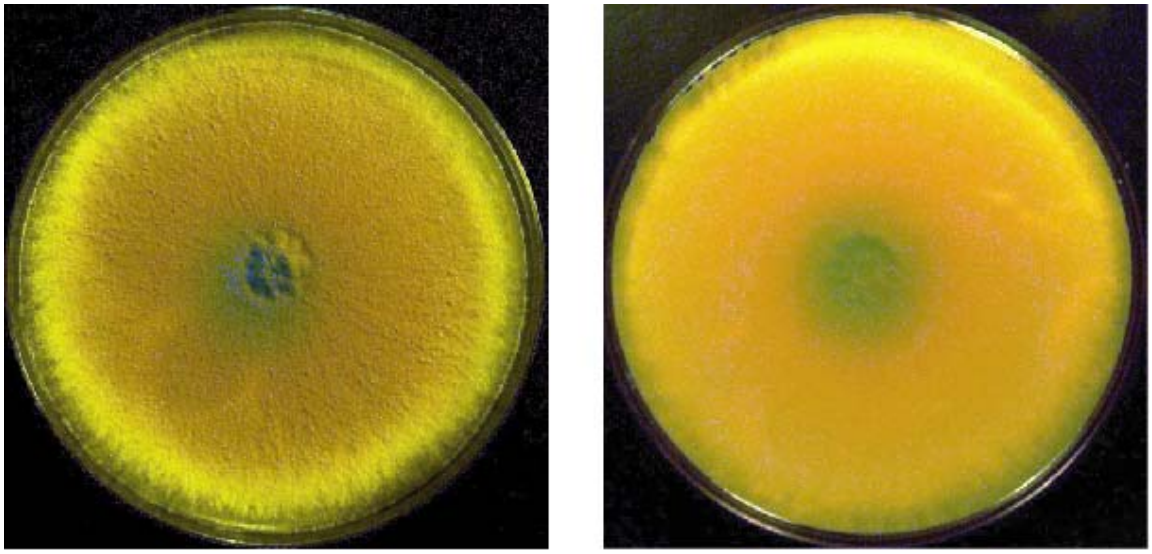

Figure 9. (A) Seven-day-old, yellowish brown to tan colored powdery colony of *Paecilomyces* on Sabouraud's dextrose agar. (B) The reverse of seven-day-old *Paecilomyces* colony on Sabouraud's dextrose agar.

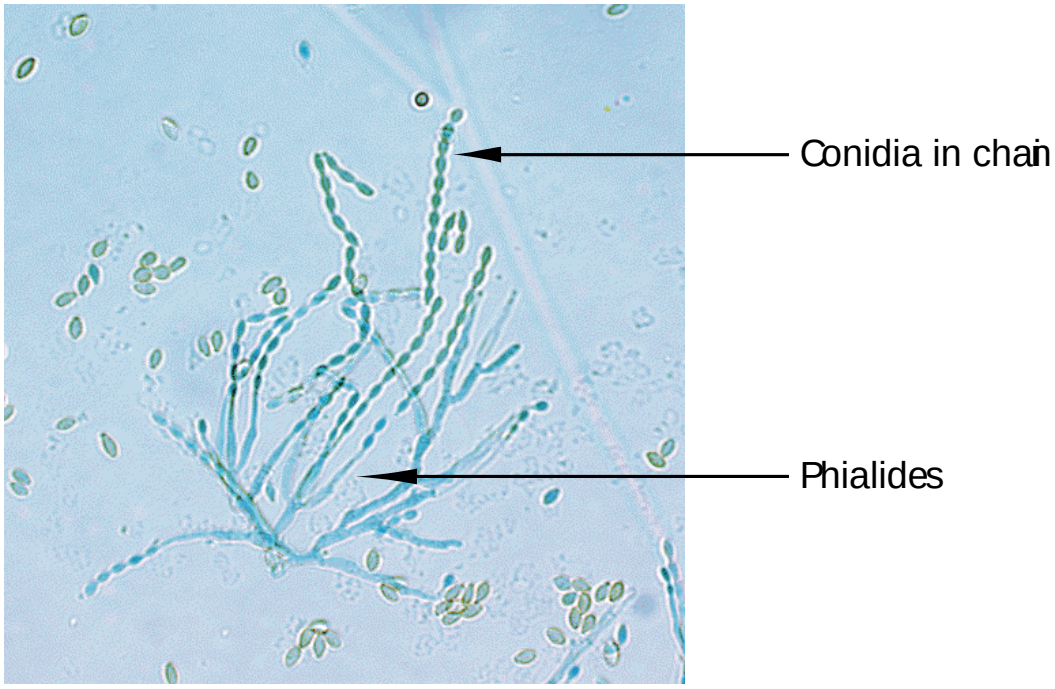

Figure 10. Microscopic morphology of *Paecilomyces.* Phialides of *Paecilomyces* was elongated and taper to a slender tube. The conidia occurred in long, unbranched chains.

YEAST DESCRIPTIONS

Y-1 *Trichosporon* spp.

Source: Nail / Urine / Tissue

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	52
Other Acceptable Answers:	
Trichosporon asahii	47
Laboratories with incorrect ID:	0
Outcome:	Validated

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

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

Laboratory Diagnosis:

1. Culture – On Sabouraud's dextrose agar, after7 days at 25°C, *T. asahii* colony was white to yellowish. The surface was wrinkled, velvety (Figure 11).
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; they originated by fragmentation of hyphae and hyphal branches (Figure 12).
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, flucytosine and azoles. Reduced-susceptibility to caspofungin is seen in some isolates.
5. Molecular tests – Sequence analysis of the ribosomal DNA intergenic spacer regions allows distinction among closely related species and clinical isolates.

Comments: In this test event, *Trichosporon asahii* was used. *T. asahii* is a new species created from *T. beigelii,* which is considered an invalidated name by Gueho and colleagues (1992).

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

```
Query      1    TCCGTAGGTGAACCTGCGGAAGGATCATTAGTGATTGCCTTTATAGGCTTATAACTATAT  60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AF444466   1    TCCGTAGGTGAACCTGCGGAAGGATCATTAGTGATTGCCTTTATAGGCTTATAACTATAT  60
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Query     61   CCACTTACACCTGTGAACTGTTCTACTACTTGACGCAAGTCGAGTATTTTTACAAACAAT  120
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AF444466  61   CCACTTACACCTGTGAACTGTTCTACTACTTGACGCAAGTCGAGTATTTTTACAAACAAT  120

Query     121  GTGTAATGAACGTCGTTTTATTATAACAAAATAAAACTTTCAACAACGGATCTCTTGGCT  180
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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   ATCGAATCTTTGAACGCAGCTTGCGCTCTCTGGTATTCCGGAGAGCATGCCTGTTTCAGT  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AF444466  245  ATCGAATCTTTGAACGCAGCTTGCGCTCTCTGGTATTCCGGAGAGCATGCCTGTTTCAGT  304

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

Query     181  TGATCGCTCGCCTTAAAAGAGTTAGCAAGTTTGACATTAATGTCTGGTGTAATAAGTTTC  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AF444466  365  TGATCGCTCGCCTTAAAAGAGTTAGCAAGTTTGACATTAATGTCTGGTGTAATAAGTTTC  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.

Further Reading:

1. Bayramoglu, G., Sonmez, M., Tosun, I., Aydin, K., and Aydin, F. 2007. Breakthrough *Trichosporon asahii* Fungemia in Neutropenic Patient with Acute Leukemia while Receiving Caspofungin. *Infection.* 36: 68-70
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5. Li H, Lu Q, Wan Z, Zhang J. 2010. *In vitro* combined activity of amphotericin B, caspofungin and voriconazole against clinical

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12. Shang ST, Yang YS, Peng MY. 2010. Nosocomial *Trichosporon asahii* fungemia in a patient with secondary hemochromatosis: a rare case report. *J Microbiol Immunol Infect.* 43: 77-80.

Figure 11. Seven-day-old, white to yellowish, wrinkled colony of *Trichosporon asahii* on Sabouraud's dextrose agar.

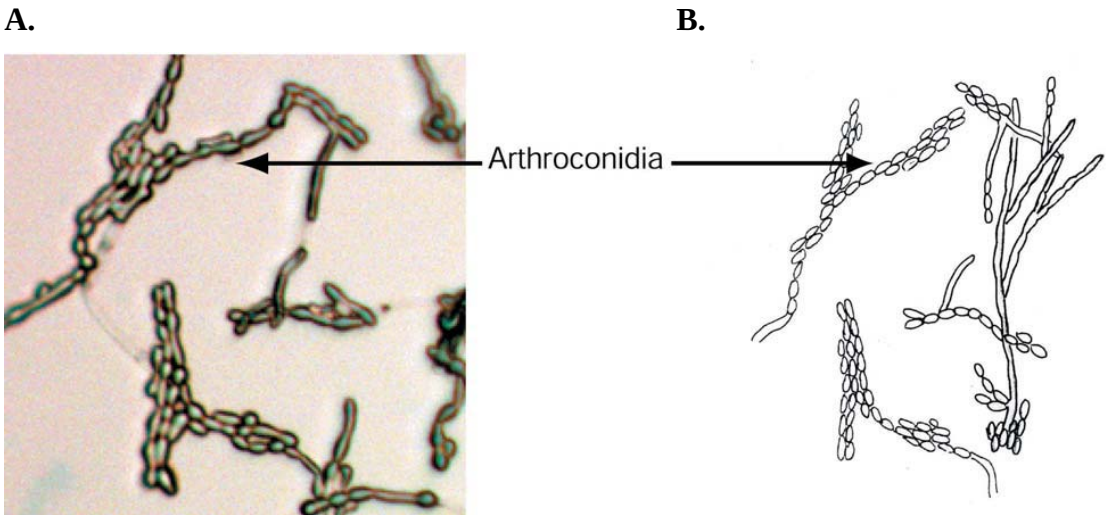

Figure 12. Microscopic morphology of *Trichosporon asahii* on corn meal agar with Tween 80 showing arthroconidia (A, 400× magnification; B, line drawing not to scale).

Y-2 *Cryptococcus albidus*

Source: Stool / Eye / Urine

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	52
Laboratories with incorrect ID:	0
Outcome:	Validated

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

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

Laboratory Diagnosis:

1. Culture – On Sabouraud's dextrose agar after 7 days at 25°C, colony was soft, mucoid, cream to pink (Figure 13).
2. Microscopic morphology – On corn meal agar with Tween 80, large, round budding yeast cells, no true hyphae or pseudohyphae are seen (Figure 14).
3. Differentiation from other yeasts – *C. albidus* does not grow on media containing cycloheximide, grows poorly at 37°C, produces urease enzyme, and assimilates nitrate. It is differentiated from *C. neoformans* by its inability to form brown colonies on niger seed agar. Although *C. terreus* is also nitrate-positive, it differs from *C. albidus* in assimilation of sorbitol and N-acetylglucosamine.
4. *In vitro* susceptibility testing – Almost all isolates are susceptible to amphotericin B, flucytosine, and azoles.
5. Molecular tests – Ribosomal DNA sequence analysis revealed diversity in *C. albidus*.

Comments: All the participating laboratories correctly identified this specimen.

Sequences alignment:

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

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

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

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

                        151                                                 200
                        TCTGTAACAA ATGTAGTCTT ATTATAACAT AATAAAACTT TCAACAACGG
                        TCTGTAACAA ATGTAGTCTT ATTATAACAT AATAAAACTT TCAACAACGG
```

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                    201                                                 250
                    ATCTCTTGGC TCTCGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA
                    ATCTCTTGGC TCTCGCATCG ATGAAGAACG CAGC
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Alignment of primary sequences of the ITS1 regions of *C. albidus* CBS 5592 and PT specimen *C. albidus* NYSDOH 0508pt.

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

                     51                                                  100
                     TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACCTTGCG
                     TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACCTTGCG

                     101                                                 150
                     CTCCTTGGTA TTCCGAGGAG CATGCCTGTT TGAGTGTCAT GAAAACCCTC
                     CTCCTTGGTA TTCCGAGGAG CATGCCTGTT TGAGTGTCAT GAAAACCCTC

                     151                                                 200
                     AACCCTAGAT TGGTTAAAAC CTCTCTTTGG TTTGGATTTG GACGTTTGCC
                     AACCCTAGAT TGGTTAAAAC CTCTCTTTGG TTTGGATTTG GACGTTTGCC

                     201                                                 250
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                     GATGATAAGT CGGCTCGTCT TAAAAGTAAT AGCTGGATCT GTCTCGCGAC

                     251                                                 300
                     ATGGTTTGAC TTGGCGTAAT AAGTATTTCG CTAAGGACAT CTTCGGATGG
                     ATGGTTTGAC TTGGCGTAAT AAGTATTTCG CTAAGGACAT CTTCGGATGG

                     301                                                 350
                     CCGCGTTGCA GGACTAAAGA CCGCTTTCTA ATCCATTGAT CTTCGGATTA
                     CCGCGTTGCA GGACTAAAGA CCGCTTTCTA ATCCATTGAT CTTCGGATTA

                     351                                                 400
                     ATACTCTTGA CATCTGGCCT CAAATCAGGT AGGACTACCC GCTGAACTTA
                     ATACTCTTGA CATCTGGCCT CAAATCAGGT AGGACTACCC GCTGAACTTA

                     401
                     AGCATATCAA TAAGCGGAGGA
                     AGCATATCAA TAAGCGGAGGA
```

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

Further Reading:

1. Burnik, C., Altintas, N.D., Ozkaya, G., Serter, T., Selçuk, Z.T., Firat, P., Arikan, S., Cuenca-Estrella, M., and Topeli, A. 2007. Acute respiratory distress syndrome due to *Cryptococcus albidus* pneumonia: case report and review of the literature. *Med Mycol.* 45: 469-73.
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immunosuppressive therapy--a case report. *Adv Med Sci*. 54: 116-119.
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7. Khawcharoenporn T, Apisarnthanarak A, Kiratisin P, Mundy LM, Bailey TC. 2006. Evaluation of *Cryptococcus laurentii* meningitis in a patient with HIV infection: a case report and review of the literature. *Hawaii Med J.* 65: 260-263.
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9. Manzano-Gayosso, P., Hernández-Hernández, F., Méndez-Tovar, L.J., Palacios-Morales, Y., Córdova-Martínez, E., Bazán-Mora, E., and López-Martinez, R. 2008. Onychomycosis Incidence in Type 2 Diabetes Mellitus Patients. *Mycopathologia.* 166:41-45
10. Narayan, S., Batta, K., Colloby, P., and Tan, C.Y. 2000. Cutaneous *Cryptococcus* infection due to *C. albidus* associated with Sezary syndrome. *Br. J. Dermatol*. 143: 632-634.
11. Wells, G.M., Gajjar, A., Pearson, T.A., Hale, K.L., and Shenep, J.L. 1998. Pulmonary cryptosporidiosis and *Cryptococcus albidus* fungemia in a child with acute lymphocytic leukemia. *Med Pediatr. Oncol*. 31: 544-546.

Figure 13. Seven-day-old, mucoid, soft colony of *Cryptococcus albidus* on Sabouraud's dextrose agar.

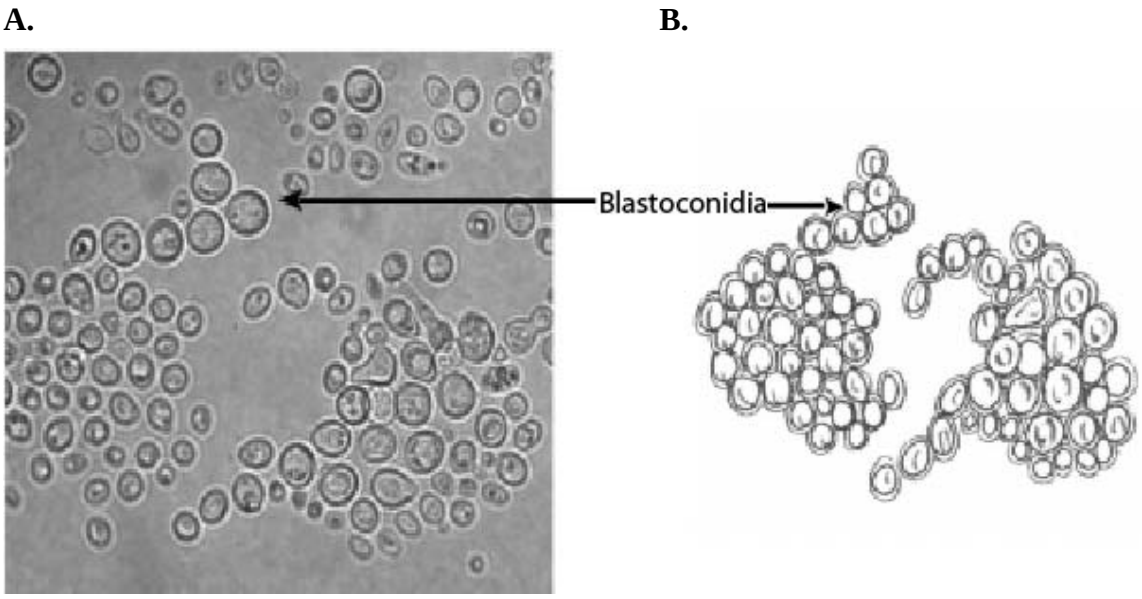

Figure 14. Microscopic morphology of *Cryptococcus albidus.* On corn meal agar culture, large, round blastoconidia are seen (A, 400× magnification; B, line drawing not to scale).

Y-3 *Rhodotorula mucilaginosa*

Source: Blood / Urine

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	42
Laboratories with incorrect ID:	2
(*Rhodotorula glutinis*)	(2)
Outcome:	Validated

Clinical Significance: *Rhodotorula mucilaginosa* is an uncommon cause of catheter-assiciated fungemia, dialysis-related peritonitis, and post surgery ventriculitis, endocarditis and meningitis.

Ecology: *R. mucilaginosa* is cosmopolitan in distribution.

Laboratory Diagnosis:

1. Culture – On Sabouraud's dextrose agar after 7 days at 25°C, colony was smooth, moist, soft, pink to coral red (Figure 15).
2. Microscopic morphology – On corn meal agar with Tween 80, oval to round yeast cells, sometimes in short chains, were seen (Figure 16). Rarely, a faint capsule and rudimentary pseudohyphae were also observed.
3. Differentiation from other yeasts – *R. mucilaginosa* does not ferment any carbohydrate, grows at 37ºC, but does not grow on media containing cycloheximide. It forms pink pigment, thereby differentiating it from other yeast species. It does not produce ballistoconidia, thus distinguishing from *Sporobolomyces* species. *R. mucilaginosa* does not assimilate nitrate or nitrite, which distinguishes it from *R. glutinis*.
4. *In vitro* susceptibility testing – *R. mucilaginosa* is susceptible to amphotericin B and 5-fluorocytosine variably susceptible to itraconazole, and resistant to fluconazole.
5. Molecular tests – Using species-specific oligonucleotide primers, PCR identification of the basidiomycetous yeasts *Cryptococcus neoformans*, *Trichosporon cutaneum*, and *R. mucilaginosa* were done from single and mixed yeast populations.

Comments: *R. mucilaginosa* is the most common cause of fungemia, followed by *R. glutinis.* Two participating laboratories have reported this isolate as *R. glutinis*; the two can be distinguished by negative nitrate assimilation test for *R. mucilaginosa.*

Sequences 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 *R. mucilaginosa* S22834 (Genebank accession number: EU871493) for ITS1 region.

```
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Sbjct  61   GAGTCCGAACTCTCACTTTCTAACCCTGTGCACTTGTTTGGGATAGTAACTCTCGCAAGA  120
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Query  121  GAGCGAACTCCTATTCACTTATAAACACAAAGTCTATGAATGTATTAAATTTTATAACAA  180
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Sbjct  121  GAGCGAACTCCTATTCACTTATAAACACAAAGTCTATGAATGTATTAAATTTTATAACAA  180

Query  181  AATAAAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAG  233
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Sbjct  181  AATAAAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAG  233
```

Alignment of primary sequence of the ITS1 regions of *R. mucilaginosa* S22834 and PT specimen *R. mucilaginosa* NYSDOH 0509.

Further Reading:

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Figure 15. Seven-day-old, smooth, moist, soft, pink to coral red colony of *Rhodotorula mucilaginosa* on Sabouraud's dextrose agar.

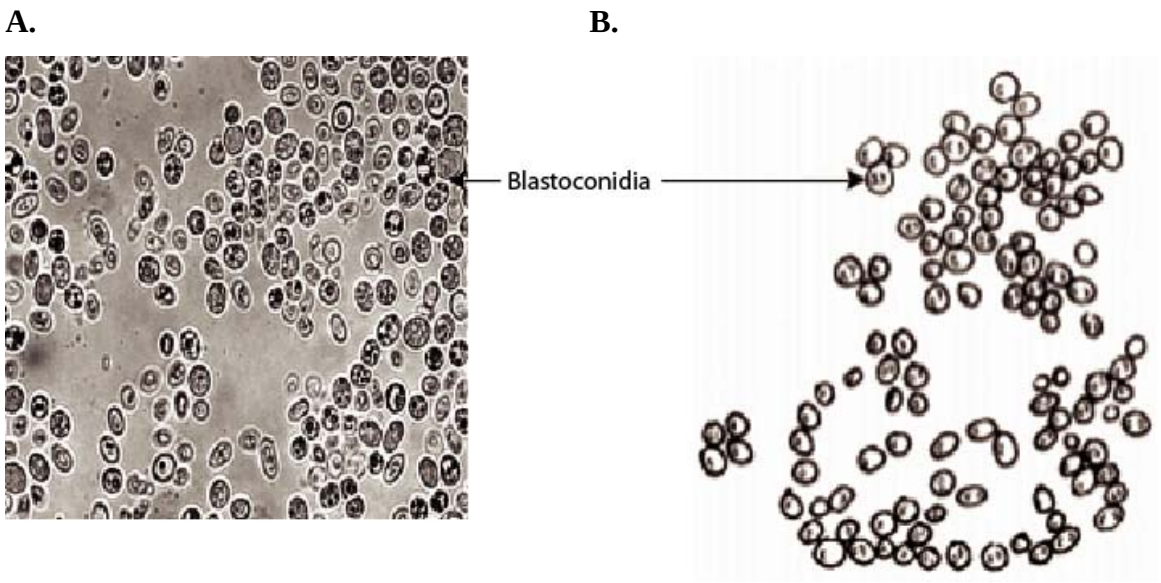

Figure 16. Microscopic morphology of *Rhodotorula mucilaginosa* on corn meal agar with Tween 80 showing oval to round blastoconidia (A, 400× magnification; B, line drawing not to scale).

Y-4 *Rhodotorula minuta*

Source: Sputum / Urine / Catheter

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	52
Laboratories with incorrect ID:	0
Outcome:	Validated

Clinical Significance: *Rhodotorula minuta* is reported as a rare/unusual causative agent of systemic 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 17).
2. Microscopic morphology – On corn meal agar with Tween 80, *R. minuta* had no pseudohyphae, round blastoconidia were seen (Figure 18).
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 – ITS sequences information is available to be used for molecular identification.

Comments: All the participating laboratories reported this specimen correctly.

Sequences alignment:

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.

```
Query     1    CCGTAGGTGAACCTGCGGAAGGATCATTAATGAATTTTAGGACGTTCTTTTTAGAAGTCC  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AF444627  2    CCGTAGGTGAACCTGCGGAAGGATCATTAATGAATTTTAGGACGTTCTTTTTAGAAGTCC  61

Query     61   GACCCTTTCATTTTCTTACACCGTGCACACACTTCTTTTTTACACACACTTTTAACACCT  120
               |||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
AF444627  62   GACCCTTTCATTTTCTTACACTGTGCACACACTTCTTTTTTACACACACTTTTAACACCT  121

Query     121  TAGTATAAGAATGTAATAGTCTCTTAATTGAGCATAAATAAAAACAAAACTTTCAGCAAC  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AF444627  122  TAGTATAAGAATGTAATAGTCTCTTAATTGAGCATAAATAAAAACAAAACTTTCAGCAAC  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.
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Figure 17. Seven-day-old, soft, smooth, pink colony of *Rhodotorula minuta* on Sabouraud's dextrose agar.

A. **B.**

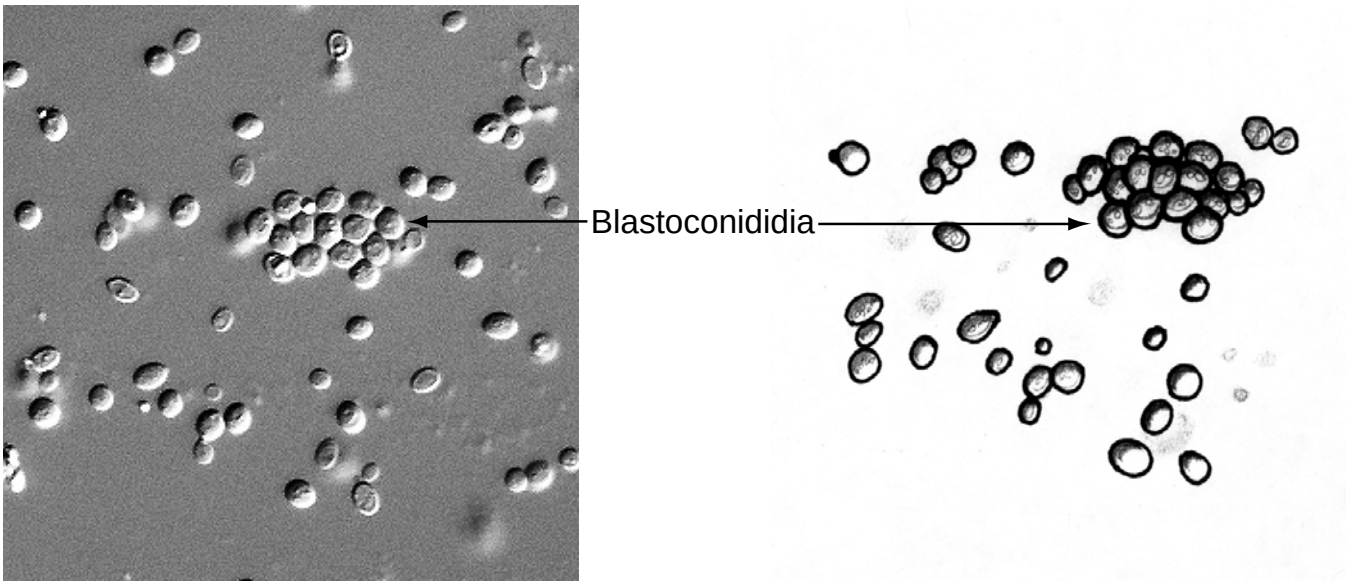

Figure 18. Microscopic morphology of *Rhodotorula minuta* on corn meal agar with Tween 80 showing round blastoconidia (A, 400× magnification; B, line drawing not to scale).

Y-5 *Cryptococcus laurentii*

Source: CSF / Urine

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	9
Laboratories with correct ID:	45
Laboratories with incorrect ID:	7
(*Malassezia furfur* complex)	(3)
(*Candida lipolytica*)	(1)
(*Candida utilis*)	(1)
(*Malassezia* sp.)	(1)
(Unable to validate)	(1)
Outcome:	Validated

Clinical Significance: *Cryptococcus laurentii* has been infrequently reported as an etiologic agent of infections in humans. Several cases ranging from fungemia to eye infections have been documented in diabetics and other immunocompromised individuals.

Ecology: *C. laurentii* is found in the environment – soil, leaves, insects, seawater, and air.

Laboratory Diagnosis:

1. Culture – On Sabouraud's dextrose agar, after 5 days at 25°C, colony of *C. laurentii* ranged from cream, yellow, tan, or pink, and the color intensified as the culture aged (Figure 19).
2. Microscopic morphology – On corn meal agar with Tween 80, *C. laurentii* showed round to oval cells (Figure 20). There was no discernible capsule.
3. Differentiation from other yeasts – *C. laurentii* shares many characteristics with the other members of the genus *Cryptococcus*. It produces urease enzyme, assimilates inositol, and does not ferment carbohydrates. It could be differentiated from *C. neoformans* by inability to form brown colonies on Niger Seed Agar.
4. *In vitro* susceptibility testing – In general, non-*neoformans Cryptococcus* species are susceptible to amphotericin B and various azoles. However, some isolates of *C. laurentii* were found to be resistant to fluconazole.
5. Molecular tests – *C. laurentii* was reported to be a heterogeneous species, based on nuclear DNA base composition and whole cell protein electrophoretic fingerprints.

Comments: A few participating laboratories reported this specimen as *Malassezia* sp; Vitek 2 yeast identification system was used by these facilities. However, other laboratories using the same system reported correct ID.

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 *Exophiala Cryptococcus laurentii* isolate ML3089 (Genebank accession number: AF410468) for ITS1 and ITS2 regions.

```
Query  1    TCCGTAGGTGAACCTGCGGAAGGATCATTAAAGATTGACCGAAAGGTCTTATCTCTATAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TCCGTAGGTGAACCTGCGGAAGGATCATTAAAGATTGACCGAAAGGTCTTATCTCTATAT  60
```

Alignment of primary sequences of the ITS1 and ITS2 regions of *Cryptococcus laurentii* isolate ML3089 and PT specimen *C. laurentii* M2341.

Further Reading:

1. Averbuch, D., Boekhoutt, T., Falk, R., Engelhard, D., Shapiro, M., Block, C., and Polacheck, I. 2002. Fungemia in a cancer patient caused by fluconazole-resistant *Cryptococcus laurentii. Med. Mycol.* 40: 479-484.
2. Bauters, T.G., Swinne, D., Boekhout, T., Noens, L., and Nelis, H.J. 2002. Repeated isolation of C*ryptococcus laurentii* from the oropharynx of an immunocompromized patient. *Mycopathologia.* 153: 133-135.
3. Cheng, M.F., Chiou, C.C., Liu, Y.C., Wang, H.Z., and Hsieh, K.S. 2001. *Cryptococcus laurentii* fungemia in a premature neonate. *J. Clin. Microbiol*. 2001 39: 1608-1611.
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6. Khawcharoenporn, T., Apisarnthanarak, A., Kiratisin, P., Mundy, L.M., and Bailey, T.C. 2006. Evaluation of *Cryptococcus laurentii* meningitis in a patient with HIV infection: a case report and review of the literature. *Hawaii Med J.* 65: 260-263.
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Figure 19. Five-day-old, white creamy colony of *Cryptococcus laurentii* on Sabouraud's dextrose agar.

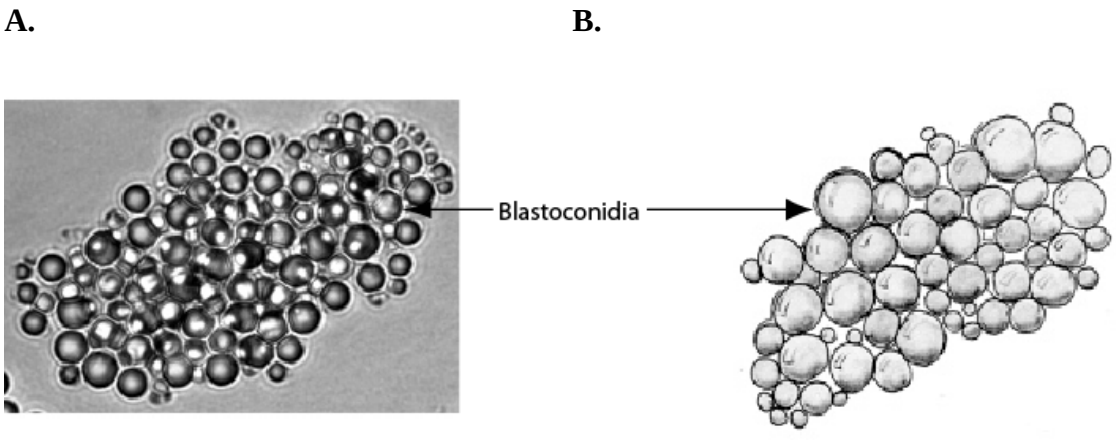

Figure 20. Microscopic morphology of *Cryptcoccus laurentii* on corn meal agar with Tween 80 showing blastoconidia (A, 400× magnification; B, line drawing not to scale).

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR YEASTS

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

Materials & Results: *Candida parapsilosis* (S-1) was the analyte in the January 26, 2011 antifungal proficiency testing event. Thirty laboratories participated in this event. The S-1 isolate was validated by all the participating laboratories. The acceptable results for antifungal susceptibility testings were based on consensus MIC values or interpretation per NCCLS/CLSI guidelines or other publications (Table 2).

Table 2. Interpretive Guidelines for *In Vitro* Susceptibility Testing of *Candida* spp.*

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

* Adapted from CLSI document M27-S3 (2008)

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

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

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

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

Summary:

Table 3. Laboratory Performance January 2011 PT Event

S- 1: *Candida tropicalis*

Acceptable Responses/Total # Laboratories (%)	
Amphotericin B	24/24 (100)
Anidulafungin	16/16 (100)
Caspofungin	21/21 (100)
Flucytosine (5-FC)	26/26 (100)
Fluconazole	30/30 (100)
Itraconazole	29/29 (100)
Ketoconzole	6/6 (100)
Micafungin	16/16 (100)
Posaconazole	17/17 (100)
Voriconazole	23/23 (100)

Table 4. Antifungal MICs (µg/ml) Reported by the Participating Laboratories

S-1: *Candida tropicalis*

Drugs (µg/ml)	Total # of labs	0.015	0.03	0.06	0.094	0.12	0.25	0.38	0.5	0.75	1	1.5	2	4
Amphotericin B	24			1			2		17	1	2		1	
Anidulafungin	16	1	4	8		2	1							
Caspofungin	21		1	10	1	5	4							
Flucytosine (5-FC)	26		3	19	1	1	2							
Fluconazole	30						1		4		16	1	7	1
Itraconazole	29	1		2	1	7	14		3		1			
Ketoconazole	6		2	2			1		1					
Micafungin	16	4	10	2										
Posaconazole	17			3		9	2	1	1				1	
Voriconazole	23		2	11		8	2							

Table 5. Antifungal Susceptibility Interpretations Reported by the Participating Laboratories

S-1: *Candida tropicalis*

Antifungal Agent	Total # of labs	Susceptible	Susceptible -dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	24	14					10
Anidulafungin	16	16					
Caspofungin	21	21					
Flucytosine (5-FC)	26	26					
Fluconazole	30	30					
Itraconazole	29	16	12	1			
Ketoconazole	6	2					4
Micafungin	16	16					
Posaconazole	17	11					6
Voriconazole	23	23					

Further Reading:

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2. Clinical and Laboratory Standards Institute. 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard - Third Edition. CLSI document M27-A3 (ISBN 1-56238-666-2).
3. Clinical and Laboratory Standards Institute. 2008. Quality Control Minimal Inhibitory Concentration (MIC) Limits for Broth Microdilution and MIC Interpretive Breakpoints; Informational Supplement - Third Edition. CLSI document M27-S3 (ISBN 1-56238-667-0).
4. Clinical and Laboratory Standards Institute. 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard – Second Edition. CLSI document M38-A2 (1-56238-668-9).
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7. Clinical and Laboratory Standards Institute. 2010. Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Approved Guideline. CLSI document M51-A (ISBN 1-56238-725-1).
8. Clinical and Laboratory Standards Institute. 2010. Performance Standards for Antifungal Disk Diffusion Susceptibility Testing of Filamentous Fungi; Informational Supplement. CLSI document M51-S1 (ISBN 1-56238-725-1).
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12. Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST definitive document Edef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. *Clin. Microbiol. Infect.* 14: 398-405.
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determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia–forming moulds. *Clin. Microbiol. Infect.* 14: 982-984.
14. Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST technical note on voriconazole. *Clin. Microbiol. Infect.* 14: 985-987.

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: Sixty-seven laboratories participated in the January, 2011 direct detection antigen test event. Two positive serum samples for cryptococcal antigen were included. The titers were 1:256 ~ 1:512 and 1:128 for Cn-Ag-1 and Cn-Ag-5 respectively. Titers within ± 2 dilutions of the reference and/or consensus results were the acceptable results for this event.

Results: Overall, the performance of 65 laboratories was satisfactory in this test event. The consensus for specimen Cn-Ag-2, Cn-Ag-3, and Cn-Ag-4 was negative, for specimen Cn-Ag-1 and Cn-Ag-5 was positive with the acceptable titer ranges 1:64 ~ 1:2048 and 1:32 ~ 1:512 respectively. One laboratory reported negative result for specimen Cn-Ag-1. One laboratory each reported positive for Cn-Ag-2 and Cn-Ag-3 respectively. One laboratory reported the titer higher than the acceptable titer ranges for both specimen Cn-Ag-1 and Cn-Ag-5. One laboratory reported the titer higher than the acceptable titer range for specimen Cn-Ag-5 only. The supplementary information on quantitative assays on *Cryptococcus neoformans* antigen test is summarized in Table 6.

Table 6. Summary of quantitative assay

Method		**Cn-Ag-1 Titers**							
	# laboratories	64	128	256	400	512	1024	2048	4098
Latex Agglutination									
Immuno-Mycologics	7			2		2	2		1
Inverness Medical	1			1					
Meridien Diagnostic	46	1	5	15	2	18	4	1	
Remel	8		1	5		2			
Total	62	1	6	23	2	22	6	1	1

Method		**Cn-Ag-5 Titers**								
	# laboratories	32	64	128	160	200	256	512	1024	2048
Latex Agglutination										
Immuno-Mycologics	7			3			1	1	1	1
Inverness Medical	1			1						
Meridien Diagnostic	46		4	17	1	1	15	7	1	
Remel	8		1	5			2			
Total	62	0	5	26	1	1	18	8	2	1

Further Reading:

1. Bennett, J.E., Hasenclever, H.F., and Tynes, B.S. 1964. Detection of cryptococcal polysaccharide in serum and spinal fluid: value in diagnosis and prognosis. *Trans. Assoc. Am. Physicians*. 77: 145-150.
2. Bloomfield, N., Gordon, M.A., and Elmendorf, D.F., Jr. 1963. Detection of *Cryptococcus neoformans* antigen in body fluids by latex particle agglutination. *Proc. Soc. Exp. Bio. Med.* 114: 64-67.
3. De Jesus, M., Hackett, E., Durkin, M., Connolly, P., Casadevall, A., Petraitiene, R., Walsh, T.J., and Wheat, J. 2007. Galactoxylomannan does not exhibit cross-reactivity in the platelia *Aspergillus* enzyme immunoassay. *Clin. Vaccine Immunol.* 14: 624-427.
4. Diamond, D. and Bennett, E. 1974. Prognostic factors in cryptococcal meningitis. *Ann. Int. Med.* 80; 176-181.
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9. Singh N, Alexander BD, Lortholary O, Dromer F, Gupta KL, John GT, del Busto R, Klintmalm GB, Somani J, Lyon GM, Pursell K, Stosor V, Muñoz P, Limaye AP, Kalil AC, Pruett TL, Garcia-Diaz J, Humar A, Houston S, House AA, Wray D, Orloff S, Dowdy LA, Fisher RA, Heitman J, Wagener MM, Husain S. 2008. Pulmonary cryptococcosis in solid organ transplant recipients: clinical relevance of serum cryptococcal antigen. *Clin. Infect. Dis.* 46: e12-18.

Summary of Molecular Tests Survey

During the January 2011 event, a survey of current practices on molecular tests was performed. A total 130 copies of survey sheets were sent out and 116 laboratories responded. The responses were as follow:

Molecular tests done in your laboratory:

PCR/RT-PCR on tissues and body fluids		10
PCR/RT-PCR on fixed tissues		3
PCR-Sequencing for fungal identifications/characterization		5
RT-PCR with species specific probes		3
Molecular typing		
Restriction Fragment Length Polymorphism (RFLP)	1	
Random Amplification of Polymorphic DNA (RAPD)	1	
Pulse Field Gel Electrophoresis (PFGE)	1	
DNA repeat probes	0	
Multi-locus Sequence Typing	1	
Other types of molecular typing	0	
DNA/RNA proves for fungal identification		1
Gen-Probes		25
In situ Hybridization/FISH		4
Others		5

Do you get request for molecular tests?

Yes	28*
No	77

*11 of those not for mycology

If yes, where these specimens are tested?

Test is done in your own lab	12
Wadsworth Center	11
NYSDOH licensed labs	18
Commercially available services	20

Do you anticipate introducing molecular testing in near future?

Yes	30
No	86

Conclusions:

Molecular testing for fungal identification will be introduced in PT event as a new sub-category in near future.

Summary of *Aspergillus* Galactomannan Antigen Test Survey

During the January 2011 event, a survey of current practices on *Aspergillus* galactomannan antigen test was performed. A total 130 copies of survey sheets were sent out and 118 laboratories responded. The responses were as follow:

1. **Do you send out *Aspergillus* Galactomannan Antigen Test specimens to reference laboratories?**

State Lab, Albany	6
Other Reference labs in New York State	18
Outside State Labs	50

2. **Do you anticipate that your facility will initiate *Aspergillus* Galactomannan Antigen Test within the next year?**

Yes	7
No	111

3. **Does your facility currently perform in *Aspergillus* Galactomannan Antigen Test?**

Yes	4
No	114

4. **If yes to item 3, please specify specimens tested:** serum
   Please specify method/kit/device used: Bio-Rad Platelia *Aspergillus* EIA

Conclusions:

A very limited number of laboratories currently perform *Aspergillus* galactomannan antigen test. For the time being, this antigen detection test may not be added to the Direct Detection panel.

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